### Oral Session 1

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### Oral Session 2

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In vitro studies suggest that the type I interferon (IFN) signature seen in SLE patients results from Fcγ receptor-mediated uptake of immune complexes consisting of nucleic acid containing autoantigens plus IgG autoantibodies and engagement of endosomal Toll-like receptors. We revisited the pathogenesis of the IFN-signature. Both wild type and FcγR-deficient mice with pristane-induced lupus developed a strong IFN-signature, which was absent in immunoglobulin (μMT), C3, or complement receptor (CD18) deficient mice. I.V. infusion of normal IgM restored the IFN signature in μMT mice, whereas the IFN signature in wild type mice was inhibited by depleting complement, suggesting that opsonization by IgM and complement is involved in IFN production. Consistent with that possibility, “natural” IgM antibodies reactive with dead cells were increased in both pristane-treated mice and SLE patients, and correlated with disease activity. Phagocytosis of apoptotic cells by macrophages involved both opsonin-dependent (complement receptor) and opsonin independent (scavenger receptor) pathways. To explore the clinical relevance, ten C4-deficient lupus patients were compared with 152 C4-intact patients and 21 healthy controls. Nephritis and anti-Sm/RNP antibodies (associated with increased IFN-I) were rare in C4-deficient patients and the IFN-signature was absent, suggesting that the IFN signature also is complement-dependent in humans. These studies define a previously unrecognized role of natural IgM, complement, and complement receptors in generating the IFN signature in lupus.
TCR signaling defect by ZAP70 mutation causes lupus like nephritis on C57BL/6 background

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[Introduction] T cell receptor (TCR) signaling defects are the risk factors for systemic lupus erythematosus (SLE). The skg gene which is a point mutation of ZAP70 gene, attenuates TCR signaling and causes autoimmune arthritis on BALB/c background. Because the mice on C57BL/6 background are more prone to develop lupus than on BALB/c background, we tried to determine whether C57BL/6, ZAP70 skg/skg (B6SKG) mouse develops lupus. [Methods] Development of lupus was assessed by the production of anti-DNA antibody and the IgG and C3 deposits in the kidney by immunohistochemistry. Follicular helper T cells (Tfh) were determined as CD4+CXCR5+Bcl-6+ PD-1+ cells, and germinal center (GC) B cells were determined as B220+AA4.1-FAS+GL-7+ cells by flow cytometry. The formation of GCs was confirmed as PNA+GL-7+CD38- area in the spleen by immunohistochemistry. [Results] After the continuous stimulation with some Toll like Receptor (TLR) agonists (ex. poly (I:C); TLR3 agonist), B6SKG mice, but not wild type C57BL/6, BALB/c, or SKG/b mice, developed lupus-like nephritis. The proportion of Tfh were higher in B6SKG mice (4-6 %) than in wild type C57BL/6 mice (1-2%) and further expanded after TLR stimulation. The proportion of GC areas in spleen was also more increased in B6SKG than wild type C57BL/6 mice. [Conclusion] TCR signaling defect caused autoimmune arthritis or lupus depending on genetic backgrounds. Because TCR signaling defects are reported in human SLE patients, B6SKG mouse can be a model to study the role of Tfh and GC B cells in the pathogenesis of SLE.
LRG promotes the differentiation of Th17 and deteriorate collagen-induced arthritis.

Hyun Lee¹, Hayato Urushima¹, Minoru Fujimoto¹, Takashi Mishima¹, Tomoharu Ohkawara¹, Hiromi Honda¹, Satoshi Serada¹, Tetsuji Naka¹

[Backgrounds] We have identified leucine-rich α2 glycoprotein (LRG) as a disease marker of rheumatoid arthritis (RA). LRG is reported to bind with TGF-β and enhance Smad2 phosphorylation. TGF-β is essential for differentiation of both Treg and Th17, the latter of which are induced with the coexistence of IL-6. In this study, using collagen induced arthritis (CIA) model, we aimed to elucidate the role of LRG in the pathogenesis of RA, with a particular focus on Th17 differentiation.

[Methods] Male C57BL/6 mice and LRG KO mice were subjected to CIA. Populations of Treg and Th17 in inguinal lymph nodes on day27 were analyzed. Naïve T cells isolated from WT or LRG KO mice were cultured under the Th17- or Treg-inducing condition to examine the effects of recombinant LRG on this process.

[Results] The arthritis scores and Th17 frequencies in lymph nodes were significantly reduced in LRG KO mice than in WT mice, with no overt difference in Treg populations. Recombinant LRG together with TGF-β enhanced Smad2 phosphorylation in naïve T cells. Recombinant LRG could also increase TGF-β-induced IL6 receptor (IL6R) expression, consistent with a previous finding that TGF-β-Smad2 pathway contributes to IL6R expression. Moreover, in LRG KO mice, IL6R expression in naïve T cells was low compared to WT mice, accounting for the finding that IL-6-induced STAT3 activation was attenuated in these cells from KO mice.

[Conclusions] LRG is critical for autoimmune arthritis by enhancing TGF-β-Smad2 pathway, increasing IL6R expression and promoting Th17 differentiation in naïve T cells.
RNA sequencing of immune cell subsets before and after biologics in rheumatoid arthritis.

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Background: Biologic response modifiers have improved functional outcomes significantly in patients with rheumatoid arthritis (RA). However, their functional mechanisms are largely unknown. To explore the immunological change after treatment, we examined peripheral blood cell transcriptome before and after biologics treatment.

Method: We performed RNA sequencing of peripheral blood cell subsets including CD4+ T cells, B cells, monocytes, dendritic cells, natural killer cells and neutrophils from RA patients before and after biologics treatments. 5 patients were treated with tocilizumab, 7 patients were treated with abatacept, and 1 patient was treated with tofacitinib. Genotyping was also performed with SNP microarray.

Result: Each treatment resulted in a different influence on gene expression in different cell subsets. Abatacept treatment had a major influence on gene expression of CD4+ T cells and neutrophils, although tocilizumab had a major influence on B cells and monocytes. Although the different gene expression patterns were observed after each treatment, changes in expression of genes associated with mitochondrial respiratory pathway were observed in common.

Conclusion: This study unveils the different modes of action of biologics. The observation of changes in mitochondrial pathway after every biologics treatment might indicate the relationship of mitochondrial dysfunction and RA pathogenesis.
Semaphorin 4D inhibits neutrophil activation and is involved in the pathogenesis of autoimmune vasculitis

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Interactions between neutrophils and vascular endothelial cells regulate local inflammatory responses. However, the molecular mechanism underlying this regulation remains unclear. Here, we show that semaphorin 4D (SEMA4D) inhibits neutrophil activation by acting as a receptor for endothelial plexin B2 and that disruption of this interaction is involved in the pathogenesis of anti-neutrophil cytoplasmic antibody (ANCA)-associated small-vessel vasculitis. We found that serum levels of soluble SEMA4D were elevated in vasculitis patients and were correlated with clinical disease scores, a possible consequence of the cleavage of SEMA4D from the surface of activated neutrophils. Indeed, inflammatory stimuli induced proteolytic shedding of SEMA4D. We also found that SEMA4D on the neutrophil cell surface bound to plexin B2 on endothelial cells, and this binding was required for the suppression of neutrophil extracellular trap (NET) formation. Furthermore, treating neutrophils with recombinant plexin B2 led to almost complete inhibition of the neutrophil oxidative burst by suppressing Rac1 activation. Collectively, our results highlight not only the regulatory function of SEMA4D in neutrophil inflammatory activation achieved through its interaction with vascular endothelium, but also the exacerbating effect of its cleavage on neutrophil-mediated autoimmune responses.
Type II collagen immune complex induce granulocyte dependent augmentation of chemokines via TLR4; a possible therapeutic target in acute onset early RA

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Anti collagen type II (CII) positive RA patients have a distinct acute onset phenotype, associated with cytokine induction by surface bound anti CII immune complexes (IC). Mononuclear cells and PMN accumulate in RA joints proximal to hyaline cartilage where anti CII IC might form.

Healthy donor PMN and PBMC were stimulated individually or together (cocultures) with surface bound IC (anti CII IC and control IC), GMCSF or LPS. Blocking and neutralizing studies were performed with antibodies against TLR4, FcgRIIa, FcgRIII and GMCSF. Cytokine and chemokine levels were analyzed with ELISA or ALBIA.

Anti CII IC induced cytokine production by PBMC, whereas PMN alone produced negligible cytokine levels. Levels of CXCL8, RANTES and MCP1 were specifically upregulated in cocultures stimulated with anti CII IC but not control IC. The CXCL8 upregulation was dependent on anti CII IC, as CXCL8 production was downregulated in cocultures stimulated with control IC or LPS. The increase of CXCL8 in anti CII IC stimulated cocultures totally depended on TLR4, partly on PMN enzymes and density of anti CII in IC. Like anti CII IC, GMCSF induced coculture dependent CXCL8 enhancement, and GMCSF neutralization diminished the anti CII IC dependent CXCL8 enhancement.

In anti CII positive RA patients, PMN can amplify accumulation of inflammatory cells and acute onset inflammation by inducing chemokines via a mechanism dependent on TLR4, PMN enzymes, GMCSF and the joint specific autoantigen CII. We now study anti CII in large scale RA studies.
RNA-binding proteins (RBPs) are known regulators of gene expression on several levels including chromatin modification, splicing, export, stability, localization and translation. How these post-transcriptional processes interconnect with one another and operate as functionally related groups of RNAs is an area of active investigation. There is abundant evidence showing that RBPs regulate these processes by using ribonucleoprotein (RNP) codes to position their RNA targets in the right time and space. An overriding principle of RNA regulation is that RNA targets are coordinated by RBPs as RNA regulons that orchestrate the production of subsets of functionally related proteins, the workhorses of the cell. In fact, RBPs are highly conserved in evolution possibly having crafted the transcriptional apparatus in early stem cells. Moreover, RNA regulons are dynamically remodeled in activated cells, and yet can be rewired over evolutionary time. I will discuss RBP autoimmune and paraneoplastic autoantigens that regulate dynamic RNA regulons and use RNP codes to coordinate the production of regulatory pathways and macromolecular complexes. While many RNA regulons of autoimmune RBPs have been discovered using different methods, I will discuss a novel set of quantitative methods that elucidate dynamically coordinated interactions of RNA regulons with their targeted mRNAs, microRNAs and IncRNAs.
Reducing false positive ANA rates by determination of anti-DFS70 antibodies

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Positive antinuclear antibodies (ANA) determined by indirect immunofluorescence using HEp-2 cells may inappropriately induce testing for or even diagnosis of a definite connective tissue disease (CTD), such as systemic lupus erythematosus (SLE), Sjögren’s syndrome (SjS), systemic sclerosis (SSc) and idiopathic inflammatory myopathies (IIM) as well as undifferentiated connective tissue disease (UCTD). The aim of this study was to determine the performance of anti-DFS70 antibodies in predicting the absence of a definite CTD in ANA positive individuals. To address this issue, we determined sera of 352 apparently healthy individuals (AHI), 1060 patients of a routine cohort (RC) positive for homogeneous ANA in the absence of CTD associated ANA specificities, 575 patients with definite CTDs (292 SLE, 171 SSc, 76 IIM, 36 SjS), 62 patients with UCTD and 618 non-CTD patients for anti-DFS70-Ab by a chemoluminescence assay (QUANTA FLASH DFS70, Inova Diagnostica, Inc., San Diego, USA). Anti-DFS70 was found in 5.1% of AHI, 19.6% of RC, 1.0% of definite CTD, 11.3% of UCTD and 1.9% of non-CTD patients. None of the 208 anti-DFS70 positive RC patients was diagnosed with a CTD up to now. Only 6 CTD patients, including 1 SLE patient, were anti-DFS70 positive, all of whom had additional CTD-specific autoantibodies. Thus, isolated anti-DFS70 had 100% specificity for the absence of a definite CTD. These data suggest that anti-DFS70 antibodies are useful for differentiating an ANA positive subset very unlikely to have a CTD from patients with definite CTDs.
Anti-DNA antibodies are internalized into live cells via cell surface DNA

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[Objective] Anti-DNA antibodies are produced in patients with systemic lupus erythematosus, but their pathogenic roles remain obscure. Normally, most antibodies do not enter live cells. It is postulated, however, that lupus autoantibodies have exceptional capacity to enter the cells, and modify cell function, or damage the cells, although the mechanism of the internalization is still unclear. Previously we observed that DNA/nucleosome is usually attached on the surface of cultured cells, therefore we hypothesized that binding to the cell surface DNA might facilitate the incorporation of anti-DNA antibodies.

[Methods] We have generated IgG mouse anti-DNA monoclonal antibodies (MoAbs) 2C10 and WB-6 which were purified by protein A column. Human vascular endothelial cell line EA.hy926 and human laryngeal epithelial cell line HEp-2 were treated or untreated with DNase I, followed by incubation with one of the MoAbs for 2 hours at 37°C. After wash, fixation, permeabilization and blocking, fluorescence-labeled second antibody was added, and analyzed by fluorescence microscopy and flowcytometry.

[Results] Anti-DNA MoAbs, but not isotype-matched IgG, entered the cytoplasm and/or reached to the nucleus of live cells. Pretreatment of the cells with DNase I significantly reduced the internalization of MoAbs, indicating that cell surface DNA fragments facilitate the internalization.

[Conclusion] Anti-DNA antibodies can be internalized into live cells after binding to the cell surface DNA. This phenomenon may be relevant to the pathogenic role of anti-DNA antibodies.
β2-glycoprotein I (β2GPI) complexed with phospholipid is recognized as a major target for autoantibodies in Antiphospholipid syndrome (APS); however, less than half of the patients with clinical manifestations of APS possess autoantibodies against the complexes. Therefore, the range of autoantigens involved in APS remains unclear. Recently, we found that HLA class II molecules transport misfolded cellular proteins to the cell surface via association with their peptide-binding grooves. Furthermore, IgG heavy chain/HLA class II complexes were specific targets for autoantibodies in rheumatoid arthritis. Here, we demonstrate that intact β2GPI, not peptide, forms a complex with HLA class II molecules. Strikingly, 100 of the 120 APS patients (83.3%) analyzed, including those whose antiphospholipid antibody titers were within normal range, possessed autoantibodies that recognize β2GPI/HLA class II complexes in the absence of phospholipids. In situ association between β2GPI and HLA class II was observed in placental tissues of APS patients but not in healthy controls. Furthermore, autoantibodies against β2GPI/HLA class II complexes mediated complement-dependent cytotoxicity against cells expressing the complexes. These data suggest that β2GPI/HLA class II complexes are a target in APS that might be involved in the pathogenesis. Because antigenicity of β2GPI complexed with HLA class II molecules may be different from that of native β2GPI, β2GPI/HLA-DR complex might have induced autoantibody production in APS as a neo-self antigen.
Background: Research on correlation between serum/saliva biomarkers and systemic damages of Primary Sjögren’s syndrome (pSS) would be beneficial to the clinical assessment and mechanism study. Novel antibodies, such as anti-CA6, anti-SP1 and anti-PSP antibodies, has showed clinical significance in the early diagnosis of pSS.

Objectives: To further investigate the roles of novel antibodies in the development of pSS.

Methods: Serum and saliva samples were collected from 88 pSS patients and 33 healthy controls. Novel antibodies level was examined by ELISA assays. Change of the antibody titer was evaluated in 16 pSS patients between onset of the disease and 2 years follow up.

Results: 1. Anti-CA6 antibody IgG, IgA and anti-PSP antibody IgG were notably correlated with ESSDAI scores ($p<0.05$). 2. All three antibodies had the highest positive rate with 5 years of disease duration. 3. The IgG levels of saliva anti-CA6, anti-SP1 and anti-PSP antibodies were remarkably elevated in pSS. 4. The titer of the three antibodies decreased significantly during their follow up visit ($p<0.05$).

Conclusions:
1. There are strong clinical correlation between novel antibodies and disease manifestations in pSS. Novel antibodies are promising serum biomarkers for the early diagnosis of pSS and can be useful for further disease assessment.
2. Measuring novel autoantibodies in the saliva offer the opportunity for further investigation of possible mechanism of localized salivary injury and the exposure of antigens in the development of pSS.
Nuclear Dense Fine Speckled ANA Pattern is Associated with a Normal Laboratory Profile

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Nuclear dense fine speckled (DFS) ANA pattern rarely occurs in systemic autoimmune diseases, but occurs in 1-9% of the general population. Would there be subclinical pathologic processes going on in apparently normal individuals with a DFS ANA test? We performed a retrospective analysis (Jan/2006-Dec/2013) of demographic data and 15 laboratory parameters in individuals with DFS ANA using the databank of a large clinical laboratory (~12,000 ANA/month). Records with DFS pattern were compared with those with negative ANA result (NR), Homogeneous (HO) and Coarse Speckled (CS) patterns regarding hemoglobin, CRP, ESR, ferritin, albumin, liver enzymes, glucose, serum complement components, and white/red blood cell count. In total, 254,840 records were eligible for analysis: DFS (7.1%), HO (0.8%), CS (0.7%), NR (91.4%). DFS pattern was associated with younger age and male gender, comparing to HO and NR ANA results. Individuals with DFS pattern had lower frequency of abnormal results in most laboratory parameters in comparison to those with HO or CS patterns, and closely resembled those with no ANA reactivity. Longitudinal analysis was performed in 5,822 individuals with multiple ANA tests. DFS pattern was stable along the years, maintaining high titer and rarely changing to NR ANA or to other patterns. ANA DFS pattern represents a temporally stable humoral response, with a laboratory profile closely resembling that of individuals with no ANA reactivity and definitely distinct from those with HO or CS ANA reactivity (especially regarding acute phase reactants and complement tests).