On September 20, 2016 under the auspices of the American Autoimmune Related Disease Association Drs. Rose and Tsokos convened 16 experts in Washington DC to discuss current issues and future directions in autoimmune diseases from the cell signaling perspective. Current aspects on T follicular helper (Tfh) cells, metabolic requirements of immune cells, regulatory T cells (Tregs) and other immune cells involved in the expression of tissue injury in autoimmune diseases were discussed.

1. Follicular T helper cells

John Mountz (Birmingham) presented new findings on the function of Tfh in autoimmune BXD2 mice. The BXD2 mouse is a multigenetic model of autoimmune and spontaneously develop well-formed germinal centers (GCs) in the spleen consisting of discrete light-zone (LZ) and dark-zone (DZ) regions [1,2]. Confocal imaging demonstrates two distinct populations of CD4 T-follicular helper (Tfh) T cells in the LZ that produce either IL-17+ or IL-21+ but not both cytokines [1]. FACS analysis gated on CXCR5, ICOS, and PD-1 confirms distinct IL-21 or IL-17 producing Tfh in BXD2 mice. Knockout of either IL-21 or IL-17 receptor A (IL-17RA) in BXD2 mice resulted in decreased GCs, autoantibodies and autoimmune disease. BXD2-II21−/− mice exhibited significantly decreased Tfh and decreased expression of IL-21 or IL-17 by these Tfh. Surprisingly, BXD2-II17ra−/− mice exhibited significantly increased Tfh, expressed high IL-17 and higher IL-21 by Tfh, but these Tfh were not localized in the GC light zone. These results are similar to previous findings from his lab that IL-17 signaling through IL-17RA results in upregulation of regulators of G-protein signaling (RGS)13 and RGS16, which decreases migration of CXCR5+ B cells to its chemokine, CXCL13, and can lead to more stable GC development [2,3]. The new results suggest that IL-17 produced by Tfh acts not only of GC B cells, but also on other Tfh’s to stabilize their localization in the light zone and promote GC development. This was confirmed by administration of a IL-17R:Fc which disburses Tfh from their location in the GC light zone. IL-17 also promotes formation of stable 2-cell doublets consisting
of CD4+ Tfh and CD19+ GC B cells [1]. Administration of an AdIl-17 can lead to regeneration of GCs by promoting B-T cell interaction, and this can occur in BXD2–II21−/− mice indicating IL-21 is not required to promote this Tfh-GC B cell interaction. The spontaneous GCs in BXD2 exhibited an IL-21-dependent increase in the Tfh to Tfr (T-follicular regulatory) cell ratio [4]. BXD2–II21−/− mice exhibited an increased number and regulatory function of Tfr cells which could be suppressed by administration of IL-21 [4]. Together, our results suggest distinctive function of IL-21 and IL-17 in promoting Tfh responses in BXD2 mice. While IL-21 directly promotes Tfh development and indirectly suppresses Tfr function and polarization, IL-17 acts through localization of Tfh cells to enhance Tfh-GC B cell cognate interactions. The well-orchestrated interactions of these two cytokines promoted the optimal spontaneous GC responses in BXD2 mice.

Peter Sage (Boston) presented novel data on T follicular regulatory cells and how they control Tfh-mediated antibody responses. Humoral immunity is initiated in germinal centers (GCs), specialized structures optimized to facilitate B cell activation [5]. A newly defined subset of T regulatory cells, called T follicular regulatory (Tfr) cells, inhibit T follicular helper (Tfh)–mediated antibody production in GCs and prevent autoimmunity [6,7]. Although inhibitory receptors such as PD-1 and CTLA-4 have fundamental roles in modulating Tfr differentiation and function, precise mechanisms by which Tfr cells exert their immunoregulatory functions are largely unknown [8–10]. Understanding mechanisms by which Tfr cells suppress antibody responses is essential for the development of new therapeutics to limit pathology associated with systemic autoimmune diseases. He reported recent studies in which he found that during Tfr inhibition of the GC reaction, Tfr cells induced a novel suppressed state in both Tfh and B cells in which global transcriptional programs were maintained, but key effector molecules (such as IL-4 and IL-21 in Tfh cells, and AID in B cells) and metabolic pathways (glycolysis, glutaminolysis, 1-carbon) were suppressed [11]. Epigenetic changes in B cells during Tfr-mediated suppression resulted in a durable suppressed state that was maintained after Tfr contact was eliminated. The cytokine IL-21 could overcome Tfr cell-mediated suppression by inhibiting Tfr cells and stimulating metabolism in B cells. Therefore, the GC reaction is a delicate balance of positive and negative signals (through Tfh and Tfr cells, respectively) that control B cell metabolism. Dr. Sage concluded that given the role of Tfr cells in controlling antibody responses in the context of autoimmune disease, understanding mechanisms of Tfr suppression will be essential for the development of new therapeutics that either promote Tfr cells, or mimic their suppression mechanisms.

Dr. Morel discussed the unique metabolic requirements of spontaneous follicular T helper cells in lupus-prone mice. The expansion of follicular helper CD4+ T (Tfh) cells correlates with disease severity in lupus patients and seropositive rheumatoid arthritis (RA) patients, as well as in mouse models of these diseases. Dr. Morel has shown that CD4+ T cells from lupus patients as well as from several lupus-prone mouse models present an elevated metabolism. Furthermore, treatments with inhibitors that normalized metabolism also normalized Tfh cell functions and reversed disease in mouse models of lupus, including the B6.Sle1.Sle2.Sle3 (TC) model. She presented data indicating that treatment with 2-DG that inhibits glucose metabolism resulted in a drastic reduction of Tfh cell frequency as well as the production of autoantibodies in several mouse models of lupus. TC Tfh cells expressed a glycolytic gene signature as compared with total CD4+ T cells. Treatment of normal or lupus-prone mice with 2-DG had however little effect on the production of T-dependent antibodies, the expansion of Tfh cells or antigen-specific germinal center B cells following immunization with a nominal antigen. In addition, 2-DG treatment had no effect on the frequency of influenza virus-specific Tfh cells in PR8 influenza virus infected B6 and TC mice. This suggests that Tfh cells supporting the production of autoAbs are quantitatively or qualitatively different from Tfh cells providing protective humoral immunity against pathogens. Overall, Dr. Morel’s results predict that targeting Tfh cellular metabolism provides an effective and safe therapeutic approach for systemic autoimmunity. [12–14].

Alexandra Zanin-Zhonov (New York) discussed recent advances on how targeted ROCK2 inhibition down-regulates the percentage and function of Tfh cells and its potential implication for SLE and cGVHD. The clinic. Rho-associated kinase 1 (ROCK1) and ROCK2 are activated by Rho GTPase and play critical in the coordination of T-cell-mediated immune responses [15,16]. While increased ROCK activity has been associated with autoimmunity through its capacity to regulate cytoskeletal proteins, only the ROCK2 isoform was shown to be physiologically activated in CD4+ T cells under T-helper 17 (Th-17) skewing conditions [17,18]. Moreover, oral administration of the selective ROCK2 inhibitor KD025 to healthy subjects attenuates the ability of T cells to secrete pro-inflammatory cytokines IL-21 and IL-17 in response to stimulation ex vivo via STAT3-dependent mechanism [19,20]. Concurrently, ROCK2 inhibition increases the suppressive function of human regulatory T cells (Tregs) through up-regulation of STAT5 phosphorylation [19]. Recently, it was shown that ROCK2 signaling is also required to induce a subset of human T follicular helper (Tfh) cells are essential in supporting B-cell maturation and antibody production, but also implicated in pathogenesis of autoimmune disorders [21,22]. Therefore, in the MRL/lpr murine model of systemic lupus erythematosus (SLE), ROCK2 targeting resulted in robust reduction in the percentage of both Tfh cells and antibody-producing plasma cells as well as a substantial improvement in histological and clinical scores. In addition, targeted inhibition of ROCK2 reversed the clinical and immunologic symptoms of an autoimmuno-like syndrome, chronic graft-versus-host disease (cGVHD), a complication of allogeneic hematopoietic cell transplantation, in 2 distinct murine models characterized by an immune-mediated fibrosis. In both SLE and cGVHD animal models the disease pathology was associated with concurrent down-regulation of STAT3 and up-regulation of STAT5 phosphorylation further confirming the immune-modulatory potential of selective ROCK2 inhibition in autoimmune setting in vivo. Finally, the results from a phase 2, open-label clinical study (NCT02317627 at ClinicalTrials.gov) demonstrated that oral administration of a selective ROCK2 inhibitor KD025 reduces clinical scores in patients with psoriasis vulgaris and normalizes skin pathology [24]. The clinical improvement was associated with simultaneous down-regulation of IL-17 and IL-23 levels, whereas the percentage of FOXP3+ Treg and levels of IL-10 were increased in KD025-treated subjects [24]. Dr. Zanin-Zhonov concluded that the findings from different experimental systems including Th17-skewing in vitro cell cultures, animal models and patients demonstrated that selective ROCK2 inhibition consistently down-regulates autoimmune-driven pathology via shifting the balance between pro-inflammatory and anti-inflammatory immune cell responses highlighting the therapeutic potential of targeting ROCK2 in autoimmune diseases.

Understanding the lymphokine, metabolic requirements as well as their biochemical requirements provides opportunities to further control their activity in autoimmune diseases. The discovery of Tfr has added a new complexity in understanding the control of GC cell activity. The prize of these studies is the fact that a ROCK2 inhibitor and anti-IL17 and IL-23 monoclonal Abs are in clinical trials in various autoimmune diseases whereas drugs controlling glycolysis should be considered given their success in preclinical experiments.

2. T cell metabolism

Mechanisms of action and clinical efficacy of mTOR blockade in lupus systemic lupus erythematosus (SLE) were elaborated by Andras Perl (Syracuse). A central role for mTOR activation is supported by widely corroborated evidence [25] that 1) mTOR activity is increased in T cells of patients [26] and mice with SLE [27]; 2) mTOR controls T cells lineage specification during development [28] and its skewing in SLE [29,30] 3) administration of rapamycin improves the clinical outcome of lupus in mice [27,31] and patients [29,32]; 4) rapamycin...
blocks the production of antiphospholipid antibodies in lupus-prone mice [33] and enhances renal allograft survival in patients with antiphospholipid antibodies [34], which represent a diagnostic criterion [35,36] and a source of co-morbidity is SLE [37]; and 5) mTOR blockade with rapamycin is known to be safe and increase overall lifespan, at least in mice [38]. Oxidative stress [26], depletion of intracellular glutathione [39], and accumulation of kynurenine have been identified as metabolic cues underlying mTORC1 activation in SLE [40]. Based on interim analysis of a prospective clinical trial (ClinicalTrials.gov Identifier: NCT00779194), the blockade of mTOR reduced disease activity in 126 ± 18 days as evidenced by well-tolerated rapamycin plasma levels of 8.7 ± 1.2 ng/ml, which was within the targeted therapeutic range of 6–15 ng/ml [29,26]. SLEDAI disease activity scores were reduced to 5.7 ± 1.0 from 11.8 ± 1.1 at baseline (p = 0.0028). Rapamycin inhibited the pro-inflammatory expansion and IL-4 production of CD4+ CD8+ double-negative (DN) T cells [29], which have been found to stimulate anti-DNA production by B cells [41,42]. Dr. Perl stressed that given that the blockade of mTORC1 abrogates disease activity in mice [31] and patients with SLE [29,32,39], it is important to further define the mechanism of mTOR pathway activation and its therapeutic efficacy in a mechanistic, randomized double-blind placebo-controlled clinical trial.

Alessandra Pernis (New York) discussed the regulation of Tfh cell expansion and systemic autoimmunity by the mTORC1–4E-BP1 axis. Precise regulation of T follicular helper cell (Tfh) numbers is critical for optimal humoral responses and aberrant expansion of Tfh cells has been associated with autoimmune diseases like Systemic Lupus Erythematosus (SLE). The transcriptional repressor Bcl6 is a lineage-defining factor for Tfh cells. Bcl6 is necessary to specify the Tfh cell program and overexpression of Bcl6 is sufficient to drive Tfh cell differentiation indicating that tight control of Bcl6 expression is essential to ensure proper regulation of Tfh cell numbers. The control of Bcl6 expression in Tfh cells has, till now, been shown to be mostly dependent on transcriptional mechanisms. The expression of Bcl6 is, however, well-known to be controlled by complex regulatory networks that fine-tune Bcl6 levels by targeting both Bcl6 mRNA and protein. Amongst post-transcriptional mechanisms, translational control plays a major role in regulating protein abundance and can influence protein levels to an extent similar to transcription. One of the key controllers of protein synthesis is mTOR, a serine/threonine kinase that exists in two distinct complexes, mTORC1 and mTORC2, distinguished by the presence of unique components such as raptor and rictor, respectively. Activation of mTORC1 occurs in response to diverse environmental cues including growth factors, energy status, and amino acid availability.

In line with the central role of mTOR in lymphocytes, mTOR has been implicated in the pathogenesis of autoimmune disorders like SLE. The pathways that lead to deregulation of mTOR activity and Tfh cell dysfunction in autoimmunity are, however, not fully understood. Def6 is a newly identified SLE risk variant, which together with its only homolog SWAP-70, comprises the SWEF family of molecules [43]. Unlike SWAP-70 that is expressed in B cells but not in naïve Tfh cells, Def6 is highly expressed in naïve Tfh cells [44]. Notably, the lack of Def6 either alone or combined with the absence of SWAP-70 (Double-knockout = DKO mice) leads to the development of lupus in mice, which similarly to human disease primarily occurs in female mice [45,46]. Autoimmunity in DKO mice results from dual abnormalities in T and B cells, whereby the lack of Def6 alone is primarily responsible for the T cell abnormalities while the absence of both Def6 and SWAP-70 contributes to the deregulated B cell responses [45]. We have now found that the robust humoral autoimmune responses observed in DKO mice are accompanied by a cell-intrinsic expansion of the Tfh cell compartment. Importantly DKO T cells exhibit aberrant control of Bcl6 protein synthesis, which occurs in an mTORC1- and eukaryotic initiation factor 4E (eIF4E)-dependent manner. Enhanced mTORC1 activation in DKO T cells is due to the dysregulated interaction of raptor with both p62 and TRAF6, critical regulators of an amino-acid sensing pathway of mTORC1 activation. We also demonstrate that Def6 controls the assembly of a raptor-p62-TRAF6 complex and that this pathway selectively regulates the abundance of a specific subset of proteins. Consistent with these findings rapamycin administration or T-cell deletion of raptor significantly decreased the accumulation of Tfh cells in DKO mice (Pernis et al. Unpublished data). Dr. Pernis concluded that abnormalities in the mechanisms by which mTORC1 regulates protein abundance can result in T cell dysfunction and contribute to autoimmunity.

Jose Crispin (Mexico City) provided some novel insights on the generation of Tcr-ab+ double negative (DN; CD4+ CD8−) T cells in SLE [41,47]. DN are expanded in SLE patients and infiltrate target organs and produce pro-inflammatory cytokines [48]. In humans and mice, Tcr-ab+ DN T cells can be generated from activated CD8 T cells [49,50] indicating the importance of events surrounding CD8 T cell activation. Using CD8 T cells with known TCR specificity, he has shown that CD8 expression is maintained during productive immune responses (e.g. during an acute infection with intracellular bacteria), but lost when T cells are exposed to cognate antigen presented as self [51]. In such context, CD8 downregulation was accompanied by the expression of high levels of PD-1 and by functional inactivation of the self-reactive cells [51]. These data suggested that PD-1+ DN T cells may represent self-reactive T cells that formerly expressed CD8. To test this hypothesis, he analyzed in vivo TCR signaling using the Nur77-GFP reporter in mice with a normal T cell repertoire. He observed that during steady state, DN T cells were comprised of two subpopulations: a PD-1low GFP+ subset and a PD-1high GFP+ subset demonstrating recent TCR signaling in PD-1+ DN T cells in the absence of external antigens (including commensal microbiota). Moreover, the abundance of PD-1+ DN T cells in autoimmune mice (Aire−/− and B6.Fas+TCRβ+) was abnormally high [52]. Dr. Crispin concluded that CD8 downregulation and generation of PD-1+ DN T cells represents a cellular tolerance response to exposure to self-antigens that may be defective in SLE and other conditions where TCR-ab+ DN T cells are expanded.

George Tsokos (Boston) presented data on the importance of Calcium/calcmodulin kinase 4 (CaMK4) in the expression of autoimmunity and kidney damage. He showed data which demonstrated that anti-CD3/TCR antibodies present in the sera of patients with SLE cause translocation of CaMK4 from the cytoplasm to the nucleus where it suppresses IL-2 production [53]. In the lupus prone MRL.lpr lupus-prone mouse, pharmacologic inhibition of CaMK4 with a small drug inhibitor suppresses autoimmunity and lupus nephritis [54] and more importantly, genetic depletion of Camk4 in the MRL lpr mouse results in suppression ofautoimmunity and mitigation of lupus nephritis probably, in addition to its effects on canonical immune cells, because it suppresses mesangial cell proliferation [55]. Next he showed data demonstrating that CaMK4 suppresses the generation of Tregs [56] and treatment of lupus prone mice with a small molecule inhibitor (KN93) expands Tregs and suppresses disease [57]. Further, CaMK4 was found to promote Th17 cell generation through the Akt/mTOR and CREMa pathways [58] and to enable their lodging to inflammatory tissues through the CCR6/CCL20 axis [59]. Because CaMK4 is important to many cells other than T cells, he showed data in which he delivered KN93 to CD11c cells using nanopipetals tagged with and anti-CD4 antibody. This targeted delivery of KN93 suppressed autoimmunity, skin and kidney disease at a 1/20th of the systemically required dose [60]. Interestingly, his group found that CaMK4 is increased in podocytes from patients with SLE and we considered that it may contribute to their failure to filter protein. Treatment of a podocytes (cell line) with IgG from patients with lupus nephritis promoted the expression of CDS6 (a costimulatory molecule) and suppressed the expression of nephrin, a protein necessary for the proper function of the podocyte slit diaphragm [61]. Collectively, he claimed that CaMK4 represents a link between the immune cells and the kidney cells. The significance of the presented work lies with the fact that it presented a novel target for the treatment of SLE and that it developed a targeted delivery of a small drug. Targeted delivery should minimize side effects.
Metabolic pathways have taken center stage in immune cells only recently although they have been well characterized for over half a century. It appears that distinct immune cell functions are dictated by known elements of metabolism. mTOR accounts for the production of IL-17 and the expansion of DN cells and so does the cAMP driven CREM/ICER pathway. The pay off of these discoveries is the identified early clinical value of mTOR inhibitors in patients with SLE.

3. T regulatory cells

An update on the regulatory T cells (Tregs) in lupus autoimmunity and potential targets for treatment were presented by Antonio La Cava (Los Angeles). CD4+ T Tregs inhibit pro-inflammatory and effector immune responses [62] and play a critical role in suppressing autoimmune reactivity in murine models of SLE [63,64] and in lupus patients [65,66]. Tregs have been reported as beneficial in SLE because of their ability to inhibit multiple pro-pathogenic events that not only include a direct suppression of effector T cells [63,66,67] but also of autoantibody-producing B cells [68]. This capacity of the Tregs to suppress effector cells in SLE manifests through mechanisms that include cell-mediated induction of anergy in the targets [63,69] and the release of suppressive factors [70,71] (11 – 12). Notwithstanding the advancement in understanding multiple aspects of the contribution of Tregs to the modulation of the pathogenesis of SLE, we still cannot fully and effectively harness the beneficial potential of these cells in the suppression of autoimmune responses. In studying the role of the environment on lupus Tregs [72,73], Dr. La Cava recently identified multiple epigenetic changes that occur in lupus Tregs concomitantly with changes of disease activity. He concluded that identification of targets of disease-driven epigenetic changes, for a restoration of the Tregs dysfunction in SLE may prove new options for treatment.

Thomas Malek (Miami) discussed the value of low-dose IL-2 in the restoration of tolerance in autoimmunity. Interleukin-2 is a critical cytokine that promotes tolerance through its role in Treg development and homeostasis and immunity by contributing to T effector cell expansion and function [74]. Importantly low levels of IL-2R signaling support many key activities of Tregs but not T effector cells [75]. This notion has led to the use of low-dose IL-2 to selectively target and expand Tregs in patients with autoimmunity to re-regulate and enhance immune tolerance [76,77]. Tregs increased in in most subjects that have been treated with low-dose IL-2, which is often accompanied by clinical improvement. Notably, there is no evidence that low-dose IL-2 reactivates autoreactive T cells [78]. Our group has been actively investigating mechanistic properties that account for the selective responsiveness of Tregs to low-dose IL-2. When compared to T effector/memory cells, human Tregs show a therapeutic window of ~10-fold for IL-2R signaling based on STAT5 activation and nearly ~100-fold for IL-2-dependent gene expression [79]. This selectively of Tregs to low-dose IL-2 is due to their increased expression of the high affinity IL-2R and to increased activity of the serine threonine phosphatase PP2A. Gene expression profiling provided preliminary indication that some individuals are high and low responders to low-dose IL-2. This finding suggests that the benefit of low-dose IL-2 therapy may vary. Dr. Malek concluded on the value of the use of low-dose IL-2 as a therapy for multiple autoimmune diseases.

Kamal Moudgil (Baltimore) discussed the modulation of Treg/Th17 balance in autoimmune arthritis. Rheumatoid arthritis (RA) is characterized by synovial inflammation and articular damage. An imbalance between the pathogenic T helper 17 (Th17) and protective T regulatory (Treg) cells has been invoked as one of the critical factors influencing RA pathogenesis [80]. This imbalance in turn is manifest in the uncontrolled activity of various pro-inflammatory cytokines, chemokines, and mediators of bone remodeling. Several anti-arthritis drugs targeting these mediators of inflammation are available for RA therapy, but their prolonged use is associated with severe adverse reactions. Accordingly, gradually increasing numbers of RA patients are using plant-derived and other natural products as adjuncts or alternatives to mainstream drugs [81]. However, despite the increasing popularity of such natural products, their mechanisms of action remain to be fully defined. Using the rat adjuvant-induced arthritis model of human RA, Dr. Moudgil has established the anti-arthritic activity of Celastrus, a traditional Chinese medicine, and its bioactive component celastrol, and then examined the immunological basis of that activity [82,83]. He presented evidence that treatment of arthritis rats with celastrol inhibited the key proinflammatory cytokines (IL-17, IL-6, and IFN-γ) in response to the disease-related antigens, reduced the levels of antibodies directed against cyclic citrullinated peptides (aCCP), decreased the activity of matrix metalloproteinase-9 and phospho-ERK, and deviated the RANKL/OPG ratio towards inhibition of osteoclastic activity [82,83]. In addition, he showed that celastrol treatment reduced Th17 cells but increased Treg cells in the inflamed joints, and it inhibited the production of Th17-differentiating cytokines and chemokines (CCL3, CCL5) [80]. The observation of altered Th17/Treg ratio in vivo was validated in vitro using the T cell differentiation assays, and it involved inhibition of pSTAT3 activation by celastrol. On the basis of above results, Dr. Moudgil suggested that celastrol/celastrol should be further considered for testing as a potential adjunct/alternative for RA therapy.

The role of regulatory cells in the control of the immune system has been discussed for almost half a century. The current emphasis on CD4 Tregs has been linked to their connection to their dependence to IL-2 and their ability to respond better to lower IL2 doses compared to their effector counterparts. Low dose IL-2 has been shown useful in early uncontrolled clinical trials but the success of ongoing controlled clinical trials may be qualified by the fact that SLE T cells display a weaker IL2 > IL2R > STAT5 response [84].

4. B cell biology, innate immunity and end organ damage

Shu Man Fu (Charlottesville) reviewed the genetics in his lupus model NZM2328. In this model, both female and male mice develop autoantibodies and immune complex (IC) mediated acute glomerulonephritis (aGN). However, female mice develop chronic GN (cGN) and end stage renal disease (ESR). Genetic studies revealed that autoantibody production, aGN and cGN are under separate genetic control. The phenotypes of the congenic strain NZM2328.C57L/Jc4 showed that lupus nephritis and ESR did not require the production of ANA and anti-dsDNA, suggesting the participation of multiple autoantibodies in the pathogenesis of lupus nephritis. He generated the congenic strain NZM2328.C57L/Jc1 which did not have autoantibodies and lupus-like renal disease. The phenotypes of these two congenic strains lead him and his colleagues to postulate a no-ANA centric hypothesis for the pathogenesis of SLE. This hypothesis states that autoimmunity and end organ damage are under separate genetic control and the interaction of these two pathway leads to SLE. This hypothesis is applicable to other autoimmune disorders.

His group has generated an informative intrachromosomal recombinant line NZM2328.C57L/Jc1R27 in which only the region controlling cGN was replaced the non-lupus prone allelic region. The female mice of this line have IC mediated AGN without progression to cGN and early mortality. Experiments involving the use of nephrotic serum against cyclic citrullinated peptides (aCCP), decreased the activity of matrix metalloproteinase-9 and phospho-ERK, and deviated the RANKL/OPG ratio towards inhibition of osteoclastic activity [82,83]. RANKL/OPG ratio towards inhibition of osteoclastic activity [82,83]. This hypothesis is applicable to other autoimmune disorders.
Dr. Marshak-Rothstein (Worchester) presented a novel rapidly-inducible model of cutaneous lupus erythematous (CLE) which depends on the loss of TLR9 and the expression of TLR7. The majority of patients diagnosed with SLE exhibit cutaneous manifestations, and in some cases patients are initially diagnosed with only CLE [91]. Common pathophysiologic mechanisms are thought to promote both CLE and SLE [92], and likely to include an inability to appropriately clear cell debris [93]. Nevertheless, tissue-specific effector mechanisms are also likely to come into play. It follows that a better understanding of the factors promoting CLE are relevant to SLE. However murine models available for the study of CLE are very limited. Dr. Marshak-Rothstein has now developed a novel inducible murine model for cutaneous lupus that may provide useful insights to human disease. The model depends on sublethal irradiation (tissue damage) as an environmental trigger, the induction of a “pseudo”-autoantigen expression by MHC class II expressing APCs, and the transfer of autoantigen-specific T cells. In line with other models of SLE [94–96], the development of cutaneous disease is completely dependent on expression of TLR7 and loss of expression of TLR9, and reinforces the notion that nucleic acid sensors can both promote and negatively regulate the development of SLE. The transferred T cells migrate to the skin and acquire a strong Th1 phenotype only in TLR9-deficient recipients, and not in TLR9/TLR7 double-deficient mice. Another unique feature of the model is that antigen expression can be regulated by doxycycline such that the disease can be turned on and off to mimic lupus flares. As discussed, this should facilitate the study of autoimmune memory subsets in both the B and T cell compartments.

Tanya Mayadas (Boston) presented novel data on capturing neutrophils in autoimmune disease Antibody-induced inflammation in the glomerulus of the kidney leading to glomerulonephritis (GN) is a leading cause of end stage renal disease and is initiated by leukocyte recruitment into glomerular capillaries disease [97–100]. The kidney glomerular capillaries are a frequent site of immune complex deposition and are one of the few sites in the body where capillaries are the major site of neutrophil influx. Yet, the mechanisms driving neutrophil recruitment into the specialized glomerular capillaries of the kidney remain largely unknown and there are no targeted therapeutics to avert this potentially most proximal event in glomerular inflammation. Dr. Mayadas and others have provided evidence that neutrophils can be directly recruited via their own FcγRs to deposited ICs [101,102]. She argued that this mechanism may be particularly relevant in the capillaries of the glomerulus [103], as the Fc portions of IgG deposited in the glomerular basement membrane (as is the case in anti-GBM GN and proliferative, lupus nephritis) are potentially accessible to circulating neutrophils via open endothelial fenestrae [104], as are anti-endothelial cell antibodies present in many forms of glomerulonephritis [105]. Studies in mice selectively expressing the human FcγRs on neutrophils of mice lacking their own FcγRs have suggested that human neutrophil FcγRIIA plays a key role in neutrophil accumulation and glomerular damage. Dr. Mayadas presented this data as well as data from 2-photon intravital microscopy analysis in the glomerulus that suggests that neutrophils can be recruited via their own FcγRs. She also presented results from a large small molecule screen for FcγRIIA inhibitors, of a drug that could block this process and the ensuing glomerular injury. Dr. Mayadas concluded that glomerular capillaries employ a unique pathway of neutrophil recruitment following IgG deposition that may be therapeutically targeted to avert glomerular injury.

Shiv Pillai (Boston) discussed new data on the pathogenesis of IgG4-related disease (IgG4-RD) which is characterized by fibro-inflammatory tumescent lesions with dense lympho-plasmacytic infiltrates and includes conditions such as autoimmune pancreatitis (type 1), Mikulicz’s disease, retroperitoneal fibrosis, Kuttner’s tumor, Reidel’s thyroiditis, as well as many other entities [106]. IgG4-RD patients with active, untreated disease show a marked expansion of plasmablasts in the circulation as well as in involved organs [107]. Th2 cells were initially thought to be associated with IgG4-RD pathogenesis, but we have revealed that single color staining for IL-4 in the tissues largely emanates from T follicular helper cells that make IL-4 and not from Th2 cells. Using an unbiased approach to characterize CD4+ T-cell subsets in patients with IgG4-RD - based on their clonal expansion and ability to infiltrate affected tissue sites - CD4+ CTLs have been identified the major CD4 + T cell subset in disease lesions as well as in circulation. These CD4+ CTLs make perforin, granzymes and pro-fibrotic cytokines such as IL-1β, TNF-α and IFN-γ. T follicular cells that express the BAFF transcription factor and secrete IL-4 are abundant in disease tissues in the vicinity of tertiary lymphoid organs and their numbers correlate plasma IgG4 levels. Finally, Dr. Pilai presented data showing that treatment with Rituxan to deplete B cells leads to a depletion of plasmablasts and CD4+ CTLs, suggesting that this disease may be driven by CD4 + CTLs that are nurtured by antigen presenting activated B cells in tissue sites [108].

Julian Ambrus presented new information of the marginal zone B cells Sjogren’s syndrome (SS) a common autoimmune disease causing significant morbidity and mortality. Because it is often picked up late in the course of the disease, animal models are needed to identify the early events of the disease and to clarify its pathophysiology [109]. The IL-14a transgenic (IIl14aTG) mouse reproduces features of Sjogren’s syndrome (SS) seen in patients in the same relative time frame [110, 111]. These include hypergammaglobulinemia, production of autoantibodies, loss of salivary and lacrimal gland function, lung disease, kidney disease and lymphoma. Salivary gland function is lost before lymphocytes are found in the salivary glands. Early destruction of the salivary glands in IL14aTG mice is dependent upon lymphotoxin (LTA). IL14aTG mice lacking LTA or treated with LTA inhibitors maintain normal salivary and lacrimal gland function. Increased LTA has been found in the serum and saliva of patients with SS [112]. Marginal zone B cells (MZB) spontaneously produce LTA in IL14aTG mice. IL14aTG mice lacking MZB are completely healthy and do not develop any features of SS. They do not produce characteristic autoantibodies, lose salivary or lacrimal gland function or produce increased amounts of type 1 interferon [113]. Similar observations were made in BAFF transgenic mice lacking MZB [114]. Type 1 interferon production is increased in IL14aTG only after the disease has progressed for several months [115]. IL14aTG mice lacking B1 cells have more severe features of SS than IL14aTG mice [113]. Dr. Ambrus concluded that SS can be divided into an early stage driven by LTA and a later stage driven by type 1 interferon. MZB are critical for SS disease manifestations while B1 cells play a role dampening the clinical features of SS. Further studies based on these observations may lead to better ways to identify SS during its early stages and to improve therapy.

It is becoming increasingly suspicious that local factors in concert with invading cells and molecules of the adaptive and the innate immune response are important for the expression for tissue injury. The R27 mouse generated by Dr. Fu may explain why some patients with lupus nephritis progress to chronic renal failure where as others respond to treatment and preserve renal function for longer periods. Studies on interaction of invading T cells with antigen presenting cells in the skin should explain why patients with SLE develop skin lesions. Someone can suspect that patients who can present autoantigen in the skin will not develop skin lesions. Along the same lines, some should assume that local factors enable CD4+ CTLs to promote fibrosis in certain tissues in patients with IgG4-related disease.

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