FRI0296

KZR-616, A SELECTIVE INHIBITOR OF THE IMMUNOPROTEASOME, BLOCKS THE DISEASE PROGRESSION IN MULTIPLE MODELS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

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Background: The proteasome inhibitor bortezomib has been used successfully to treat patients with SLE. The immunoproteasome is a distinct class of proteasome found predominantly in immune effector cells. KZR-616 is an analogue of ONX 0914, Nature Medicine 2009) that selectively targets the LMP7 and LMP2 subunits of the immunoproteasome.

Objectives: To examine the therapeutic potential of KZR-616 in preclinical models of SLE and lupus nephritis (LN).

Methods: Cytokines were measured in human PBMCs stimulated with endotoxin and in CD4⁺T cells stimulated with antibodies to CD3 and CD28. Human B-cells were stimulated with IL-21 and antibodies to CD40 and IgM to induce plasmablast differentiation. Immunoproteasome inhibition was measured in KZR-616 treated human PBMCs and in mice by measuring proteasome active site occupancy. The therapeutic effect of KZR-616 alone or in combination with mycophenylate mofetil (MMF) was evaluated in the NZB/W F1 model of SLE. T-dependent antibody responses (TDAR) were measured in mice and monkeys following 1, 4, or 13 weekly administrations.

Results: At a concentration resulting in inhibition of LMP7 and LMP2 by 89% and 59%, respectively, KZR-616 induced a decrease in pro-inflammatory cytokine production in human PBMCs, including TNF- α , GM-CSF, IL-6, and IL12/IL-23 p40. In lymphocytes, KZR-616 blocked T-cell production of IFN- γ , TNF- α and GM-CSF, and the differentiation of B-cells to plasmablasts. KZR-616 administration to mice resulted in selective inhibition of LMP7 and LMP2 similar to levels *in vitro*. KZR-616 treatment in diseased mice resulted in a complete resolution of proteinuria and significant reductions in autoantibody production and renal IgG deposition. The halt in disease progression was durable as proteinuria levels did not significantly increase 8 weeks after treatment discontinuation. Histologic analysis following 12 weeks of treatment revealed a complete prevention in glomerular nephritis and sclerosis. Administration of KZR-616 in combination with MMF resulted in significantly greater disease inhibition and prolonged survival compared to either treatment alone. Levels of activated T- and B-cells and short- and long-lived plasma cells were effectively depleted in diseased animals following KZR-616 treatment. KZR-616 had no significant effect on TDAR in mice or monkeys and did not affect the number of circulating lymphocytes in monkeys.

Conclusions: KZR-616 is a novel and selective covalent inhibitor of the immunoproteasome that potentially blocks inflammatory cytokine production *in vitro* and disease progression in mouse models of SLE. Durable disease remission in animals was achieved at well tolerated doses without affecting normal T-cell dependent immune responses. KZR-616 is currently being developed for the treatment of LN and Phase 1 safety and pharmacokinetics results are presented elsewhere at this meeting.

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FRI0297

MACROPHAGE-DERIVED LCN2 CONTRIBUTES TO DEVELOPMENT OF LUPUS NEPHRITIS

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Background: Renal involvement is a major concern in systemic lupus erythematosus (SLE). Many findings have indicated that plasma and urinary LCN2 is a potential marker of the presence and severity of renal involvement in SLE. However, whether LCN2 has a pathogenic or protective role in lupus nephritis (LN) is still unknown.

Objectives: In this study we analysed the expression of LCN2 in lupus patients and determined whether neutralisation of LCN2 with a monoclonal antibody or injection of recombinant LCN2 affects LN in mice.

Methods: Plasma and urine LCN2 was quantified by ELISA in SLE patients and healthy controls (HCs). The correlations between the levels of LCN2 and clinical features were analysed by Spearman's correlation test. CD14⁺ monocytes were isolated from peripheral blood of HCs and SLE patients. We cultured human monocytes for 7 days with macrophage colony-stimulating factor (M-CSF) to generate macrophages. To investigate the production of LCN2 in lupus mice during progression of LN, plasma and urine LCN2 levels were measured by ELISA, mRNA and protein levels of LCN2 in different tissues were measured by qPCR and western blot respectively at different stages of disease. To investigate whether increased LCN2 levels contribute to the development of LN, the 16-week-old mice received weekly intraperitoneal of anti-LCN2 antibody or recombinant LCN2 for 4 weeks and analysed 1 week after the last injection. The renal pathology, proteinuria, spleen index were evaluated. The frequency of T lymphocyte subpopulation, B cells and plasma cells in spleen and lymph node was analysed by flow cytometry. Bone marrow derived macrophages (BMDMs) were isolated from tibias and femurs in mice. The expression of LCN2 in human macrophages and BMDMs were detected by qPCR.

Results: Plasma and urine LCN2 levels were markedly increased in SLE patients, and plasma LCN2 was positively correlated with urine LCN2. The levels of LCN2 in both plasma and urine were positively correlated with serum creatinine and 24 hour urine protein. The levels of LCN2 in the patients with LN were significantly higher than those without LN. At 10 weeks, when mice do not show any signs of systemic lupus, plasma LCN2 levels were low but increased significantly in 15- and 20-week-old mice. The increased plasma LCN2 was correlated with proteinuria and deposition of IgG in glomeruli. The mRNA level of LCN2 was increased in different tissues including liver, kidney and spleen. The same results were found in protein levels. Administration of LCN2 aggravated the disease with significantly higher renal activity scores and more IgG deposition. Neutralisation of LCN2 significantly improved renal pathology and reduced the glomerular deposition of IgG. Furthermore, anti-dsDNA antibody and proteinuria were significantly lower in the anti-LCN2 group. Flow cytometry revealed a significant decrease of T cells, B cells and Th1 cells in the anti-LCN2 group. While LCN2 significantly increased T cells, plasma cells and Th1 cells. LCN2 mRNA level in macrophages was significantly increased in SLE patients. And its expression was also increased in BMDMs with the progression of disease in lupus mice.

Conclusions: These findings suggested that LCN2 over-production by macrophages plays an important role in the pathogenesis of SLE and the progression of lupus nephritis. Thus, blockade of LCN2 may represent a new strategy for treatment of LN.

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FRI0298

ALTERED FREQUENCIES OF CIRCULATING INNATE LYMPHOID CELLS AND ITS SUBSETS ARE CORRELATED WITH DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

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Background: Systemic lupus erythematosus (SLE) is a chronic, multisystem autoimmune disorder with a heterogeneous clinical manifestations that results from abundant immunological abnormalities. Innate lymphoid cells (ILCs), one subset of the innate immune cells, are divided into cytotoxic ILCs (namely natural killer cells) and non-cytotoxic ILCs (namely helper-ILCs). Non-cytotoxic ILCs are composed of three groups, group 1 ILCs, group 2 ILCs and group 3 ILCs, based on depended transcription factors and producing cytokines. So far, alterations of ILCs and their subsets have been reported in some autoimmune diseases except SLE.

Objectives: To visualise differences in frequencies of ILCs and their subsets in the peripheral blood of patients with SLE and healthy controls in Chinese Han population.

Methods: Peripheral blood mononuclear cells (PBMCs) was obtained from twenty-five SLE patients and twelve healthy controls and were stained with antibodies to CD45, lineage (CD3, CD19, CD123, CD11c, CD14, CD16, CD34, CD94, and Fc ϵ R1 α), CD4, CD8, CD127, CD117, CRTH2 and NKp44. Circulating total ILCs and its subsets were identified by flow cytometry. The associations between disease activity and all the detected cells were evaluated using the Pearson or Spearman correlation coefficient.

Results: Increased frequencies of ILC2 and ILC3 were observed in patients compared to controls, while decreased frequency of ILC1 was found in patients compared with controls ($p=0.008$, $p=0.004$, and $p=0.006$, respectively). We also found the expression of T cell surface markers, CD4 or CD8, on ILCs and their subsets. The results showed that decreased frequencies of CD4⁺CD8⁺ ILCs, CD4⁺CD8⁺ILC1, CD4⁺CD8⁺ ILC2, CD4⁺CD8⁺ ILC2, and CD4⁺CD8⁺CD336⁺ ILC3 were found in patients compared with healthy controls ($p=0.001$, $p=0.017$, $p=0.001$, $p=0.004$, and $p=0.002$, respectively). Furthermore, frequencies of CD4⁺CD8⁺ ILCs and CD4⁺CD8⁺ ILC2 were positively correlated with the SLEDAI-2000 score ($r=-0.548$, $p=0.005$ and $r=-0.613$, $p=0.001$, respectively). Frequencies of CD4⁺CD8⁺ ILCs and CD4⁺CD8⁺ ILC1 were positively related with serum C3 level ($r=0.519$, $p=0.008$ and $r=0.528$, $p=0.007$, respectively), and were positively related with serum C4 level ($r=0.623$, $p=0.001$ and $r=0.643$, $p=0.001$, respectively).

Conclusions: In the present study, we demonstrated that frequencies of circulating ILCs and its subsets were altered in SLE patients and some subpopulations were negatively correlated with SLE disease activity.

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FRI0299 MUCOSAL-ASSOCIATED INVARIANT T CELL DEFICIENCY IN SYSTEMIC LUPUS ERYTHEMATOSUS IS REALTED TO AN INTRINSIC DEFECT IN THE CA2+/CALCINEURIN/NFAT1 SIGNALLINGPATHWAY

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Background: Mucosal-associated invariant T (MAIT) cells contribute to protection against certain microorganism infections and play an important role in mucosal immunity. However, the role of MAIT cells remains enigmatic in autoimmune diseases.

Objectives: Here, we examined the level and function of MAIT cells in patients with rheumatic diseases.

Methods: Patients with systemic lupus erythematosus (SLE; $n=54$), rheumatoid arthritis (RA; $n=66$), Behçet's disease ($n=9$), ankylosing spondylitis ($n=21$), and healthy controls ($n=136$) were enrolled in the study. MAIT cell, cytokine and programmed death-1 (PD-1) levels were measured by flow cytometry.

Results: Circulating MAIT cell levels were significantly reduced in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) patients. In particular, this MAIT cell deficiency was more prominent in CD8⁺ and double-negative T cell subsets, and significantly correlated with disease activity, such as SLE disease activity index (SLEDAI) and 28-joint disease activity score (DAS28). Interestingly, MAIT cell frequency was significantly correlated with natural killer T (NKT) cell frequency in SLE patients. IFN- γ in MAIT cells was impaired in SLE patients, which was due to an intrinsic defect in the Ca²⁺/calcineurin/NFAT1 signalling pathway. In SLE patients, MAIT cells were poorly activated by α -galactosylceramide-stimulated NKT cells, thereby showing the dysfunction between MAIT cells and NKT cells. Notably, an elevated expression of PD-1 in MAIT cells and NKT cells was associated with SLE. In RA patients, MAIT cell levels were significantly higher in synovial fluid than in peripheral blood.

Conclusions: Our study primarily demonstrates that MAIT cells are numerically and functionally deficient in SLE. In addition, we report a novel finding that this MAIT cell deficiency is associated with NKT cell deficiency and elevated PD-1 expression. These abnormalities possibly contribute to dysregulated mucosal immunity in SLE.

Disclosure of Interest: None declared

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FRI0300 POLYMORPHISM OF 5,10-METHYLENETETRAHYDROFOLATE REDUCTASE (C677T) IN PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME, ITS ASSOCIATION WITH CARDIOVASCULAR LESIONS

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Background: Many studies have been conducted to determine the role of genetic polymorphism in the occurrence of cardiovascular diseases. The pathogenetic significance of MTHFR polymorphism is the subject of intensive research, especially its connexion with lesions of the cardiovascular system. The frequency of C677T polymorphism in the 5,10-MTHF gene is poorly known in patients with antiphospholipid syndrome (APS), and its relationship with vascular lesions has not been studied yet.

Objectives: The present study aimed to analyse the C677T mutation of the MTHFR gene and its association with endothelial dysfunction and clinical manifestations of cardiovascular lesions in APS.

Methods: We studied 82 patients with APS, including 34 (41.6%) with primary antiphospholipid syndrome (PAPS) and 48 (58.4%) with secondary antiphospholipid syndrome (SAPS). The analysis of the MTHFR C677T mutation was performed by PCR followed by digestion according to Frosst et al. All patients were assessed for the endothelium-dependent vasodilatation of brachial artery (EDVD), the thickness of the intima-media of the common carotid artery (IMT), the presence of atherosclerotic plaque (AP) and clinical manifestations of cardiovascular lesions

Results: There were 10.8% of homozygotes (677-TT), 37.8% of heterozygotes (677-CT) and 51.4% of homozygotes (677-CC) in the control group, and the frequency of T-alleles amounted to 29.7%. The incidence of T-alleles was higher among the patients with APS than in the control group and was 35.4%. The prevalence of homozygotes (677-TT), heterozygotes (677-CT) and homozygotes (677-CC) was not significantly different between the PAPS and SAPS groups (44.1%, 38.2%, 17.7% and 45.8%, 39.6%, 14.6% respectively $p<0.05$). The frequency of T-alleles was higher in PAPS group than in SAPS group (36.8% against 34.4%, respectively $p<0.05$). The analysis of structural and functional vascular lesions in homozygotes (677-CC), homozygotes (677-TT) and heterozygotes (677-CT) did not reveal significant differences in both mean values and the proportion of individuals with IMT thickness (0.86 ± 0.03 mm, 0.88 ± 0.05 mm, 0.90 ± 0.03 and 35.3%, 38.5%, 51.7% respectively $p<0.05$) with decrease of EDVD (7.09 ± 0.49 , 6.32 ± 1.0 , 6.92 ± 0.58 and 47.0%, 53.8%, 48.3% respectively $p<0.05$) and the presence of AP (26.5%, 23.1%, 48.3% respectively $p<0.05$). Although there was a tendency of IMT thickness increase and EDVD decrease for T-carriers. The proportion of persons with IMT thickness (>0.90 mm) and the decrease of EDVD BA ($\leq 8.0\%$) among the homozygote 677-TT was 3%–6.5% higher than that of the 677-CC homozygote. The frequency of clinical manifestations of cardiovascular lesions (myocardial infarction, stroke, TIA) was in 1.2–1.8 times more often among the homozygotes 677-TT than 677-CC homozygote.

Conclusions: The mutation of the C677T of the MTHFR gene is not a risk factor for the development of atherosclerotic vascular damage in patients with APS, due to the lack of associative interrelationships between the decrease of EDVD, increase of IMT, clinical manifestations on the one hand, and the MTHFR polymorphism on the other

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FRI0301 SERUM EXOSOMES INVOLVED IN THE PROGRESSION OF LUPUS NEPHRITIS

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Background: Systemic lupus erythematosus (SLE is a chronic autoimmune disease that systemically affects several important organs).¹ Lupus nephritis (LN is one of the most severe complications of SLE).² Exosomes are important mediators of biological information and play a part in the occurrence and development of various diseases including LN.³

Objectives: The aim of study was to find whether exosomes participate in the pathogenesis of lupus nephritis.