murine colonization testing for immune modulatory properties of individual strains.

References:

Disclosure of Interests: None declared, Alexander Alekseyenko: None declared, Jing Deng: None declared, Zhi Li: None declared, David Fenyo: None declared, Alexander Alekseyenko: None declared

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THU0240

SELECTIVE DEPLETION OF AUTOREACTIVE B CELLS USING A PEPTIDE VACCINATION APPROACH

R. Singh 1; UCLA, Autoimmunity and Tolerance Laboratory, Department of Medicine / Rheumatology, Los Angeles, United States of America

Background: B cell depletion is currently approved or being tested in clinical trials for the treatment of many autoimmune diseases including rheumatoid arthritis, myositis, systemic sclerosis, and lupus. We previously demonstrated that the heavy chain variable regions (VH) of anti-DNA antibodies contain epitopes that can bind MHC class I molecules. 1 Vaccination of autoimmune-prone NZB/NZW F1 mice with plasmid DNA vectors carrying minigenes that encode such epitopes induced CD8+ cytotoxic T lymphocytes (CTL) that killed anti-DNA antibody-producing B cells, reduced serum anti-DNA antibody levels, retarded the development of nephritis, and improved survival. 2 Treatment with peptides alone did not induce such CD8+ T-cells, as we and others have reported impaired CD8+ regulatory and CTL responses in mice and humans. 2,3 Here, we ask if we could overcome such impairment using synthetic oligonucleotides containing unmethylated cytidine-phosphate-guanosine dinucleotides (CpG-ODN) that can enhance innate and adaptive immunity.

Objectives: To determine if peptides representing MHC class I binding epitopes in the autoantibody VH regions conjugated to CpG-ODN will elicit CD8+ CTLs that will ablate autoantibody-producing B cells, reduce serum anti-DNA levels, and retard the development of autoimmune disease.

Methods: We first screened VH regions of anti-dsDNA and other autoantibodies for MHC class I binding epitopes using various bioinformatic approaches, and then verified the binding using cellular binding approaches. We immunized lupus-prone (NZB/NZW F1) mice that develop lupus that mimics human lupus with CpG-ODN or a control ODN, and assessed CTL responses. We then treated these mice with CpG-ODN conjugated to MHC class I binding VH epitopes. We monitored lupus mice for proteinuria, serum anti-dsDNA antibody levels, and survival.

Results: Immunization with CpG-ODN corrected the impairment in peptide-specific CTL responses in lupus-prone mice. The CpG-ODN conjugated with MHC class I binding, anti-dsDNA antibody VH-derived epitopes induced potent peptide-specific CTL responses against autoreactive B cells that expressed the respective epitopes, and against B cells from diseased lupus-prone mice. The animals treated with CpG-ODN-peptide conjugates had reduced serum anti-DNA antibody levels and proteinuria, significantly delayed development of nephritis, and improved survival as compared to animals injected with a control ODN.

Conclusion: Selective ablation of autoreactive B cells by CTLs induced by peptide vaccines conjugated to CpG-ODN represents a novel approach to treat autoimmune-mediated diseases.

References:

Disclosure of Interests: None declared

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THU0241

ALTERATIONS OF THE FECAL MICROBIOTA ASSOCIATED WITH EXACERBATION OF LUPUS ACTIVITY BY HCMV PP65 422-439 IMMUNIZATION IN NZBW/F1 MICE

Y. C. Tsai 1; A. H. Hsieh 1; L. C. Wang 1; J. J. Chou 2,3; W. Y. Tseng 4,5, K. H. Yu 1; S. F. Luo 1; C. F. Kuo 1, 1, 1; Chang Gung Memorial Hospital, Division of Rheumatology, Allergy and Immunology, Taoyuan, Taiwan, Republic of China; 1 Chang Gung Memorial Hospital, Division of Paediatric Neurology, Taoyuan, Taiwan, Republic of China; 1 University of Nottingham, Division of Clinical Neurology, School of Medicine, Nottingham, United Kingdom; 1 University of Oxford, Kennedy Institute, Oxford, United Kingdom; 1 Chang Gung Memorial Hospital, Division of Rheumatology, Allergy and Immunology, Keelung, Taiwan, Republc of China; 1 Chang Gung Memorial Hospital, Center for Artificial Intelligence in Medicine, Taoyuan, Taiwan, Republic of China

Background: Systemic lupus erythematosus (SLE) is a multifactorial disease. Gut microbiota is an important environmental factor for SLE. 1 The perturbation of gut microbiota is often observed at onset or during the disease course. The fragment of HCMV phosphoprotein 65 (HCMVpp65) 2 containing B cell epitopes has been reported to elicit humoral immunity and accelerate the autoimmune response in murine lupus. 3, 4 However, little is there to know about the interplay between viral trigger for SLE and the change of gut microbiota during lupus progression.

Objectives: By using a murine lupus model with NZB/W F1, we investigated the differential alteration in gut microbiota associated with the progression of lupus disease in HCMVpp65-immunized mice and control mice.

Methods: Ten-week-old NZB/W F1 mice were given or not given an intraperitoneal injection of 100-μg HCMVpp65 422-439 peptide biweekly for four times. Fecal samples, urine and blood of mice were collected once every two weeks followed by 16S rRNA genes sequencing and ELISA tests. The pathological investigation of renal tissue from sacrificed mice was conducted at 24 weeks of mice age. Statistical analysis for dynamics and alteration of the gut microbiota as well as functional prediction of bacterial communities related to the progression of lupus-like activity was performed.

Results: HCMVpp65 422-439 immunization results in the onset of lupus-like activities in NZB/W F1 mice with a higher titer of anti-dsDNA antibody, creatinine and proteinuria, and severe glomerular damage (Figure 1). Also, higher diversity and increased family abundance of several bacterial species were observed in HCMVpp65-immunized mice (Table 1 and Figure 2a). The predicted metagenomic taxonomic profile in NZB/W F1 mice showed statistically significant enrichment of flagellar assembly, bacterial motility, and chemotaxis (Figure 2b). Spearman’s correlation analysis revealed that a significant association between the increased relative family abundance for Saccharimonadaceae, Marinilaceae, Desulfovibrioaceae, and Rikenellaceae and HCMVpp65 422-439-induced lupus-like activity in NZB/W F1 mice (Figure 2c).

Table 1. Significant test of microbial community structure between two groups

<table>
<thead>
<tr>
<th>Group</th>
<th>MRPP</th>
<th>Adonis</th>
<th>Anosim</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E-4</td>
<td>P</td>
<td>R</td>
</tr>
<tr>
<td>Pre-disease</td>
<td>0.43</td>
<td>0.1</td>
<td>0.77</td>
</tr>
<tr>
<td>Control</td>
<td>0.45</td>
<td>0.013</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Conclusion: Our results demonstrated that HCMVpp65 422-439 immunization induced the change in gut microbiota composition and suggested the association of gut microbiota alteration with lupus-like activity in NZB/W F1 mice.

References:

Disclosure of Interests: None

REGULATORY ROLE OF TRANSCRIPTION FACTOR BLIMP-1 IN SJÖGREN’S SYNDROME

R. Wu1, L. Wang1, X. Zheng1, Y. Wang2, G. Wang1, X. Li1, X. Li1.

1The First Affiliated Hospital of China University of Science and Technology, Hefei, China; 2Westmead Institute for Medical Research, the University of Sydney, NSW, Australia

Background: The pathogenesis of primary sjögren’s syndrome (pSS) is multifactorial. Self-antigen-driven responses perform a vital function in the development of autoimmune diseases [1]. B cells, only 20-25% of total infiltrating cells in labial glands, are the cellular basis for spontaneous antibody production [2]. Genome-wide association studies (GWAS) have identified Blimp-1 as a susceptibility gene for autoimmune diseases and played an important role in the pathogenesis of autoimmune diseases [3].

Objectives: To investigate the expression and effect of B lymphocyte induced maturation protein 1 (Blimp-1) in pSS and the correlation of Blimp-1 with B cell subsets and clinical features.

Methods: The PRDM1 mRNA expression in B lymphocyte and labial gland were examined by RT-PCR. The levels of B cell subsets were examined by flow cytometry. Hematoxylin-eosin (HE) staining and immunohistochemistry (IHC) were used to examine the invasion degree of lymph cell and Blimp-1 distribution, respectively. The correlation of PRDM1 mRNA with B cell subsets and clinical indicators were also analyzed.

Results: The levels of PRDM1 mRNA expression of B cells were significantly higher in SS than in healthy controls (HC) and which were also significantly higher in the high immunoglobulin (Ig) group than that in normal Ig group (P<0.02, Fig. 1a-b). The number of CD19+B cells and CD138+ plasma cells(PC) have increased while the CD27- cells decreased in SS(P<0.05). The percentage of PC and PC/B is positively correlated with PRDM1 mRNA(=0.380, P=0.002; r=0.317, P=0.009, Fig. 1c-d). Blimp-1 expression level showed a positive correlation with invasion degree of lymph cell in histology (Fig. 2a-c), Ig levels and ESSDAI score and an inverse correlation with the glucocorticoids usage (Fig. 3c).

Conclusion: Blimp-1 displayed high expression in SS, which could affect pSS disease activity. SS activity is suppressed by glucocorticoid which might be through inhibition of Blimp-1.

References:

Table 1. Clinical characteristics of pSS and HC.

<table>
<thead>
<tr>
<th></th>
<th>HC(n=17)</th>
<th>pSS(n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (Male/Female)</td>
<td>0/17</td>
<td>0/50</td>
</tr>
<tr>
<td>Age(exs)</td>
<td>45.2±18.55</td>
<td>46.8±11.05</td>
</tr>
<tr>
<td>Xerostomia(positive/negative)</td>
<td>0/17</td>
<td>43/7</td>
</tr>
<tr>
<td>Keratoconjunctivitis sicca</td>
<td>0/17</td>
<td>35/15</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>0/17</td>
<td>32/18</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0/17</td>
<td>18/32</td>
</tr>
<tr>
<td>ESSDAI(exs)</td>
<td>-</td>
<td>2.78±1.61 (0–7)</td>
</tr>
<tr>
<td>ESSPRI(exs)</td>
<td>-</td>
<td>3.3±1.39 (1–6)</td>
</tr>
<tr>
<td>ANA(positive/negative)</td>
<td>-</td>
<td>49/1</td>
</tr>
<tr>
<td>SSA</td>
<td>-</td>
<td>49/1</td>
</tr>
<tr>
<td>SSB</td>
<td>-</td>
<td>18/32</td>
</tr>
</tbody>
</table>

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Disclosure of Interests: This study was supported by the grants from the National Natural Science Foundation of China (81877127).

Fig. 1 (a-b) RT-PCR showed that PRDM1 mRNA expression in SS patients and HC. (c-d) Correlation between PRDM1 mRNA expression and PC and PC/B.

Fig. 2 (a) Expression of Blimp-1 in labial glands of sjoå¡gren’s syndrome. (b) PRDM1 mRNA levels in different invasion degree of lymph cell group. (c) Correlation between PRDM1 mRNA expression and invasion degree of lymph cell.

Fig. 3 (a-b) RT-PCR showed that PRDM1 mRNA expression in different usage of glucocorticoids. (c) Correlation between PRDM1 mRNA expression and different glucocorticoid usage.