factors such as age, sex and cell preparation date and differential expression of markers between groups was calculated via t-test. In parallel, PBMC B cell receptor (BCR) repertoires were investigated in 13 ACAPS pos. RA patients and six age-matched healthy donors using the Illumina MiSeq platform and PCR multiplex amplicon libraries with a molecular barcode strategy to generate full variable region coverage. Sequences were filtered using pRESTO, annotated by IMGT and Change-O, finally generating 587 000 unique V-regions. Total serum IgM levels were screened by sandwich ELISA in 157 population controls, 193 ACAPS pos. and 50 ACAPS neg. RA patients. Variable gene frequency was analyzed by Chi-square with Yates correction and serum IgM levels with Kruskal-Wallis test.

Results: Several B-cell phenotypes were found to be significantly different in ACAPS pos. RA compared to controls including an increase in HILA-DR across subsets, CD11c in IgA memory and CD22 expression in clusters of naive IgM positive B cells. Moreover, we could see lower circulating cell counts in ACAPS pos. RA in IgM memory (p<0.01) and trends for elevation in an CXCR5/CCL6 high transitional B cell cluster (p<0.06), with parallel lower number of transitional B cells with lower CCR6 expression (p<0.06). Notably, ACAPS neg. RA generally had an intermediate phenotype between healthy controls and ACAPS pos. RA. Several significant shifts in the RA BCR repertoire could be observed, including an expected higher frequency of VH N-linked glycosylation in mutually blocked (p<0.0001). Yet, the most striking difference was a significantly higher frequency of VH with low somatic hypermutation (SHM) levels in RA-derived B cells (<5 mutations, p<0.0001 14.7% vs 8.7). This was seen in all sequences, both IgM and class-switched, but was especially prominent in IgM1 rearrangements (9.6% vs 18.8% low mutation, p<0.0001 OR=2.2 C1:2.0-2.35). In line with an IgM and low mutation profile in RA, we also observed that both ACAPS pos. (p<0.0001) and ACAPS neg. (p<0.001) RA patients have a significant increase in total IgM levels compared to controls (1.4 ± 0.7; 1.3 ± 0.7; 1.0 ± 0.6 mg/ml, respectively).

Conclusion: Previous studies have shown that anti-citrulline autoreactivity in RA is primarily originates from memory B cells and characterized by high somatic mutations and N-glycosylation sites. However, here the largest B cell distortions in ACAPS positive RA are observed in the naive B cell population that have not undergone germinal center responses. These differences could reflect baseline shifts and elevated natural autoreactivity as an underlying mechanism in RA pathogenesis.

Disclosure of Interests: Yan Wang: None declared, Katy A. Lloyd: None declared, Ioannis Melas Employee of: Employed by UCB Pharma, Daniel Ramskold: None declared, Diana Zhou: None declared, Karin Lundberg: None declared, Lars Klareskog: None declared, Ioannis Melas Employee of: Employed by UCB Pharma, Daniel Ramskold: None declared, Diana Zhou: None declared, Karin Lundberg: None declared, Lars Klareskog: None declared, Ioannis Melas Employee of: Employed by UCB Pharma, Daniel Ramskold: None declared, Diana Zhou: None declared, Karin Lundberg: None declared, Lars Klareskog: None declared, Ioannis Melas Employee of: Employed by UCB Pharma, Daniel Ramskold: None declared, Diana Zhou: None declared, Karin Lundberg: None declared, Lars Klareskog: None declared, Ioannis Melas Employee of: Employed by UCB Pharma, Daniel Ramskold: None declared, Diana Zhou: None declared, Karin Lundberg: None declared, Lars Klareskog: None declared, Joeng Gao2, LI Xiaofeng1, Caihong Wang1.

OBJECTIVES

To compare the effects of new immunoregulatory therapy and conventional DMARDs therapy on disease remission and immune homeostasis in RA patients, and to investigate the efficacy of immunoregulatory therapy in the reconstruction of immune tolerance.

Methods: The study included 184 patients with RA (meeting the diagnostic classification criteria for RA revised by ACR in 1987). According to the therapeutic regimen they have received, there were assigned to the immunoregulatory (144 patients, Sirolimus capsule, 0.5 mg twice a week and Tretinoin tablets, 10 mg once a day, for 12 weeks) and conventional DMARDs group (40 cases, Leflunomide tablets, 10 mg once a day for 12 weeks). Other drugs are similar between the two groups. The absolute numbers of Th17 and Treg cells in peripheral blood were measured by Flow Cytometer (FCM).

Results: 1. There was no statistically significant difference in DAS28 score (p>0.05) between the two groups. 2. In the immunomodulatory group, there were statistical differences in the number of Treg compared with normal controls (p<0.05). After immunoregulatory therapy, the absolute number of Treg cells was increased, leading to ratio of Th17/Treg decreased (Table1, Figure1). 3. Conventional DMARDS therapy reduced, significantly the number of Treg cells (p<0.05), leading to an increase in the ratio of Th17/Treg (Table 1, Figure 2).

Conclusion: The results of this study show that the remission rates of immunomodulation group and conventional DMARDS group were 37.39% and 36.00%, respectively. There was no significant difference between the two groups. But as for the effects on their immune cells, immunomodulatory therapy can increase the level of Treg cells compared with conventional DMARDS. It also shows that immunomodulatory therapy may play an important role in restoring immune tolerance in patients with RA, which may help us find new ideas for treating RA. However, we need to enlarge the sample size and extend the treatment time to observe the long-term efficacy of the immunomodulatory therapy in the population.

REFERENCE


Table 1. The number of Th17 and Treg cell (cell/ul), ratio of Th17/Treg in immunomodulatory therapy and Traditional DMARDS therapy.

<table>
<thead>
<tr>
<th>Immune Cells</th>
<th>Immunomodulatory n=144</th>
<th>Traditional DMARDS n=40</th>
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</thead>
<tbody>
<tr>
<td>Before therapy</td>
<td>After therapy</td>
<td>Before therapy</td>
</tr>
<tr>
<td>Th17</td>
<td>(6.8643, 4.2411, 10.000)</td>
<td>(9.2359, 3.2539)</td>
</tr>
<tr>
<td>Treg</td>
<td>(15.900, 36.270)</td>
<td>(31.900, 43.120)</td>
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<tr>
<td>Th17/Treg</td>
<td>(0.1990, 0.490)</td>
<td>(0.1720, 0.341)</td>
</tr>
</tbody>
</table>

Figure 1. The number of Treg cells and the ratio of Th17/Treg before and after immunomodulatory therapy.
The study of CD4+ T cell subsets in recurrent polychondritis

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Background: Relapsing polychondritis (RP) is an rare inflammatory disease of unknown causes, characterized by recurrent inflammation in cartilaginous tissues of the whole body(1). The histologic features of the chondritis include loss of basophilic staining of the cartilage matrix, perichondrial inflammation, cartilage destruction with replacement by fibrous tissue, and perivascular cellular infiltration with plasma cells and lymphocytes. Additional clinical features of the disease include ocular inflammation, vasculitis, audiosteviolar dysfunction, myocarditis, cardiac valvular insufficiency, and nonerosive inflammatory arthritis. Many studies have shown that the imbalance of helper T cell 17(Th17) and regulatory T cell (Treg) is involved in the pathogenesis of autoimmune diseases such as SLE and RA. But little is known about the roles of peripheral immune cell subsets peripheral in RP patients. Up to now, just few studies focus on this issue.

Objectives: We aimed to analyse the distribution and phenotype of CD4+ T cell subsets peripheral in RP patients. Up to now, just few studies focus on this issue.

Methods: The proportion and absolute counts of circulating immune cells were assessed in 14 patients diagnosed as RP and 14 healthy controls. CD4+ T cell subsets were also analysed in 9 untreated RP patients and 9 healthy volunteers by flow cytometry. All statistical analyses were performed with SPSS v. 22.0. Continuous variables were reported as median. For all study variables, comparison among controls and RP subjects was based on the non-parametric Wilcoxon Mann-Whitney exact test. For all analyses, we used two-sided tests, with p-values <0.05 denoting statistical significance.

Results: Proportion and absolute counts of Treg cells were significantly reduced in RP patients in comparison with controls (proportion, 3.78% vs. 5.66%, p=0.008; absolute counts, 27.36/ micrometer cubed vs. 46.56/ micrometer cubed, p<0.001). But there were no significant difference between the percentage and number of Th17, Th1 or Th2 cells in patients with RP and healthy controls. Thus, the ratio of Th17/Treg increased in RP patients (0.25 vs. 0.14, p<0.001), as did the ratio of Th2/Treg (0.28 vs. 0.22, p=0.001) and Th1/Treg(2.75 vs. 1.92, p=0.019)(Figure 1). Similarly, the proportion and absolute counts of Treg cells in untreated RP patients were significantly lower than that in healthy controls (proportion, 3.78% vs. 5.66%, p=0.008; absolute counts, 32.24/ micrometer cubed vs. 50.76/ micrometer cubed, p=0.001).And the ratio of Th17/Treg also increased in untreated RP patients (0.25 vs. 0.15, p=0.003), as did the ratio of Th1/Treg (2.35 vs. 1.88, p=0.014)(Figure 2).

Conclusion: Our data suggested that the immune-inflammation in RP patients may be related to the depletion of Treg cells and the imbalance of Th17 or Th1 or Th2 and Treg cells. Reduction of peripheral Treg cells may exacerbate the disease progression by not being inhibited Th cells.

REFERENCES

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