decreased in patients with active disease (mean ± SD in HC 5.5 ± 1.6% vs. active 3.7 ± 2.6%). Furthermore, the percentage of CD19+CD24hiCD27+ cells was decreased in both remission and active patients when compared to HC (HC 14.0 ± 9.3% vs. remission 6.3 ± 4.2% vs. active 5.7 ± 6.0%). B cell capacity to produce IL-10 upon stimulation with CpG-ODN was comparable in patients and HC. B cells from HC suppressed TNF-α production in monocytes (median inhibition 43%, range –24 to 53).

Conclusion.-- The percentage of CD19+CD24hiCD38hi cells is decreased in AAV patients with active disease, whereas the CD19+CD24hiCD27+ population is diminished in AAV patients independent of disease activity. The ability of B cells to produce IL-10 is not compromised in patients. Studies in progress will determine whether B cells from AAV patients suppress monocyte activation to the same extent as HC.

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References

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P175
Epitope analysis of anti-myeloperoxidase antibodies in patients with ANCA-associated vasculitis
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Introduction.-- Increasing evidences have suggested the pathogenic role of anti-neutrophil cytoplasmic antibodies (ANCA) directing myeloperoxidase (MPO) in ANCA-associated vasculitis (AAV). The current study aimed to analyze the association between the linear epitopes of MPO-ANCA and clinicopathological features of patients with AAV.

Methods.-- Six recombinant linear fragments, covering the whole length amino acid sequence of a single chain of MPO, were produced from E. coli. Sera from 77 patients with AAV were collected at presentation. Thirteen out of the 77 patients had co-existence of serum anti-GBM antibodies. Ten patients also had sequential sera during follow up. The epitope specificities were detected by enzyme-linked immunosorbent assay using the recombinant fragments as solid phase ligands.

Results.-- Sera from 45 of the 77 (58.4%) patients with AAV showed a positive reaction to one or more linear fragments of the MPO chain. The Birmingham Vasculitis Activity Scores and the sera creatinine were significantly higher in patients with positive binding to the light chain fragment than that in patients without the binding. The epitopes recognized by MPO-ANCA from patients with co-existence of serum anti-GBM antibodies were mainly located in the N-terminus of the heavy chain. In five out of the six patients, whose sera in relapse recognize linear fragments, the reactivity to linear fragments in relapse was similar to that of initial onset.

Conclusion.-- The epitope specificities of MPO-ANCA were associated with disease activity and some clinicopathological features in patients with ANCA-associated vasculitis. The present study may suggest a role of “immunological memory” for inducing relapse in AAV.

Further readings

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P176
Investigating the microRNA signature of ANCA associated vasculitis
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Introduction.– MPO positive and PR3 positive ANCA associated vasculitis (AAV) are now established to be distinct genetic and clinical entities. However, the underlying differences in pathogenesis are not fully understood. Epigenetic studies have enabled the discovery of novel pathways involved in disease mechanism and potential therapeutic targets in other diseases. The most abundant epigenetic control mechanism is the production and function of microRNAs (miRs).

We sought to investigate the miR signature of AAV, focusing on differences between MPO and PR3 positive disease.

Methods.– We used pooled plasma from PR3 positive and MPO positive patients in both active and quiescent disease states (total 40 samples) as well as pooled normal plasma. Total RNA was isolated and reverse transcribed to produce cDNA. Real time PCR was performed and results analysed using miScript miRNA Array Data Analysis software. This process was repeated using the TaqMan Array Human microRNA cards for validation of results.

The resulting data was examined in silico using mirBase and associated sites to identify predicted gene targets of the differentially expressed miRNAs along with literature review to identify potentially relevant functions of the identified miRs.

Results.– Several differentially expressed miRs were identified in MPO active versus PR3 active disease (validated in comparisons with healthy controls) (table I). The majority of these have roles in T cell regulation.

Table I
MicroRNAs differentially expressed in MPO active versus PR3 active disease

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>Up or down regulated</th>
<th>Gene targets top 10 predicted</th>
<th>Relation to T cell function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsa-miR-424</td>
<td>Up</td>
<td>TBP1l, LY9, PLAG1, FGZ2, OBE2, RASGEF1B, PAPPAS, SYT4, TLF1, PHF19</td>
<td>Mir-424 expression has been identified as discriminative of T-lineage versus B-lineage in ALL. Expression increased in patients with TB.</td>
</tr>
<tr>
<td>Hsa-miR-620</td>
<td>Up</td>
<td>FIGN, LIN28, TRIM71, IGDC3, ARGMAP28, ENSG00000217865, COL1A2, IGCG, OBE21, BACH1</td>
<td>Predominantly expressed in naive CD8 T cells. Involved in control of fate of memory T cells.</td>
</tr>
</tbody>
</table>

Further readings