Dynamic Reshaping of Lymphocyte Repertoires in Multiple Sclerosis Patients Treated with Alemtuzumab: Insights into Secondary Autoimmunity

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INTRODUCTION AND AIM OF THE STUDY

Alemtuzumab (ALEM), a recombinant, humanized IgG1 monoclonal antibody targeting the surface molecule CD52 expressed on T- and B-lymphocytes. After depletion of the former “diseased multiple sclerosis (MS) immune repertoire”, a new, “healthy immune repertoire” is assumed to form. Although beneficial for many MS patients, considerable side effects have been described, in particular secondary autoimmune disorder (SAID) occurring in about one-third of alemtuzumab treated patients.

This study aimed to comprehensively characterize the longitudinal changes in lymphocyte repertoires pre- and post-ALEM treatment, shedding light on both its therapeutic benefits and potential side effects, particularly secondary autoimmunity.

METHODS

- Peripheral blood mononuclear cells (PBMCs) acquired from twelve MS patients (up to 4 time points each) and 5 untreated controls (2 time points each)
- Defined numbers of T cells (CD8+ and CD4+) and B cells (naïve and memory) were FACS sorted and processed further for high-throughput (HT) immune repertoire sequencing
- Sequencing data were analyzed using custom R, Python scripts and different bioinformatics tools

RESULTS

TCR and BCR repertoire changes with specific temporal dynamics are associated with secondary autoimmunity:

We compared data from five alemtuzumab-treated MS patients with SAID to seven patients without SAID.

In CD8+ and CD4+ T cell population-
- persisting top 100 clones expanded and occupied significantly larger proportions of the repertoires in SAID patients as compared to non-SAID patients (representative example Fig. 2a and 2b).
- non-persisting clones dominated CD8+ T cell repertoires in non-SAID patients, whereas expanded persisting clones were predominant in SAID patients (representative example Fig. 2a).

In untreated controls- clonal composition was relatively unaltered over time in both CD8+ and CD4+ T cell repertoire (representative example Fig. 2a and 2b).

Antigen specific CD8+ T cell clones profoundly expanded over the treatment only in SAID patients:

- In SAID patients: antigen specific CD8+ T cell clones persisted and underwent further expansion over the ALEM treatment, occupying a larger proportion of the repertoire volume compared to non-SAID patients (Fig. 3).
- In untreated controls: antigen specific CD8+ T cell clonal composition remained highly stable over time (Fig. 3).

At baseline, in both CD8+ and CD4+ T cell population-
- the top 10 and top 100 clones occupied a significantly (p<0.05) larger proportion of the repertoire space in SAID patients compared to non-SAID patients (Fig. 4).

At baseline, certain clonal lineages dominate the B cell repertoire only in SAID patients:
- Certain clonal lineages (the most abundant clonal lineage C1) which dominated the memory B (MB) cell repertoire were detected only in SAID patients (Fig. 5).
- The memory B cell repertoire volume in SAID patients compared with non-SAID patients (bar plot) was significantly higher in SAID patients (p<0.05).

CONCLUSION

- Expansion of persisting CD8+ T cell clones in SAID >> in non-SAID patients.
- Expansion of persisting antigen specific CD8+ T cell clones in SAID >> in non-SAID patients.
- Already at baseline, higher tendency of the expansion of CD8+ T cell clones, certain V-J pairs and clonal lineages dominate the B cell repertoire in SAID patients -> may be predictive for the development of SAID with alemtuzumab treatment

REFERENCES