Myasthenia Gravis Ig gene lineage trees and mutation analyses

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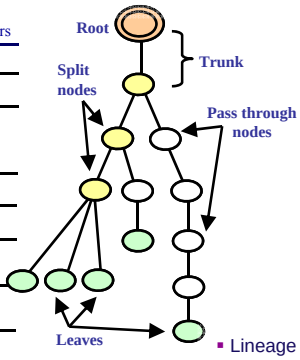
Abstract

Much information about hypermutation and antigen-driven selection during the humoral immune response is contained in the shape of immunoglobulin (Ig) gene mutational lineage trees deduced from the responding B cell clones. We microdissected germinal centres from the pathological ectopic germinal centres seen in the thymus of patients with Myasthenia Gravis (MG) and analysed the Ig gene characteristics to determine whether the ectopic germinal centres behave in a similar fashion to normal germinal centres from secondary lymphoid tissue. Lineage tree analysis demonstrated similar diversification and mutations per cell compared to the normal control trees, in spite of the chronic nature of the MG response. Mutation analyses revealed that most replacement mutations were observed in the framework regions, responsible for the structural integrity of the B cell receptor (BCR); these mutations were mostly conservative or neutral, confirming that a functional BCR is conserved in MG. Analysis of replacement and silent mutation patterns revealed selection against replacement mutations in the complementary determining regions (CDRs), responsible for antigen binding. This might indicate that MG clones are highly specific for the antigen, and thus most CDR replacement mutations, which may be affinity-reducing, disappear during selection. Somatic hypermutation targeting motifs in MG were similar to those of the normal controls. However, sequence mutation spectrum in the MG in-frame genes slightly deviated from that of the MG out-of-frame and the normal controls. This may be a result of, as yet unknown, selective processes. Overall, these results suggest that B cells in the ectopic germinal centres in MG seem to undergo normal diversification and selection in spite of the chronic nature of the response.

Methods

- Patient sequences were aligned with germline genes using ClustalW (www.ebi.ac.uk/clustalw).
- Lineage trees were generated from the alignments using IgTree© (Barak *et al.,* 2008).
- Tree properties were measured by MTree©, a program developed at the Mehr lab (References below).
- Tree properties were statistically analyzed to compare between trees from MG and normal controls (Shahaf *et al.,* 2008).
- Somatic hypermutation motifs were plotted and presented as pictograms, where the influence of each nucleotide on the motif is depicted by its size.
- Analysis of replacement (R) and silent (S) mutations was performed to estimate antigenic selection.
- Amino Acid (AA) substitution analysis was performed to estimate the nature of the substitution and its effect.

Tree variable	Correlation to 2nd B cell response dynamic parameters
(T) Trunk length	Affinity ↓
(PL-min) Path length	Affinity ↓ Selection ↓
(DLFSN-avg) Average distance from a leaf to the first split node	Affinity ↓ Selection ↓
(OD-avg) Outgoing degree	Affinity ↑ Selection ↑ Mutation ↓
(RootD) Root's outgoing degree	Affinity ↑
(DASN-min) Distance between adjacent split nodes	Affinity ↓ Selection ↓
(DRSN-min) Distance from the root to any split node	Affinity ↓ Selection ↓

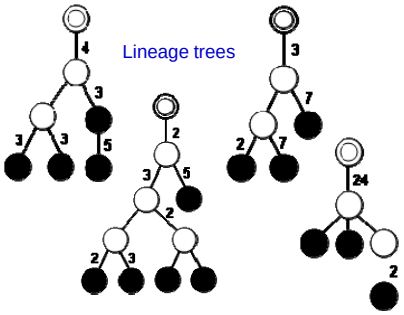


Previous work with lineage trees

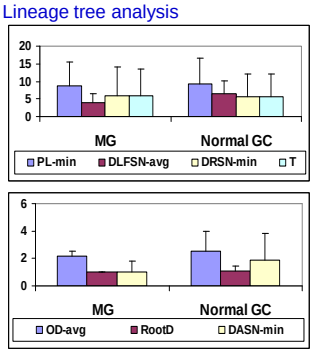
- Abraham *et al.,* Novel Analysis of Clonal Diversification in Blood B Cell and Bone Marrow Plasma Cell Clones in Immunoglobulin Light Chain Amyloidosis. *J. Clin. Immunol* 27(1):69-87 (2007).
- Manske *et al.*, Quantitative analysis of clonal bone marrow CD19+ B cells: use of B cell lineage trees to delineate their role in the pathogenesis of light chain amyloidosis. *Clin. Immunol* 120(1): 106-20, 2006.
- Steiman-Shimony *et al.*, Immunoglobulin variable-region gene mutational lineage tree analysis: application to autoimmune diseases. *Autoimmun Rev* 5(4):242-51, 2006.
- Mehr *et* al., Analysis of Mutational Lineage Trees from Sites of Primary and Secondary Immunoglobulin Gene Diversification in Rabbits and Chickens. *J. Immunol.* 172:4790-4796, 2004.
- Dunn-Walters *et al.*, Immune System Learning and Memory Quantified by Graphical Analysis of B-Lymphocyte Phylogenetic Trees. *BioSystems* 76:141-155, 2004.
- Dunn-Walters et al., The dynamics of germinal centre selection as measured by graph-theoretical analysis of mutational lineage trees. *Dev. Immunol.* 9 :233-245, 2002.
- Banerjee et al., Age and tissue specific differences in human germinal center B cell selection revealed by analysis of IgVH gene hypermutation and lineage trees. *Eur. J. Immunol.* 32:1947-1957, 2002.

Results

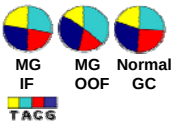
Lineage trees



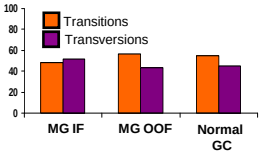
Lineage tree analysis



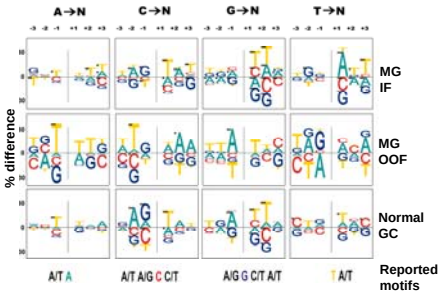
Mutation spectrum



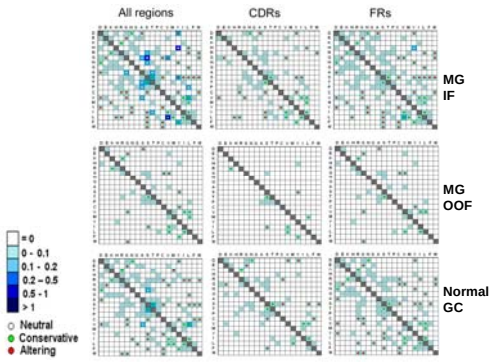
RS analysis



Motif analysis



AA substitutions



Conclusions

- Lineage tree analysis →
similar diversification and mutation per cell in MG and normal controls.
- AA substitution analysis →
Conserved/Neutral R mutations in FRs confirmed conservation of functional BCR.
- RS analysis →
Selection against R mutations in CDRs (may be affinity-reducing, disappear during selection?), high specificity for Ag.
- SHM targeting motifs analysis → Similar to normal controls.
- Mutation spectrum analysis →
MG IF deviated from MG OOF and normal control – an unknown selective process? Or just a small sample effect?

➡ B cells in ectopic GCs seem to undergo normal diversification and selection, in spite of the chronic nature of MG.