

IGOME GRAPHS SUGGEST THE CHANGES IN THE IGM AND IGG REPERTOIRES IN ANTIPHOSPHOLIPID SYNDROME ARE IN PART IDIOTYPICALLY DEFINED

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Abstract

Antiphospholipid Syndrome (APS) is characterized by the persistence of high-affinity antiphospholipid antibodies, manifesting clinically as pregnancy complications, thrombosis, or neurological symptoms. This study explores the changes in the IgM and IgG antibody repertoires in APS patients using igome graphs, which represent antibody repertoires through peptide libraries selected from phage display on the complete immunoglobulin fraction of the serum. Serum samples from healthy women and APS patients were processed to isolate IgM and IgG fractions, which were then used for phage selection and next-generation sequencing. Peptide sequences were analyzed using graph representation, clustering, and spectral embedding to identify significant differences between the repertoires. The study revealed that while IgM repertoires in APS patients exhibit a loss of public reactivities, the IgG repertoires do not follow this trend. Three orthogonal sequence motifs were identified in the IgM and IgG repertoires, suggesting potential idiotypic interactions. These findings highlight the intricate mechanisms of antibody selection and cross-reactivity in APS, offering insights into potential therapeutic approaches. The study underscores the importance of system-level analysis in understanding immune mechanisms and suggests that idiotypic interactions, though complex, could play a crucial role in APS pathogenesis.

Key words: antiphospholipid syndrome, antibody repertoires, IgM, idiotype

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Introduction. RYVKIN et al. [1] coined the term *igome* for antibody repertoire images in the form of peptide libraries selected from phage display on the complete immunoglobulin fraction of the serum. Persistence of high affinity antiphospholipid antibodies is a sign of APS. The typical clinical manifestation of antiphospholipid syndrome (APS) are pregnancy complications, thrombosis, or neurological symptoms [2]. In a previous report we have shown that the *igome* image of the IgM repertoire in APS has signs of loss of public reactivities in the patients [3]. Here the relationship between the *igome* images of the IgM and the IgG repertoires in the same patients is further examined.

Materials and methods. Serum samples, immunoglobulin isolation, deep panning. The details of the recruitment and the IgM isolation have been described previously [3]. Like for IgM, the first step of fractionation was ultrafiltration of 0.4 ml serum through a 100 kDa cut-off membrane which was followed by a HiTrap Protein G High Performance affinity column (GE Healthcare). This yielded IgG and IgM fractions from healthy women ($n = 20$) and women with APS ($n = 24$). The fractions were pooled by diagnosis group and used for phage selection. The deep panning technique has been previously described [3]. It follows roughly the *igome* technique [1, 4]. Briefly, it consists in panning a 7-amino acid residue long random peptide library (New England Biolabs) on the pooled immunoglobulin fractions of the two diagnostic groups followed by single phage amplification step, DNA isolation and PCR amplification of the peptide inserts and next generation sequencing (NGS).

Data processing and other data sources. The peptide sequences were extracted from the fastq files from the sequencing using the scripts published by [5] with some corrections and using high accuracy (Q score > 32). Any subsequent data processing was done using the R software environment (packages *igraph*, *stringdist*, *pROC*, *parallel*, *stringi*, *Biostrings*, *qualV*, etc.). The libraries were labelled and pooled together with a library from a previous analysis of the IgM public *Igome* of healthy donors [6]. Sequences in the current library that met the criteria of copy number in the pooled library between 3 and 10 [6] were retained. The SAROTUP [7] algorithm was used to filter potential artifactual target unrelated sequences. The selected peptides are by definition mimotopes (mimics of the epitopes) of the public antibody reactivities (the private ones are diluted too much after pooling) in the repertoires studied but also collectively referred to as *igome*. The protein BLAST algorithm for short sequences of the NCBI server (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) was used to detect homologous sequences in the human proteome.

Generating the *igome* graph and finding clusters. To model the similarities between the sequences in the *igome*, the data was analyzed using graph representation. The selection of a criterion for predicting that a pair of peptides are isospecific for an antibody has been described previously [3]. The selected

string metric was LCS (longest common subsequence) distance and the criterion for connecting two sequences was a LCS of 5, 6 or 7 letters. The degree, eigen-centrality, etc. graph parameters as well as the visualizations were calculated and produced using the *igraph* package. The visualization was based on spectral embedding and UMAP [8]. The community structure of the graphs was determined using the Leiden clustering algorithm [9] with modularity. The resolution parameter was adjusted to yield cluster number and size distribution appropriate for visualization or motif extraction. χ^2 test ($p < 0.05$ after correction for multiple testing) was used to determine clusters with differential expression of reactivities. A graph of clusters was defined as a complete graph of the clusters in the initial graph. In the cluster graph, the edge weights (as distances) were set equal to the modularity of the induced subgraph containing the respective pair of clusters from the initial graph with the cluster membership as grouping. The maximal edge length in the minimal spanning tree of the cluster graph was used as criterion to prune its longer edges so that the remaining maximally simplified graph still represents a single connected component to be used as the ultimate cluster graph. All data and scripts are available at <https://github.com/ansts/aPLIgG>.

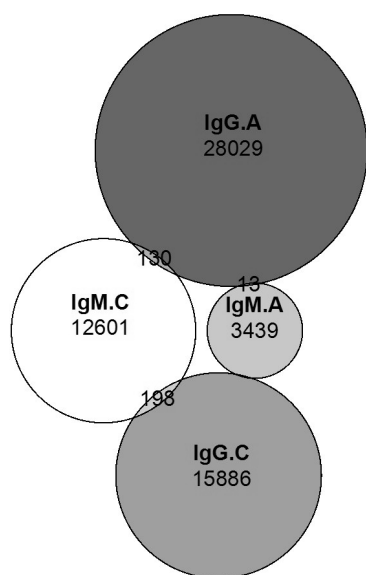


Fig. 1. Venn diagram of the distribution of the mimotopes found in clusters with a significant difference in the representation between the 4 libraries. A – APS, C – Control

Results. The 4 igome libraries of mimotopes representing the repertoires studied (IgM – 109 617 from clinically healthy women and 297 028 from APS patients, IgG – resp.: 318 637 and 196 940) were labelled and pooled by immunoglobulin isotype to compare the repertoires by diagnosis separately for IgM and IgG. Two graphs were produced, respectively, of sizes 41 263 592 and 62 358 579 edges. The graphs were clustered in an appropriate number of clusters (1983 for IgM and 1955 for IgG) so that the cluster size distribution had the same median as

the degree distribution. Thus, the clusters reflected the graph topology in detail comparable to the individual neighbourhoods. For each graph, the frequencies of control or APS derived mimotopes in the different clusters were compared and clusters with significantly different than expected distribution were determined using χ^2 test ($p < 0.05$). The mimotopes found in significant clusters from all four categories were compared to determine the sizes and the overlap of the libraries (Fig. 1). While for the IgM the significant clusters contained 11.495 % of the mimotopes from the controls and 1.154 % of those from APS, for IgG the two subsets were proportional to the sizes of the libraries (8.066 % for the controls and 8.797 % for APS). Negligible overlap between the libraries was observed.

To compare the reactivities between all conditions, the mimotopes in the significant clusters from the 4 libraries were pooled and used to construct a new graph. Thus, clusters of mimotopes could represent similar reactivities that could be overlapping between groups more than the individual sequences. This third graph was clustered in 88 clusters which were used to construct a fourth one – a simplified graph of the clusters.

The graph of clusters was embedded using the eigenvalue decomposition of the normalized Laplacian in a dimensionality informed by the difference of the smallest eigenvalues. Since this embedding was in 5 dimensions, it was further reduced to 3 dimensions using the UMAP algorithm. The resulting layout can be seen in Fig. 2. The distribution of the mimotopes from the original libraries was mapped to the graph and compared to the degree and eigencentality of the vertices (here representing original clusters). Sequence motifs were determined from the 2 clusters of highest degree (clusters 25 and 83) and the eigencentral cluster 51 using suffix trees of the LCS characterizing the respective edges in the primary graph between the mimotopes. The motifs defined were readily identifiable in the junctional regions (CDR3) of other human immunoglobulin molecules when compared to the human proteome sequences in the NCBI database (HFGLGQH, HFGLG, and HENVHE for cluster 83, KTITSST for cluster 25, and GSNSMN, MSNSM, and ISNSMN for cluster 51).

Notably, clusters 83 and 51 were over represented by mimotopes of IgM in the controls while cluster 25 showed intriguing relation between the IgG in the APS and the IgM lost in APS (observed as control group associated) (Fig. 3).

Discussion. The igeome libraries from the public IgG and IgM repertoires of clinically healthy women and APS patients diagnosed because of habitual abortions were compared to find specificity relations. As previously described [3], the IgM repertoire in APS was characterized mostly by loss of public reactivities. As expected, here it was shown that this is not true for the IgG repertoire.

Furthermore, the IgM reactivities found in excess in the controls, respectively, lost in APS, contained a small subset (3.5%) with homologies to most of the other clusters irrespective of the prevalence of the libraries in the latter. Thus, aggregating the large igeome graphs based on modularity clustering, cen-

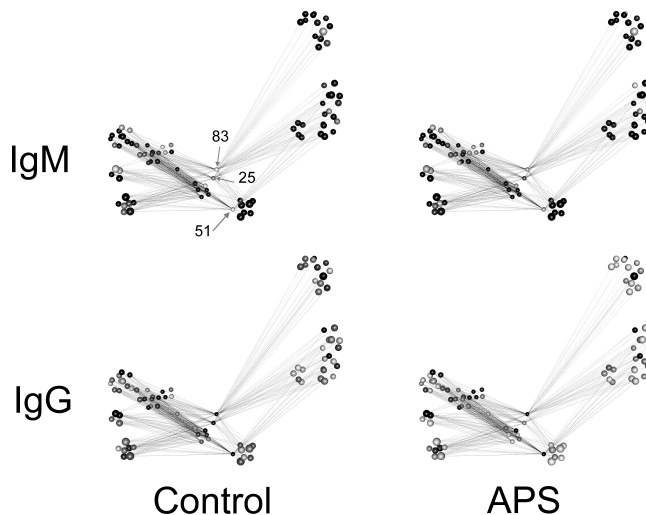


Fig. 2. Spectral embedding of a graph of the 88 clusters of the disease associated mimotopes. The original libraries were first represented as graphs of the IgM and the IgG sets of sequences. In each of these graphs, clusters with disproportionate (χ^2) representation of the control and the APS libraries were detected. The mimotopes they contained were pooled and used to generate a graph of the disease specific mimotopes. This third graph was clustered in 88 clusters shown above – each vertex is a cluster with the radius proportional to its size. Top and lower panels – the proportion of mimotopes from the library indicated in each cluster is gray colour coded (white is high)

trality measures, and a better visualization helped extract deep cross-reactivity dependencies. Spectral embedding as mathematically tractable and consistent algorithm was instrumental in this.

The three main hub clusters were not connected between themselves meaning that the graph analysis extracted 3 orthogonal sequence motifs which could be found in 69/88 (cluster 25), 55/88 (cluster 51), and 74/88 (cluster 83) of the clusters. Finding of these motifs in immunoglobulin junctional regions (HCDR3) suggests that most of APS related IgM or IgG reactivities, although different by specificity and direction of change in the disease, might be cross-reactive with antibody clones carrying very limited set of paratope determinants. Cluster 51 defines polyserine motifs which have been found previously to characterize APS associated B cell receptors especially in the catastrophic form of this disease [10].

This antibody vs. antibody reactivity has been traditionally labelled idiotypic and long back several reports insisted that idiotypic interactions play a role in APS and can be even tapped as a potential therapeutic approach [11–13]. Due to the

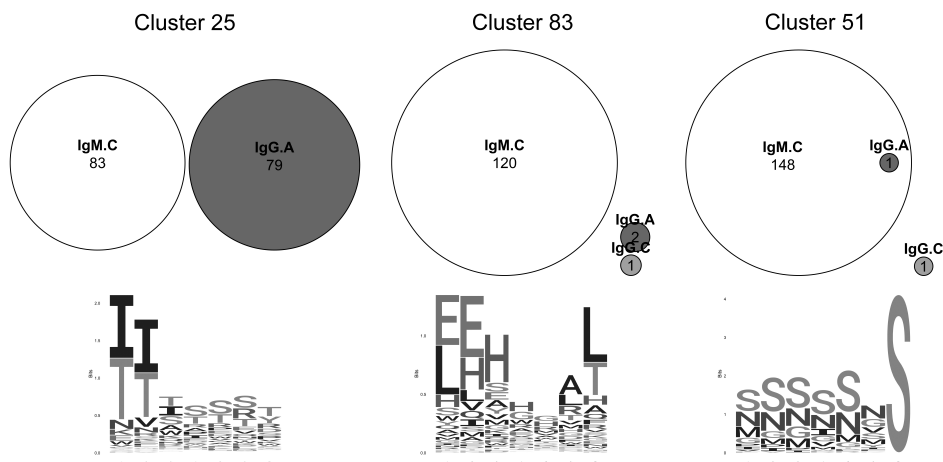


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complexity of these interactions, the lack of appropriate experimental approaches for a better mechanistic explanation, as well as the inadequacy of the theoretical tools, usually applied to these findings, idiotype has been largely abandoned as lacking explanatory power. This, though, does not serve science as numerous credible findings of idiotypic interactions [14] remain unexplained but could lead potentially to understanding of ill understood immune mechanisms. For instance, antiidiotypic interactions may not be due to persistent immune responses to one's own antibodies but rather to an intricate mechanism of selection which imprints on the emerging IgM repertoire certain complementarities to parts of the existing antibody repertoire. System level analysis applying approaches like igome graphs could help move closer to an explanation.

REFERENCES

- [1] RYVKIN A., H. ASHKENAZY, L. SMELYANSKI et al. (2012) Deep Panning: steps towards probing the IgOme., PLoS One, **7**(8), e41469.
- [2] D'ANGELO C., O. FRANCH, L. FERNÁNDEZ-PAREDES et al. (2019) Antiphospholipid Antibodies Overlapping in Isolated Neurological Syndrome and Multiple Sclerosis: Neurobiological Insights and Diagnostic Challenges, Front. Cell. Neurosci., **13**, <https://doi.org/10.3389/fncel.2019.00107>.

- [3] PASHOVA S., L. BALABANSKI, G. ELMADJIAN et al. (2022) Restriction of the Global IgM Repertoire in Antiphospholipid Syndrome, *Front. Immunol.*, **13**, <https://doi.org/10.3389/fimmu.2022.865232>.
- [4] WEISS-OTTOLENGHI Y., J. M. GERSHONI (2014) Profiling the IgOme: meeting the challenge, *FEBS Lett*, **588**(2), 318–325.
- [5] MATOCHKO W. L., K. CHU, B. JIN et al. (2012) Deep sequencing analysis of phage libraries using Illumina platform, *Methods*, **58**(1), 47–55.
- [6] PASHOV A., V. SHIVAROV, M. HADZHIEVA et al. (2019) Diagnostic Profiling of the Human Public IgM Repertoire with Scalable Mimotope Libraries, *Front. Immunol.*, **10**(2796), 1–14.
- [7] HE B., H. CHEN, N. LI, J. HUANG (2019) SAROTUP: a suite of tools for finding potential target-unrelated peptides from phage display data, *Int. J. Biol. Sci.*, **15**(7), 1452–1459.
- [8] MCINNES L., J. HEALY, J. MELVILLE (2018) UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction, *arXiv:1802.03426 [stat.ML]*.
- [9] TRAAG V. A., L. WALTMAN, N. J. VAN ECK (2019) From Louvain to Leiden: guaranteeing well-connected communities, *Sci. Rep.*, **9**(1), 5233.
- [10] SWAIDANI S., S. CHATURVEDI, X.-Z. PAN et al. (2021) Abundance of B Cell Receptors Harboring Elongated Polytyrosine and Polyserine Rich Motifs within Their Heavy Chain CDR3 Distinguishes Catastrophic and Antiphospholipid Syndrome, *Blood*, **138**(Suppl. 1), 2117.
- [11] BLANK M., L. ANAFI, G. ZANDMAN-GODDARD et al. (2007) The efficacy of specific IVIG anti-idiotypic antibodies in antiphospholipid syndrome (APS): trophoblast invasiveness and APS animal model, *Int. Immunol.*, **19**(7), 857–865.
- [12] MASON A. N., I. J. HARMER, R. A. MAGEED, C. G. MACKWORTH-YOUNG (1999) The BH1 idiotypic defines a population of anticardiolipin antibodies closely associated with the antiphospholipid syndrome, *Lupus*, **8**(3), 234–239.
- [13] CACCAVO D., F. VACCARO, G. M. FERRI et al. (1994) Anti-idiotypes against antiphospholipid antibodies are present in normal polyspecific immunoglobulins for therapeutic use, *J. Autoimmun.*, **7**(4), 537–548.
- [14] KOHLER H., A. PASHOV, T. KIEBER-EMMONS (2019) The Promise of Anti-idiotypic Revisited, *Front. Immunol.*, **10**, <https://doi.org/10.3389/fimmu.2019.00808>.

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