AID-ing B-Cell Differentiation

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Hyper-IgM syndrome (HIGM) is a rare immunodeficiency disorder that suppresses the ability to produce certain antibodies, consequently heightening susceptibility to infections.

Enhancing our understanding of B-cell development and HIGM2 genetics is crucial in improving diagnostics and counselling for affected individuals.

Issue: The autosomal recessive form of this disorder, HIGM2, has proven challenging in understanding its genetic and molecular causes, resulting in limited diagnostic measures.

Question: Do mutations in the human form of AID (huAID) in patients with HIGM2 cause defects in B-cell differentiation, including class switch recombination (CSR) and somatic hypermutation (SHM)?

- **CSR** - switching of antibody types
- **SHM** - improves antibody specificity

HIGM Syndrome

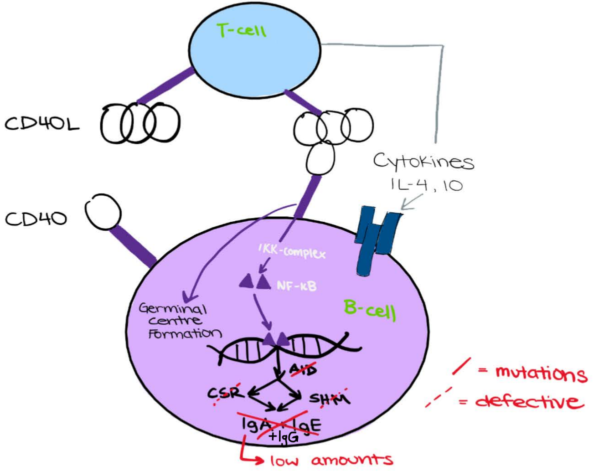


Figure 1: Representation of the mutations and defects present in HIGM syndrome.

Materials and Methods

Study included 18 HIGM2 affected patients from 12 families

Ficoll-Hypaque density centrifugation

- Isolated peripheral blood mononuclear cells (PBMC)

Immunofluorescent for DNA analysis

- Visualizing molecules present on B-cells using specific antibodies marked by dyes
- DNA from blood samples map which chromosomes the genes for HIGM2 were located

Gene sequencing

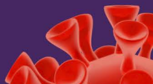
- The huAID gene was also sequenced and analyzed for mutations

Cell stimulation

- Stimulated blood cell cultures with soluble CD40L (a protein that aids in CSR and SHM) and IL-4 (helps in B-cell differentiation) to assess IgE switch (an antibody produced in immune responses)

Immunohistochemical analysis

- Patients' lymph nodes and tonsil tissues were examined and stained to identify the cell types of protein locations in these tissues



Overview of Findings

Researchers observed that the HIGM2 patients had low levels of IgG and IgA antibodies, but normal or increased levels of IgM antibodies.

- Due to a lack of antibodies, defective CSR and SHM, these patients also commonly experienced recurrent bacterial infections in respiratory and gastrointestinal tracts

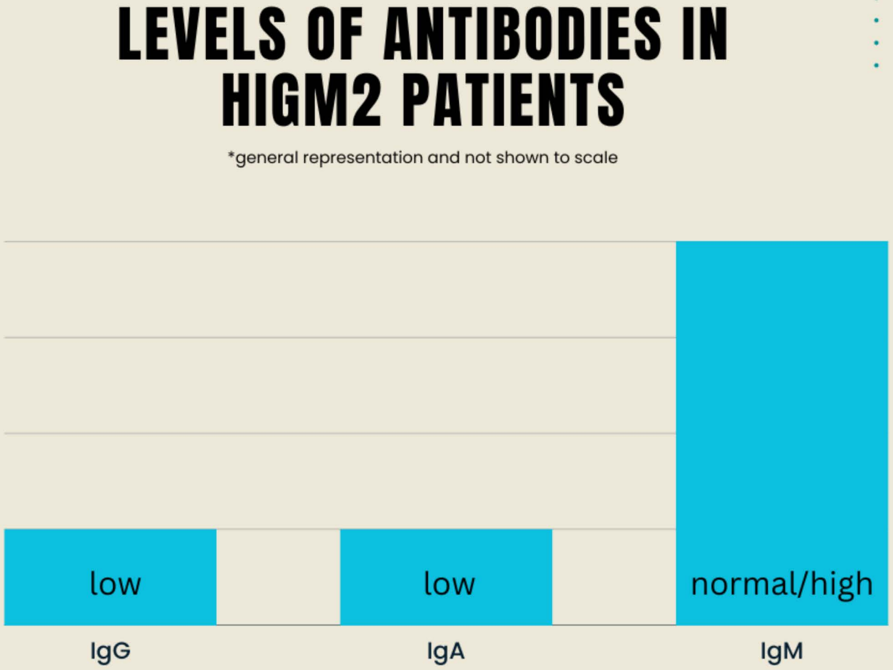
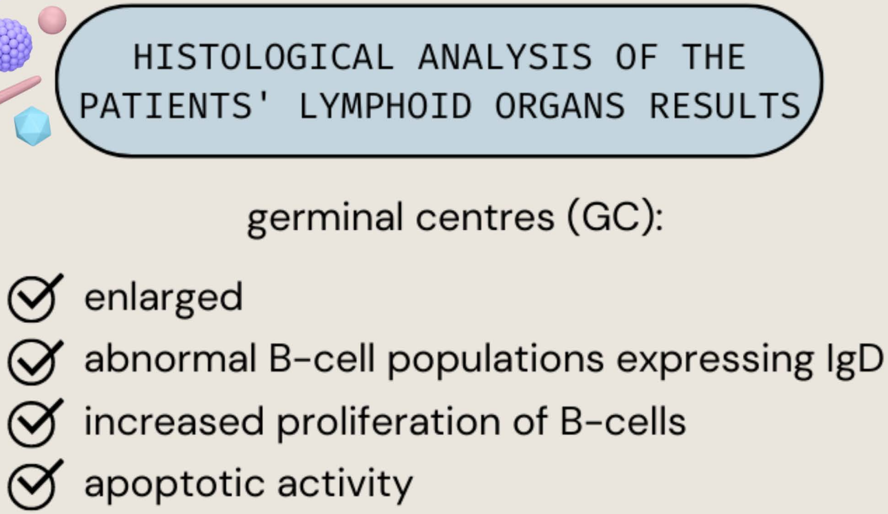


Figure 2: General representation of antibody levels in HIGM2 patients.

A notable characteristic included lymphoid hyperplasia, enlargement of lymphoid organs, with a few patients needing tonsillectomy.

Histological analysis of the patients' lymphoid organs showed enlarged germinal centers with abnormal B-cell populations expressing IgD, increased proliferation, and apoptotic activity, were also observed.



HISTOLOGICAL ANALYSIS OF THE PATIENTS' LYMPHOID ORGANS RESULTS

germinal centres (GC):

- ☑ enlarged
- ☑ abnormal B-cell populations expressing IgD
- ☑ increased proliferation of B-cells
- ☑ apoptotic activity

Figure 3: The germinal centre findings from histological analysis of patients' lymphoid organs.

Major finding: Genetic mapping identified a specific region on chromosome 12p13 associated with HIGM2 and mutations in the huAID gene in all patients

- 10 different mutations were revealed
- Impaired IgE antibody production and reduced somatic mutations in the V3-23 Ig V gene

Discussion/Conclusion

HuAID Gene

- Provided significant insights into the role of the huAID gene in HIGM2 syndrome and B-cell differentiation: identified defects in molecular mechanisms involved in class-switch recombination, somatic mutation generation, and germinal centre formation
  - These findings were replicated in subsequent studies, including Kefei (2022)

Evidence of Causation

- Chromosome mapping and identification of harmful mutations in huAID in HIGM2 patients confirms its causative role in the disease
  - Subsequent studies have supported this finding and this study is cited in Etzioni & Ochs (2004)

These discoveries have implications for the diagnosis, treatment, and management of HIGM2 syndrome, as well as for broader research in immunology and antibody production.

Bibliography:

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