Alterations in Immunophenotype of Autoimmune-prone Hypomorphic RAG-deficient Patients with CID-G/AI Phenotype

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Introduction

- Recombination-activating genes 1 or 2 (RAG1/2) are instrumental in V(D)J recombination and generation of T and B cell repertoire
- *RAG* mutations in humans have **heterogeneous clinical phenotypes** (**Figure 1**)

1. Complete lack of Rag activity: severe combined immunodeficiency (SCID)
- absence of mature B and T cells (T- B- SCID)

2. Partial RAG activity; Hypomorphic *RAG* mutations

A. Leaky SCID (LS) or Omenn Syndrome (OS)
- Rag activity is low but present (<5%) with limited generation of T and B cells
- tendency for infections (LS) or immune dysregulation/autoreactive cells (OS)

B. Combined immunodeficiency withgranuloma and autoimmunity (CID-G/AI)
- more preserved Rag activity (>10%) with more preserved T and B cells
- milder phenotype with survival into late childhood or adulthood
- granuloma formation, and/or **autoimmunity**

- **Immunological phenotype: (Figure 1, highlighted in red)**

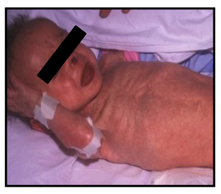
Figure 1. Clinical spectrum of RAG deficiency

INFECTION → AUTOIMMUNITY

SCID ($T^-B^-NK^{+}$)
Early (4-6 months)
Severe infections
Failure to thrive
↓↓/absent T and B cells
↓↓/absent Ig

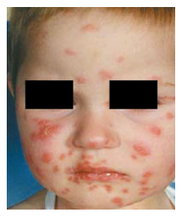
LS/OS
High inflammatory state
Oligonal autoreactive repertoire of T/B cells
↑ IgE/ ↓ IgG

CID-G/AI
Later onset
Less severe infections
Granulomas, AI cytopenias
↓ to normal T/B cells
↓ to normal Ig

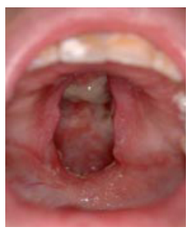


Courtesy of
Dr. Luigi Notarangelo

Puzenat (2007)
Eur J Derm



Schuetz (2008)
NEJM



De Ravin (2010)
Blood

Objective

We evaluate patients with RAG deficiency and CID-G/AI phenotype by
- Comprehensive characterization of B and T cell populations
- Focus on autoreactive-prone subsets

Methods

Multiparametric flow cytometry approach (Figure 2A and 3A)

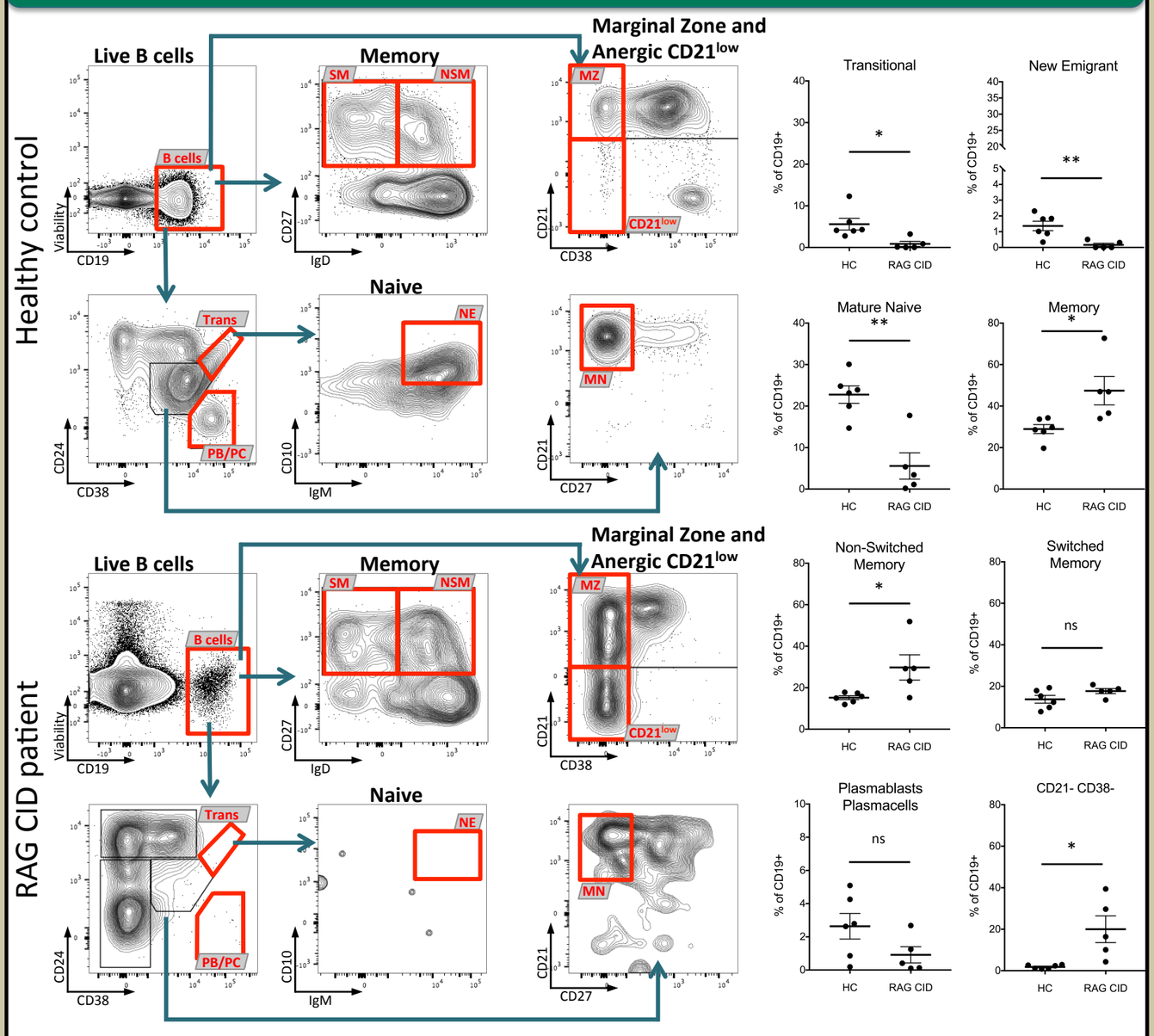
Major B cell subsets were identified as **transitional** ($CD24^{high}CD38^{high}$), **mature naïve (MN)** ($CD24^{mid}CD38^{mid}CD27^{-}IgM^{+}$, **non-switched memory (NSM)** ($CD27^{+}IgD^{+}$), **switched memory (SM)** ($CD27^{+}IgD^{-}$), **anergic** ($CD38^{low}CD21^{low}$), **marginal zone (MZ)** ($CD38^{low}CD21^{high}$), **plasmablasts/plasmacells (PB/PC)** ($CD38^{high}CD24^{-}$)

Regulatory T cells (Treg): $CD3^{+}CD4^{+}CD25^{high}CD127^{low}$

Follicular helper T cells (Tfh): $CD3^{+}CD4^{+}PD\text{-}1^{+}CXCR5^{+}$ cells

Follicular regulatory T cells (fTreg): $CD3^{+}CD4^{+}PD\text{-}1^{+}CXCR5^{+}$ $CD25^{high}CD127^{low}$

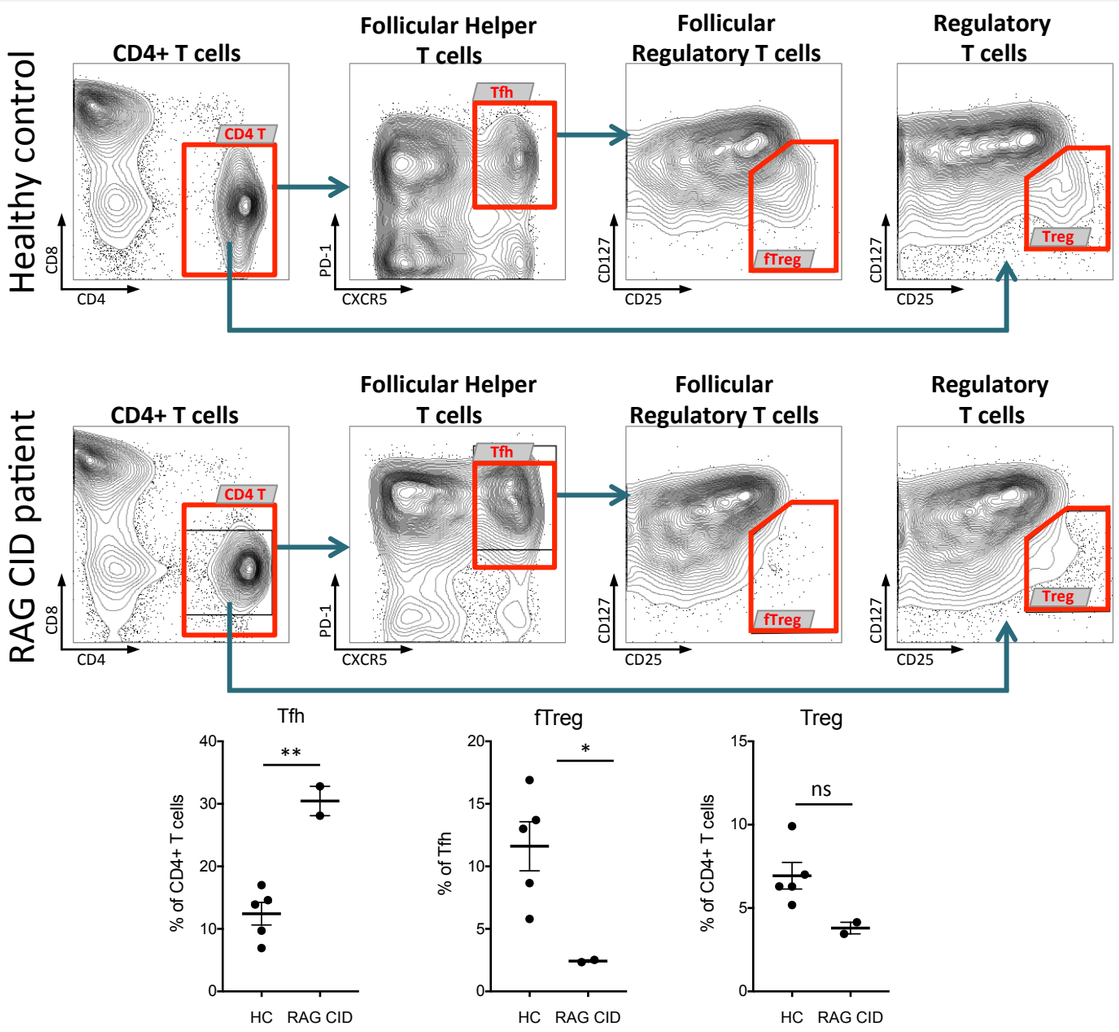
Figure 2. B cell phenotyping



Results

In peripheral blood of patients with partial RAG deficiency and CID-G/AI phenotype:
- Mild B cell lymphopenia and skewed B cell development with significant decrease in fraction of T, NE, MN and PB/PC subset (**Fig. 2**) significant increase in fraction of MZ, NSM, $CD21^{-}CD38^{-}$ anergic B cells (**Fig. 2**)
- Increase in frequency of Tfh cells and decrease in Treg and follicular Treg (**Fig 3**)

Figure 3. T cell phenotyping



Conclusions and discussion

- CID-G/AI patients have skewed B and T cell repertoire with expansion of autoreactive B and T cells and decrease in regulatory T cells
- This may result in peripheral B and T cell tolerance that promotes autoimmune disease

References and Funding

Walter JE. Expansion of immunoglobulin-secreting cells and defects in B cell tolerance in Rag-dependent immunodeficiency. J Exp Med. 2010 Jul 5;207(7):1541-54. doi: 10.1084/jem.20091927.

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