Analysis of Lymphocyte and Clinical Profile in Nonmonogenic Common Variable Immunodeficiency Patients With and Without Class Switch Recombination Defect

Tofighi Zavareh F[1,2,3,4], Mirshafiey A[1,4,5], Yazdani R[1,3,6], Keshtkar AA[7], Abolhassani H[1,8,9], Kiaee F[10], Parvaneh N[11], Shariat M[11], Rezaei N[1,3,12], Aghamohammadi A[1]

[1]*Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
[2]*Central Research Laboratory, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[3]*Primary Immunodeficiency Diseases Network (PIDNet), Universal Scientific Education and Research Network (USERN), Tehran, Iran*
[4]*Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[5]*Livonian Biotech Millennium Ltd, Riga, Latvia*
[6]*Department of Neurology, Thomas Jefferson University, Philadelphia, USA*
[7]*Department of Health Sciences Education Development, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[8]*Division of Immunology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden*
[9]*Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska Institute at Karolinska University Hospital Huddinge, Stockholm, Sweden*
[10]*Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[11]*Department of Pediatrics, Division of Allergy and Clinical Immunology, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
[12]*Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

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Common variable immunodeficiency (CVID) is the most common heterogeneous inborn error of immunity (IEI). It is characterized with reduced class-switched memory B cells (SMBs), hypogammaglobulinemia, respiratory infections, autoimmunity, enteropathy, and lymphoproliferation [1]. Several monogenic defects are associated with the CVID phenotype [2,3], although 50%-80% of patients with no known monogenic IEI [4] develop nonmonogenic CVID. Class switch recombination (CSR) is a DNA rearrangement of the immunoglobulin (Ig) heavy-chain locus that switches the Ig isotype from IgM to IgG, IgA, and IgE, generating SMBs or plasma cells [5]. Defects in genes involved in CSR will cause CSR defect (CSR-D), leading to monogenic CVID, although CSR-D has rarely been observed in nonmonogenic CVID patients [6]. CSR-D is associated with dramatically reduced SMB levels and specific clinical manifestations in CVID patients [7]. We aimed to evaluate alterations in B- and T-lymphocyte subpopulations and clinical manifestations in genetically unresolved cases of CVID with and without CSR-D to identify distinctive immunological and clinical phenotypes for this group.

We included 30 genetically unresolved cases of CVID from the Iranian Immunodeficiency Registry. Using whole-exome sequencing, we excluded known monogenic IEI [8], as previously described [4,9]. Based on clinical diagnosis [10], patients were classified into different groups, namely, infection only, autoimmunity, chronic enteropathy, and lymphoproliferation. We also included 30 age- and sex-matched healthy controls. The study was approved by the ethics committee of Tehran University of Medical Sciences (IR.TUMS.CHMC.REC.1399.003), and patients gave their written informed consent.

Based on serum Ig levels, we classified patients into 2 groups, CSR-D and non–CSR-D. CVID patients with decreased IgG and IgA but normal IgM levels were considered CSR-D. The remaining patients were categorized as non–CSR-D. The laboratory methods have been described elsewhere [11]. In brief, extracellular and intracellular flow cytometry procedures were conducted to detect all B- and T-cell subsets. IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp) was used for the statistical analyses.

Among the CVID patients (Table S1), 16.67% (5 out of 30) had a CSR-D. In the CSR-D group, IgM levels were significantly higher, and delayed diagnosis and pneumonia were significantly more common (Figure S1 and Table S2). Of note, all cases with bronchiectasis belonged to the non–CSR-D group, suggesting the functional role of residual IgM in CSR-D patients. Among CSR-D patients, 80% were infection only and 20% were the noninfectious phenotype (exclusively chronic enteropathy). In the non–CSR-D population, we also found infection only to be the most frequent clinical phenotype (56%) (Table S2).

All gating strategies for lymphocyte subsets have been reported elsewhere [12]. We observed no statistically

significant differences in lymphocyte subsets between CSR-D and non–CSR-D (Figure S2). However, in comparison with healthy controls, significant decreases were observed in the non–CSR-D group for the following: total, marginal zone, and IgM-only memory B cells (IgM only MB); SMB; plasmablasts; total, naive, central memory, and regulatory (Treg) $CD4^+$ T cells; and naive $CD8^+$ T cells. Significant increases were observed in total, effector memory (EM), terminally differentiated effector memory (T_{EMRA}), and activated and cytotoxic $CD8^+$ T cells. The same reduction in SMB, IgM only MB, and plasmablasts was observed for CSR-D compared with healthy controls (Table S3). Normalization of B cells and marginal zone B cells was in line with higher levels of IgM and the phenotype of CSR-D. Based on the calculated cut-off values, we observed a higher percentage of patients with decreased SMB, IgM only MB, type 1 helper T cells (T_H1), Treg, and naive $CD8^+$ T cells, in addition to a higher percentage of patients with increased $CD21^{low}$ and transitional B cells, central memory $CD4^+$ T cells, and T_{EMRA} T cells among CSR-D than among non–CSR-D (Table S4).

Observation of CSR-D among CVID patients with no known monogenic defects suggests that defects in the other genes involved in CSR may have a potential role in the pathogenesis of CVID rather than the well-known genes. Some of these potential genes have been predicted to cause CVID [6]. Regarding detection of CSR-D among 17% of our nonmonogenic CVID patients, we expected lower SMB levels in the CSR-D group than in the non–CSR-D group. Nevertheless, this reduction was observed among 100% of CSR-D and 96% of non–CSR-D patients, with no statistically significant differences. It seems that CSR-D in nonmonogenic CVID patients is too mild to cause significant alterations in SMB levels or result in other immunological and clinical features.

The cut-off values showed that a defect in CSR had noticeable effects on some lymphocyte subsets, leading to a markedly higher number of CSR-D patients having fewer memory B cells, T_H1, Treg, and naive $CD8^+$ T cells and more $CD21^{low}$ B cells, transitional B cells, $CD4^+$ T cells, and T_{EMRA} T cells than non–CSR-D patients. Abnormalities in some of the above-mentioned lymphocyte subsets, irrespective of whether they are CSR-D, have been shown to be associated with particular clinical phenotypes and to play a role in prediction of CVID in children with hypogammaglobulinemia [12,13]. This finding would strongly support the prominent role of abnormalities in $CD21^{low}$ B cells, T_H1, Treg, and naive and T_{EMRA} T cells in the development of more severe features such as autoimmunity and chronic enteropathy among CSR-D patients later in their lives.

We expected much more severe clinical complications among CSR-D patients [14]. However, to our surprise, despite the higher rate of infection, pneumonia, and delayed diagnosis, all patients with bronchiectasis fell into the non–CSR-D group, indicating the presence of immune dysregulation in the development of bronchiectasis in CVID patients. Having no genetic defects in CVID-related exomes seems to alleviate the adverse impacts of defects in CSR and lead to a milder form of the disease. In summary, we can conclude that observation of CSR-D in genetically unresolved CVID could be a result of defects in predictive genes, epigenetic changes, and environmental or host-intrinsic factors leading to mild modification of B- or T-cell response pathways, which in turn fail to cause severe clinical conditions or considerable alterations.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Abbas Mirshafiey, Asghar Aghamohammadi

Farzaneh Tofighi Zavareh
https://orcid.org/0000-0003-0402-132X
Research Center for Immunodeficiencies
Children's Medical Center
Tehran University of Medical Sciences
62 Qarib St., Keshavarz Blvd.
Tehran 14194, Iran
E-mail: mirshafiey@tums.ac.ir

