

# Modulation of Adaptive Immune Response following Intravenous Immunoglobulin Therapy in Common Variable Immunodeficiency

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Common variable immune deficiency (CVID) is the most frequent symptomatic primary immune deficiency in adults with a prevalence of approximately 1 in 25,000 in the general population [1]. Indeed patients frequently become symptomatic later in life; however, their clinical history may reveal features of the disease dating back to early childhood. The disorder is characterized by recurrent and/or severe infections and may be associated with autoimmunity and increased risk of lymphoid malignancies. CVID probably represents a heterogeneous group of disorders culminating in late-onset antibody failure. The genetic basis of CVID has been identified in only a minority of patients; it is likely that in the majority of patients the disease may have a polygenic origin [2]. Since the cause of CVID is unknown and the clinical features variable, there is no universally accepted definition of the disorder and several diagnostic criteria have been proposed [3]. The diagnostic criteria of the ESID (European Society of Immunodeficiencies)/PAGID (Pan American Group for Immune Deficiency) comprise three aspects: (i) hypogammaglobulinemia with immunoglobulin G (IgG) levels 2 standard deviations below the mean value, (ii) impaired vaccine responses or absent isohemagglutinins, and (iii) exclusion of other causes of hypogammaglobulinemia.

The typical defect in CVID is the failure of B lymphocytes to differentiate into switched memory B cells and plasma cells [4]. Several abnormalities of T cells have also been described in CVID, including oligoclonal expansion of CD8+T cells and decreased numbers of CD4+T cells [5]. Moreover, T lymphocytes show an impaired secretion of several soluble mediators. The current standard of care for patients with CVID is lifelong replacement with intravenous immuno-

globulin preparations (IVIg) that reduce the frequency of infections and the progression of complications, including suppurative lung disease. IVIg use is increasing rapidly, given its efficacy also in patients with autoimmune and inflammatory disorders. IVIg therapeutic effect seems to be related not only to antibody replacement but also to active modulation of immune responses [6]. Such immunomodulatory effects have been hypothesized but never proven. The aim of our study was to address this issue by using a gene expression approach.

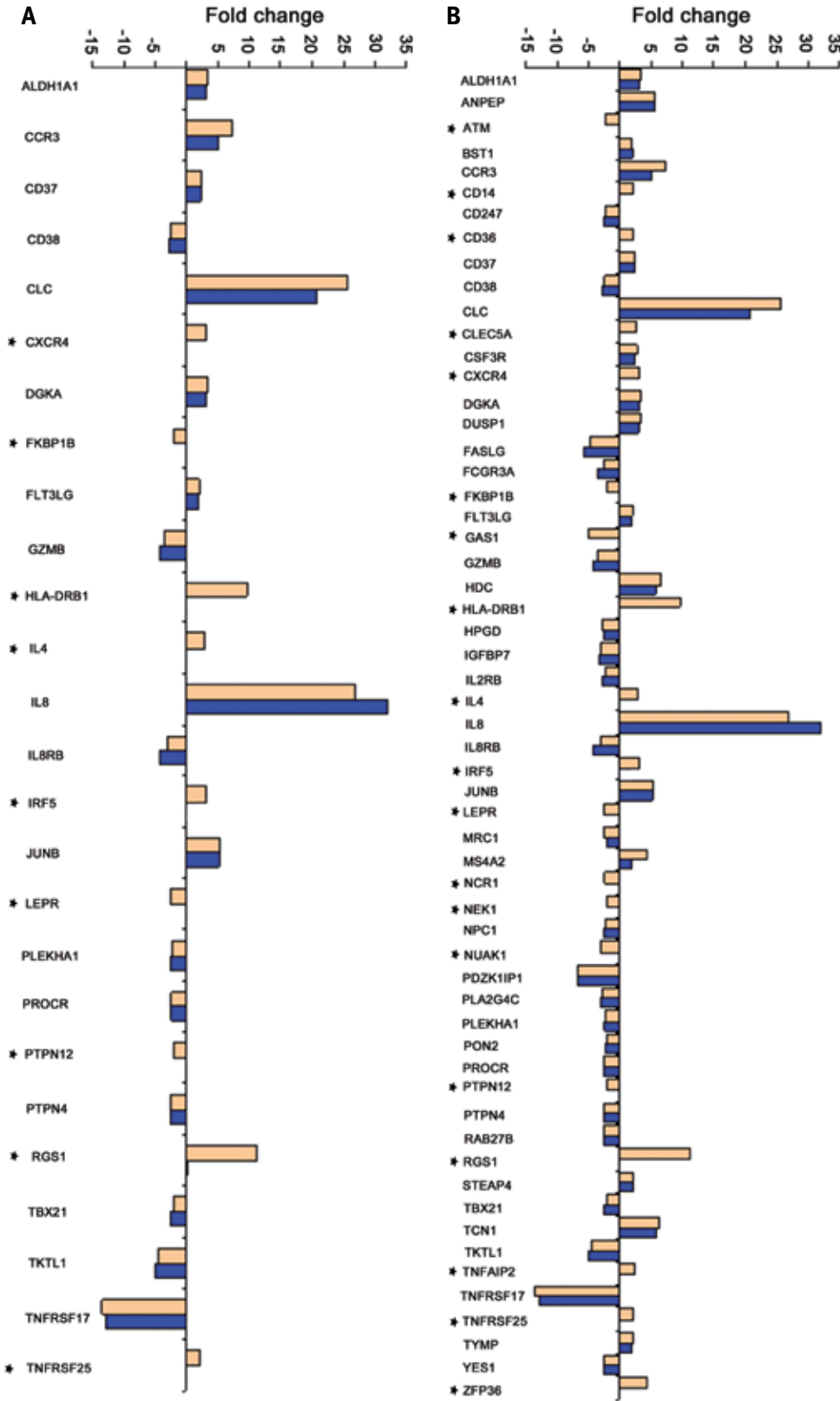
## METHODS

We analyzed the effect of IVIg treatment in 10 patients with CVID by evaluating the gene-expression profiles from Affimetrix HG-U133A [7]. The gene array results were validated by real-time polymerase chain reaction (RT-PCR) and by the detection of soluble mediators by enzyme-linked immunosorbent assay. Moreover, we performed fluorescence-activated cell sorting (FACS) analysis of blood mononuclear cells from the patients enrolled in the study.

## RESULTS

Seventy-seven genes were differentially expressed in CVID patients before IVIg therapy, including the CD14 molecule (CD14, FC 2), leptin receptor (LEPR, FC 22.5), CD38 molecule (CD38, FC 22.4), RGS1 (FC 11.3), TNFRSF25 (FC 2.2), interleukin-4 (IL-4, FC2.9), CXCR4 (FC 3.1), CCR3, (FC 7.4), and IL-8 (FC 26.9) [Figure 1A]. The vast majority (26/77) of these modulated transcripts belong to the innate and acquired immune response gene categories [Figure 1B]. We then studied the effect of IVIg infusion on the immune response in CVID patients by analyzing the gene expression profiles obtained from the same CVID patients 3 days after IVIg therapy. Interestingly, 23 of 77 genes returned to an expression level similar to that of normal controls [Figure 1 A & B].

RT-PCR of selected genes and serum levels of soluble mediators (IL-4, IL-8 and CXCR4) before and after therapy changed



**Figure 1.** Gene expression profiles of modulated genes and flow cytometric analysis of T and B cell populations in CVID patients before and after IVIG treatment  
**[A]** Global representation of genes related to innate and adaptive immune responses  
**[B]** Representation of genes involved in T and B cell immune response.  
 Figure shows FC in gene expression in peripheral blood mononuclear cells (PBMC) isolated from 10 patients with CVID before (orange bars) and after (blue bars) IVIG infusion. Y axis represents the fold change level of gene modulation. Asterisks indicate genes returned to the level of healthy controls after IVIG infusion

**[C,D,E]** The histograms show the percentages of CD4+, CD8+ T and CD19+CD5-CD23-CD27-IgM-IgD- B (centrocytes) cell populations before (blue bars) and after (red bars) IVIG infusion, respectively.  
 Data are representative of the mean values obtained for all the 30 subjects studied.

**C**

**D**

**E**

according to gene array results. Moreover, FACS analysis demonstrated a marked decrease of CD8+T cells and an increase of CD4+T cells following treatment [Figure 1 C & D]. Interestingly, we also observed an increase of centrocytes (CD23- CD27- IgM- IgD- B cells) [Figure 1 E]. The phenotypic modifications observed with the FACS analysis are in agreement with the gene expression data. Indeed, the expression of the *TNFRSF25* gene, able to promote CD8+T cell survival, was up-regulated before therapy and returned to baseline levels after IVIg infusion, in accordance with the reduction of CD8+T cells. The *LEPR* gene, which has a crucial role in CD4+T cell proliferation, was down-regulated before infusion returned to baseline levels after the treatment. Therefore, the modulation of *TNFRSF25* and of *LEPR* genes may be related to the decrease of CD8+ cells and to the increase of CD4+ T cells after IVIg. Similarly, modulation of genes involved in B cell development such as *RGS1* may result in modification of the B cell phenotype induced by IVIg infusion, i.e., increased number of switched memory B cells.

## CONCLUSIONS

Our results provide further support to the hypothesis that the benefits of IVIg therapy [8-10] are not only related to antibody replacement but also to its ability to modulate the immune response in common variable immunodeficiency.

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