

Letter to the Editor

Decreased IL-10 production by EBV-transformed B cells from patients with VODI: Implications for the pathogenesis of Crohn disease

To the Editor:

Veno-occlusive disease with immunodeficiency (VODI) is an autosomal recessive disorder characterized by B- and T-cell immunodeficiency and hepatic vascular occlusion and fibrosis. Patients present within the first year of life with opportunistic infections and hepatic dysfunction. Patients with VODI have normal numbers of circulating T cells, but there is decreased production of IFN- γ , IL-2, IL-4, and IL-10 in response to stimulation with phorbol myristate acetate and ionomycin. The B-cell deficiency in patients with VODI is characterized by the absence of memory (CD19⁺, CD27⁺, and IgD⁺) B cells and plasma cells and severe hypogammaglobulinemia. The gene responsible for VODI was mapped to chromosome 2q37.1, and it was shown that these patients lack functional Sp110, a putative transcription factor.¹ The mechanisms by which Sp110 mutations produce the T- and B-cell abnormalities in VODI are unknown.

IL-10, produced by B and T cells, is a growth and differentiation factor for B lymphocytes.^{2,3} To further investigate a potential association between Sp110 deficiency and impaired IL-10 production, we tested EBV-transformed B-cell lines from patients with VODI for the ability to produce IL-10. The cell lines were derived from 3 patients with the homozygous c.642delC mutation, which causes a frameshift in Sp110 exon 5. The absence of Sp110 from these cell lines was confirmed by indirect immunofluorescence, whereas Sp110 was readily detected in each of the 3 control cell lines derived from relatives of patients with VODI (Fig 1).

Reverse transcription and PCR were used to measure IL-10 mRNA levels in each of the cell lines. IL-10 mRNA levels in EBV-transformed cells from patients with VODI were 24% of

those in control cells ($P < .002$; Fig 2, A). By using a solid-phase immunoassay, IL-10 levels in the supernatant of VODI cell lines were found to be much less than those in supernatants of control cells (23.5 ± 7.0 pg/mL vs 693 ± 313 pg/mL, respectively; $P = .003$; Fig 2, B). For comparison, there was no significant difference in the relative level of IL-8 mRNA in control and VODI B-cell lines (control vs VODI: 1.0 ± 0.85 vs 0.92 ± 0.26 ; $P = .90$). There was also no difference in the amount of IL-8 secreted by the control and VODI B-cell lines (51 ± 22 pg/mL vs 49 ± 10 pg/mL; $P = .84$).

To further characterize the control and VODI B-cell lines, we determined the relative levels of CD27, CD38, IL-6, IL-12, and IL-19 mRNA. There was no difference between control and VODI B-cell lines in the level of mRNA encoding CD38 (control vs VODI: 1.0 ± 0.60 vs 0.93 ± 0.39), IL-6 (1.0 ± 0.09 vs $1.06 \pm .24$), IL-12 (1.0 ± 0.21 vs 0.98 ± 0.08), or IL-19 (1.0 ± 0.12 vs 1.3 ± 0.15). There was, however, a significant difference between control and VODI B-cell lines in the level of mRNA encoding CD27 (control vs VODI: 1.0 ± 0.47 vs 0.08 ± 0.06 ; $P = .001$). The decreased level of CD27 mRNA in VODI B-cell lines is consistent with the previously reported decreased number of memory B cells in patients with VODI.¹

The extremely low level of IL-10 produced by EBV-transformed VODI B-cell lines is remarkable because EBV transformation usually induces B lymphocytes to produce IL-10. The viral small non-polyadenylated RNAs (EBERs), which are expressed during viral latency, have been shown to induce IL-10 expression in transformed cells.⁴

IL-10 has an important role in inducing the differentiation of B cells. In the presence of IL-10, activated B cells undergo isotype switching and express high levels of IgG and IgA as well as IgM.⁵ We used immunoblot and antisera directed against IgG or IgM heavy chains to determine the immunoglobulin isotype produced

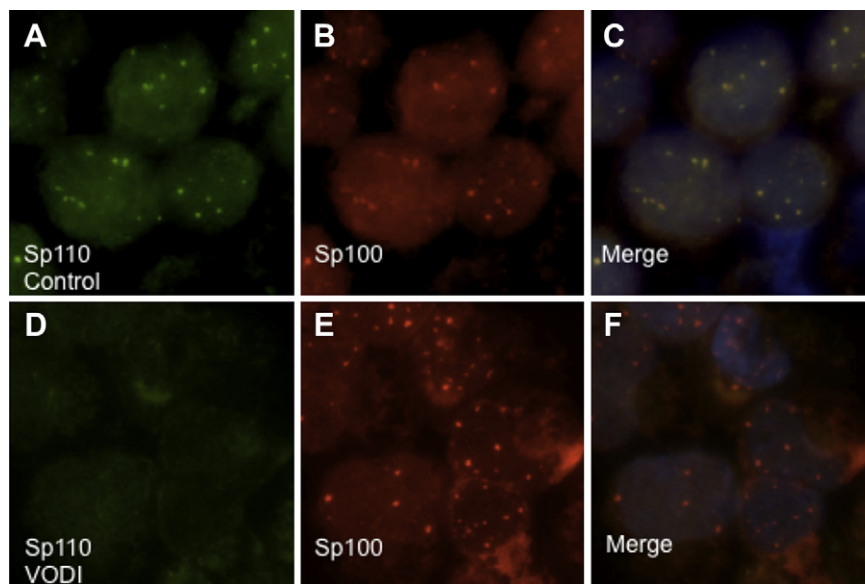


FIG 1. Sp110 (green) (A) and Sp100 (red) (B) were detected in nuclear bodies in control, EBV-transformed B-cell lines. Sp100 (E), but not Sp110 (D), was detected in nuclear bodies in cell lines derived from patients with VODI. Merging of panels A and B and of panels D and E is shown in panels C and F, respectively. 4'-6-Diamidino-2-phenylindole dihydrochloride staining (blue) in C and F indicate the location of nuclei.

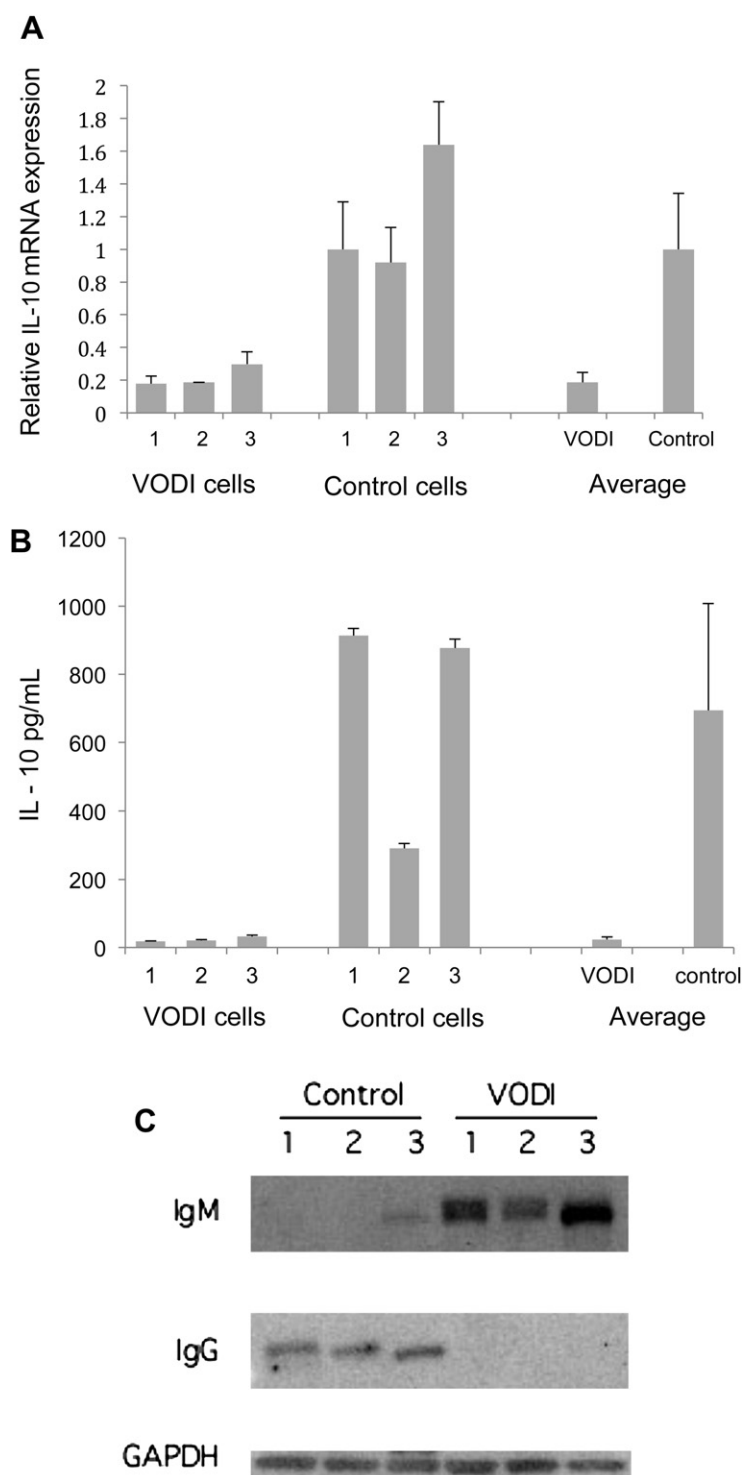


FIG 2. IL-10 production is decreased in EBV-transformed VODI cell lines. Compared with control cell lines, VODI B-cell lines produced significantly less IL-10 mRNA (**A**). Immunoreactive IL-10 was markedly decreased in the supernatant of VODI, compared with control cell lines (**B**). Control B-cell lines produced IgG while VODI B-cell lines produced IgM (**C**), consistent with a defect in isotype switching. *GAPDH*, Glyceraldehyde 3-phosphate dehydrogenase.

by each B-cell line. The 3 control B-cell lines produced IgG. In contrast, the 3 VODI B-cell lines produced IgM (Fig 2, C). These results are consistent with an isotype switching defect that might result from decreased IL-10. Impaired production of IL-10 by T

and B cells may explain, in part, the hypogammaglobulinemia seen in patients with VODI.

IL-10 also has potent antiinflammatory effects and dampens the immune response to infection. Mice that lack IL-10

develop colitis, which has been attributed to an exaggerated response to intestinal flora. IL-10-deficient mice serve as a model for inflammatory bowel disease.⁶ A recent genome-wide meta-analysis described 71 loci that may predispose to the development of Crohn disease. Two of the newly identified genomic loci contain the genes encoding Sp110 (at 2q37) and IL-10 (at 1q32).⁷

Children with defects in the IL-10 signal transduction pathway, such as inactivating mutations in the IL-10 receptor, develop inflammatory bowel disease at an early age.⁸ In contrast, despite decreased IL-10 production, inflammatory bowel disease has not been reported in patients with VODI. It is possible that the low (but detectable) level of IL-10 combined with other environmental and genetic factors is sufficient to prevent or delay the onset of inflammatory bowel disease in patients with VODI.

In summary, IL-10 production is markedly reduced in B cells from patients with germline mutations in Sp110. Decreased production of IL-10 may contribute to the hypogammaglobulinemia that is seen in these patients. In view of the potential link between Sp110 deficiency and decreased IL-10 production, the role of IL-10 deficiency in inflammatory bowel disease, and the presence of Sp110 and IL-10 in Crohn disease susceptibility loci, it is tempting to speculate that polymorphisms that affect the expression or function of Sp110 may be associated with decreased IL-10 production and contribute to the risk of developing inflammatory bowel disease.

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