An inherited immunoglobulin class-switch recombination deficiency associated with a defect in the INO80 chromatin remodeling complex

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Background: Immunoglobulin class-switch recombination defects (CSR-D) are rare primary immunodeficiencies characterized by impaired production of switched immunoglobulin isotypes and normal or elevated IgM levels. They are caused by impaired T:B cooperation or intrinsic B cell defects. However, many immunoglobulin CSR-Ds are still undefined at the molecular level.

Objective: This study's objective was to delineate new causes of immunoglobulin CSR-Ds and thus gain further insights into the process of immunoglobulin class-switch recombination (CSR).

Methods: Exome sequencing in 2 immunoglobulin CSR-D patients identified variations in the *INO80* gene. Functional experiments were performed to assess the function of *INO80* on immunoglobulin CSR.

Results: We identified recessive, nonsynonymous coding variations in the *INO80* gene in 2 patients affected by defective immunoglobulin CSR. Expression of wild-type *INO80* in patients' fibroblastic cells corrected their hypersensitivity to high doses of γ-irradiation. In murine CH12-F3 cells, the INO80 complex accumulates at Sα and Eμ regions of the IgH locus, and downregulation of *INO80* as well as its partners Reptin and Pontin impaired CSR. In addition, Reptin and Pontin were shown to interact with activation-induced cytidine deaminase. Finally, an abnormal separation of sister chromatids was observed upon *INO80* downregulation in CH12-F3 cells, pinpointing its role in cohesin activity.

Conclusion: *INO80* deficiency appears to be associated with defective immunoglobulin CSR. We propose that the INO80 complex modulates cohesin function that may be required during immunoglobulin switch region synapsis. (J Allergy Clin Immunol 2015;135:998-1007.)

Key words: *Chromatin remodeling, class-switch recombination defect, CSR synapse, cohesin*

Immunoglobulin CSR defects (CSR-Ds) are rare primary immunodeficiencies characterized by impaired production of switched immunoglobulin isotypes and normal or elevated IgM levels.[1] Indeed, the analysis of CSR-Ds caused by impaired T:B

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Abbreviations used
AID: Activation-induced cytidine deaminase
ChIP: Chromatin immunoprecipitation
CSR: Class-switch recombination
CSR-Ds: CSR defects
DAPI: 4′,6-Diamidino-2-phenylindole
GLT: Germ-line transcript
MMR: Mismatch repair
NHEJ: Non-homologous end joining
TCR: T-cell receptor
wt: Wild type

cooperation[2] or intrinsic B cell defects has provided a better understanding of the complex mechanisms underlying antibody maturation in humans. The description of patients with an activation-induced cytidine deaminase (AID) deficiency revealed this enzyme's master role in both CSR and somatic hypermutation.[3] The identification of a CSR-D caused by mutations in the *uracil-N glycosylase* gene also demonstrated that AID had DNA editing activity.[4] Furthermore, the identification of mutations in CSR-D patients has shown that several proteins involved in DNA repair—such as non-homologous end joining (NHEJ) factors and mismatch repair (MMR) enzymes—also have roles in CSR.[5-7] However, many immunoglobulin CSR-Ds remain still undefined at the molecular level, and their delineation, now possible through the use of whole exome (or genome) sequencing, affords a better understanding of the complex mechanisms involved in CSR.

In the present study, we report the identification of 2 CSR-D patients with recessive, nonsynonymous coding variations in the *INO80* gene and show that *in vitro* downregulation of INO80 complex subunits impairs CSR. Our results also suggest that *INO80* is involved in the conformational modification of the immunoglobulin locus required for the S-region-specific recombination process in CSR, possibly through modulation of cohesin activity. We also found that the INO80 complex subunits Reptin and Pontin interact with AID—suggesting that AID's known role in S-region synapsis[8,9] occurs through its interaction with the INO80 complex.

A role for a chromatin remodeling complex in CSR is not unexpected, because CSR is achieved by a DNA recombination between two S regions. The S regions need to be accessible and transcribed, and DNA's interactions with most nuclear factors is restricted when the chromatin is highly condensed, suggesting the requirement of chromatin modification. Chromatin dynamics are regulated by (i) post-translational modifications of the core histones and (ii) ATP-dependent chromatin remodeling.[10] Histone phosphorylation, ubiquitination, methylation and acetylation have all been implicated in immunoglobulin CSR.[11-15]

Four structurally related families of ATP-dependent chromatin remodeling complexes (SWI/SNF, INO80, CHD, and ISWI) have been described, each being defined by its characteristic catalytic core ATPase from the SWI2/SNF2 superfamily.[16] The complexes' biological functions include the disruption of histone-DNA contact within nucleosomes and the cis and trans movements of histone octamers that facilitate access to nucleosomal DNA for transcription factors and restriction endonucleases.

The INO80 chromatin remodeling complex has 3′-5′ helicase activity and contains the SNF/SWI2 ATPase INO80.[17] The INO80 ATPase binds to actin, 3 actin-related proteins (ARPs, with ARP5 and ARP8 specifically present in the INO80 complex), and 2 AAA^+-ATPases (RUVBL1 and RUVBL2, also known respectively as Reptin and Pontin).[18] The INO80 complex is conserved from budding yeasts through to humans and has functional roles in DNA replication, DNA repair, the regulation of transcription, chromosomal segregation, and telomere maintenance.[19]

METHODS

A detailed description of materials and methods is provided in this article's Online Repository available at www.jacionline.org. The study was performed in accordance with the precepts of the Declaration of Helsinki.

RESULTS

Immune system defects in CSR-deficient patients

Patient 1 (P1) was the unique child born from a Turkish nonconsanguineous family. He presented with severe, recurrent bacterial infections at the age of 5 years. No opportunistic infections were noticed. A serum immunoglobulin assay revealed normal IgM levels (0.7 g/L) but decreased IgG (4.7 g/L) and IgA (0.09 g/L) levels. P1 received prophylactic antibiotics with no immunoglobulin substitution. During follow-up, the IgG levels (including IgG isotypes) and IgA levels rose progressively but remained lower than normal at 10 years of age (Table I). No specific antibody response to antigenic challenge could be studied.

Patient 2 (P2) was an English-born male not related to P1 who first suffered from severe and recurrent upper respiratory infections at the age of 18. No susceptibility to opportunistic infections was reported. At diagnosis, he presented with depressed IgG levels (0.70 g/L) and IgA levels (0.03 g/L) but had normal IgM levels (0.87 g/L). At the time of evaluation, P2 was 67 years old and had chronic obstructive pulmonary disease following 35 years of smoking. Immunoglobulin assays revealed a lack of serum IgA but a slight decrease of serum IgM; IgG (and specific antibody response) could not be evaluated because of the patient's regular immunoglobulin replacement therapy (Table I). The patient responded well to immunoglobulin replacement therapy, with the exception of the chronic obstructive pulmonary disease.

In both patients, the presence of mutations in genes already known to be involved in CSR was ruled out through sequence analysis (*AID* and *UNG)* or the observation of normal expression (CD40L and CD40) and CD40-mediated B cell proliferative responses. Total B cell counts were normal, but the number of IgM^- IgD^- $CD19^+$ $CD27^+$–switched memory B cells was low. T cell counts were within the normal range. Likewise, the T-cell receptor (TCR) beta chain and the BCR repertoires were within the normal range, as assessed by amplification of V-J rearrangements (data not shown). Analysis of B cell function revealed a normal frequency of somatic hypermutations in the VH3-23 region of IgM in P1. The nucleotide substitution pattern was normal, suggesting that AID activity was unaffected (data not shown). *In vitro* $CD40L^+$ IL4-induced CSR to IgE was consistently found impaired in peripheral blood lymphocytes from both patients, when compared with age-matched controls (Table I). An *ex vivo* analysis of Sμ-Sα junctions revealed that blunt junctions were less frequent in P1 and P2 than in age-matched controls. In contrast, junctions based on 4 to 9 nt microhomologies were more frequent in the patients (Fig 1, *A*).