Immunodeficiency Canada Satellite Symposium
4th Annual sCID Symposium

CALL FOR ABSTRACTS – Fellows Lighting Talks

Attention: Program Directors and Trainees.

June 16, 2016

Immunodeficiency Canada is pleased to announce a call for abstracts for the 4th Annual Immunodeficiency Canada sCID Satellite Symposium. The symposium will take place on September 29th, 2016 at the CSACI Annual Meeting, Montreal.

Fellows are invited to submit a 2-3 page structured abstract (approx 1,500 words). Figures and references should be included. Please refer to the attached sample abstract for further details. Abstract must be send to network@immunodeficiency.ca.

The deadline for abstract submission is Monday 15th August, 2016.

As in previous years, the top three abstract submissions will be recognized. Selected abstracts will be presented during 10-15 minute lighting speaker sessions, and all abstracts can be published online and in print in LymphoSign Journal.

Thank you for your contribution and we look forward to another successful symposium!

Sincerely,

Chaim M. Roifman. MD, FRCP.  
Scientific Director  
Immunodeficiency Canada
<SAMPLE ABSTRACT>

CD40 DEFICIENCY: MORE THAN A CLASS SWITCH RECOMBINATION AND SOMATIC HYPERMUTATION DEFECT. A REVIEW AND REPORT OF AN ADULT PATIENT WITH A PARTICULAR HYPER IMMUNOGLOBULIN M SYNDROME

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Background

CD40 deficiency is a rare autosomal recessive combined primary immunodeficiency characterized by defects of immunoglobulin class switch recombination (CSR) and somatic hypermutation (SMH) that is part of an expanding group of diseases collectively known as Hyper immunoglobulin M syndromes [1]. This defect not only affects immune cells. Signaling through CD40 is important to endothelial cells and neurons, among others [2-4].

CD40/CD40 Ligand interaction in B and T cells occurs after antigen-mediated activation and it is an absolute requirement to initiate CSR and SHM by promoting germinal center development in secondary lymphoid organs, the location where these processes take place. This cross-linking induces germinal centers to proliferate and to be suitable for CSR and SHM but also protects B cells from undergoing apoptosis and generates long-lived plasma cells [5,6].

Following cross linking of CD40 on the B cell membrane, and given that this molecule lacks kinase activity on its cytoplasmic domain, intracellular signaling happens through the activation of TNF receptor associated factors (TRAFs), particularly TRAF-2, -3, -5, and -6. These factors function as adaptor molecules to recruit different kinases (e.g. mitogen-activated protein kinases or MAPKs) resulting in activation of transcription factors such as NF-κB [7,8].

Clinical manifestations of the disease usually begin early in life with recurrent sinopulmonary bacterial infections and susceptibility to opportunistic organisms. Management of CD40 deficient patients consists of aggressive antibiotic treatment of infections and immunoglobulin replacement therapy [7]. In contrast with CD40 Ligand deficiency, where the defect resides mainly on T cells, treatment with hematopoietic stem cell transplantation (HSCT) for CD40 deficiency is theoretically not a complete cure. It has been attempted in four patients with successful immune reconstitution in three [9-11].

Since its description in 2001 [12] only fourteen patients with CD40 deficiency from ten unrelated families have been reported to date, and with the exception of one patient of Italian descent, the rest are from Middle Eastern countries [9,10,13,14]. We report an additional adult patient of Iranian/French Canadian decent
with a novel mutation in CD40, presenting later in life and with a milder phenotype. Also, in contrast with the patients reported previously, our patient’s mutation allows CD40 expression in the cell membrane and adds 37 amino acids to the cytoplasmic domain of the protein, which seems to affect one of the two known TRAF2 binding sites linked to the MAP-kinases pathway while the NF-κB pathway appears to activate normally (preliminary data).

**Methods**

Clinical data was collected by retrospective chart review and directly from the patient. Informed consent was obtained. Genomic DNA was extracted from whole blood samples obtained from the patient. Promoters, exons, and both the 5’ and 3’ untranslated regions of CD40 were amplified by polymerase chain reaction for forward and reverse sequencing using the previously designed primers. RNA was also extracted from his Epstein-Barr virus (EBV)-transformed B cell line. CD40 expression on B cells was assessed by flow cytometry. Western blot analyses were performed following standard protocols. The complete manuscript, currently in preparation, will describe methods in more detail.

**Results**

**Clinical features**

The patient is a now a 20 year-old man born to non-consanguineous parents. His father is from Iran and his mother is French-Canadian. The patient was reported to have had motor developmental delay during his first 2 years of life and aversion to foods of unclear etiology requiring gastrostomy tube feedings until he was 3 years of age. His development improved afterwards. He first presented to us at the age of 15 years with a history of three documented pneumonias and one episode of septic arthritis, all presenting after he was 10 years of age. He had a history of frequent acute otitis media between the ages of 1 and 4 years that resolved afterwards without the need of tympanostomy tubes or other measures. He was healthy until the age of 10 years when he had the first episode of pneumonia. The second and third pneumonias occurred at the ages of 12 and 13 years, respectively. Also at the age of 13 years he experienced an episode of right knee septic arthritis for which we do not know the causative organism. He recovered fully from all of these infections after completing adequate antibiotic courses.

**Immunological Features**

When he was assessed at the age of 15 years he was found to have undetectable (<0.1 g/L) titers for both IgG and IgA (normal ranges of 4.5-14.3 and 0.2-1.0 g/L, respectively) and elevated IgM of 19.2 g/L (0.2-1.8 g/L). He did not have protective titers of specific antibodies for the vaccines that he had received despite a complete immunization schedule. He had normal numbers of B cells and T cells (CD19+ 454, CD3+ 2526, CD4+ 1670, CD8+ 721, NK cells 161) and his lymphocyte proliferation assays to both mitogens and antigens were also normal. He was then commenced on monthly intravenous immunoglobulin replacement therapy and has continued until now without any other episodes of severe or recurrent infections. As there was never any evidence of opportunistic infections he was not started on prophylactic antibiotics. He has remained clinically well.
Mutation Analysis

Molecular analysis showed normal CD40 Ligand and AID gene sequences. Analysis of CD40 gene revealed a novel homozygous 1 base pair deletion in Exon 9 of both genomic and complementary DNA. The mutation c.823delG results in p.E275RfsX38 that translates into a frame shift that adds 37 extra amino acids into the protein.

CD40 Expression

CD40 is expressed in the surface of the patient’s EBV-transformed B cell line as shown by figure 1.

![Figure 1](image1.png)

**Figure 1.** Flow cytometry analysis showing expression of CD19 and CD40 on both the patient and a healthy control. FMO: Fluorescence Minus One control.

Western blot analyses

Expression of CD40 protein in our patient’s EBV-transformed B cells was also confirmed by Western blot analysis. Preliminary results show normal activation of the NF-κB pathway. (Figure 2)

![Figure 2](image2.png)

**Figure 2.** A) Western blot analysis of CD40 protein comparing the expression in our patient with that of a healthy control. B) Normal nuclear translocation and activation of NF-κB pathway when assessing expression of RelA (involved in NF-κB heterodimer formation, nuclear translocation, and activation) after 10 minutes stimulation with CD40 Ligand.
Conclusions

We report an adult patient with CD40 deficiency that presented to us at a later age and manifested with a milder phenotype as compared with previously reported patients. Also, in contrast with these patients, our patient has a novel mutation that allows expression of CD40 molecule in the surface of his B cells and adds 37 amino acids to the intracellular domain of this receptor likely affecting one of the two known TRAF-2 binding sites linked to the MAP-kinases signaling pathway ultimately resulting in the CSR and SMH defects. Until fully understanding the action of CD40 in lymphocytes and other cell types, we must remain sensible to identify all the possible phenotypes, and thus the importance of reporting our patient and his particularities as we learn more from the disease.

References