

Cell mediated immunity to the influenza virus

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Abstract: Background: In patients with Common Variable Immune Deficiency (CVID) it is generally not recommended to give the Influenza vaccine. This recommendation is due, in part, to the inability of patients with CVID to generate protective antibody responses. However, it is not known if these patients are capable of generating a cell mediated response to the vaccine. Objective: To measure lymphocyte antigen stimulation, pre and post Influenza vaccine, in patients with CVID compared with normal patients. Methods: Blood was drawn from 4 patients CVID and 5 normal patients pre and post vaccination with Fluzone (the 2004-2005 Influenza vaccine). Lymphocyte antigen stimulation was then measured immediately prior to and 4 weeks after vaccination. Results: A significant difference was observed in the lymphocyte response to undiluted Influenza vaccine in the normal group. No significant response was seen in the CVID group to Influenza vaccine or to positive control. However, lymphocytes from 2 patients with CVID did have a robust response to the vaccine. Conclusion: Although the number of patients in our study is small, these data support the variable nature of lymphocyte function in patients with CVID.

Background: Common variable immunodeficiency (CVID), also called acquired hypogammaglobulinemia, adult-onset hypogammaglobulinemia, or dysgammaglobulinemia, is a heterogeneous group of disorders involving both B-cell and T-cell immune function, the predominant manifestation of which is hypogammaglobulinemia. CVID is characterized by recurrent bacterial infections, decreased serum Ig levels, and abnormal antibody responses. The variable in CVID denotes variability in the age at presentation (eg, early childhood, adolescence, or as young adults) and variability in the degree and type of hypogammaglobulinemia as well as T lymphocyte abnormalities. (1) In one study of 176 patients with CVID done by Cunningham Rundells, T cell abnormalities were common. 40% of those 176 had sub-normal lymphocyte proliferative responses to one or more mitogens. (2). The average age of onset of symptoms in CVID is 25 years, and the average age at diagnosis is 28 years.(2) In a subsequent study by Cunningham-Rundles and Bodian, (3). the mortality rate over a 25-year period was 24%, mostly because of lymphoma (18%) and chronic pulmonary disease (11%). (3) In patients with CVID it is generally not recommended to give the Influenza vaccine. This recommendation is due in part to the inability of patients with CVID to generate protective antibody responses. However, it is not known if these patients are capable of generating a cell mediated response to the vaccine.

Methods: Patients were selected based on a diagnosis of common variable immune deficiency. This was defined as clinical history of recurrent infections, 2 classes of Immunoglobulin that were 2 standard deviations below the mean and demonstration of functional antibody deficiency. Patients signed informed consent for their blood to be drawn. All subjects both normal and CVID, had not received the Influenza vaccine at entry into our study. All consented to receiving the Fluzone after initial blood draw. Lymphocyte response to antigen stimulation with dialyzed Fluzone undiluted and 1:5 dilution was performed using a standard technique.(4,5) All participants' lymphocytes were stimulated with antigen pre and post vaccination with both Influenza 1:5 and undiluted as well as Streptolysin O at 1:20 and 1:10 dilutions. Streptolysin O was used as a positive control and unstimulated wells with culture media and lymphocytes were used as a negative control. Correlations were sought between pre and post vaccination lymphocyte responses in both delta cpm (Post cpm-Pre cpm) and delta stimulation index, $SI = \text{cpm of stimulated cells} / \text{cpm of non-stimulated cells}$.(SI post- SI pre). (7)

Immunological studies

10 cc's of whole blood was collected in a heparinized tube. A 1:1 mixture of blood and RPMI was prepared. 8 ml of diluted blood was layered over Ficoll-Hypaque. Each tube was centrifuged and washed with HBSS. Lymphocytes were counted . Cells were cultured with 15% HIHPS (heat inactivated human plasma solution) and antigens. Streptolysin O at concentration of 1:20 and 1:10 was used as a positive control and un-stimulated cells were used as the negative control. Cells were incubated for 6 days and pulsed with tridiated thymidine. Cells were harvested on Day 7 and counted.(4,5)

Statistical analysis

A *P* value of <.05 was considered to be statistically significant; Prism software was used in these analyses.

Results:

Figures 1-8 represent the change in lymphocyte responses from pre to post Influenza vaccination expressed in change in cpm (counts per minute). These results show no statistically significant difference from pre and post vaccination lymphocyte antigen stimulation for Influenza in patients with CVID. However, a statistically significant change was seen in the change in cpm in Normal patients with undiluted Influenza vaccine.

Figures:

Figure 1

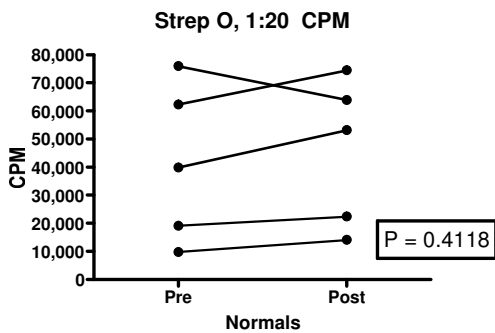


Figure 2

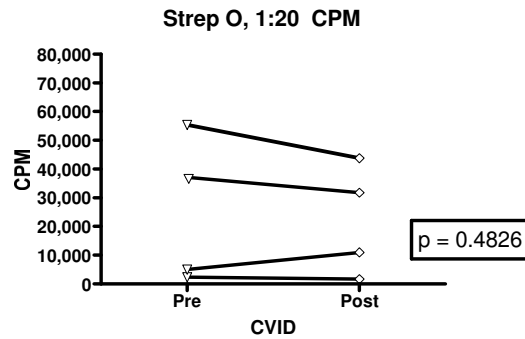


Figure 3

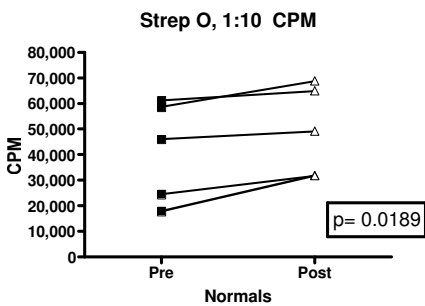
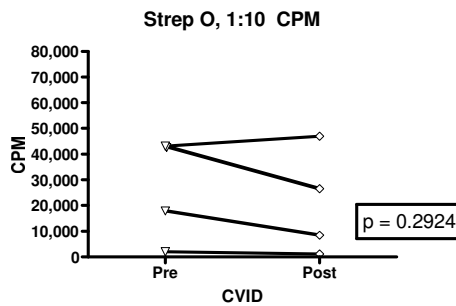


Figure 4



Figures Cont.:

Figure 5

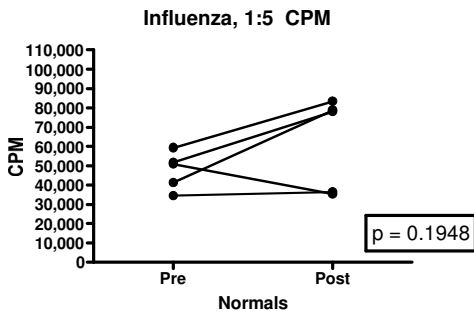


Figure 6

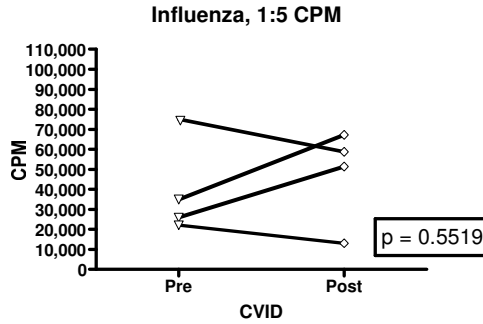


Figure 7

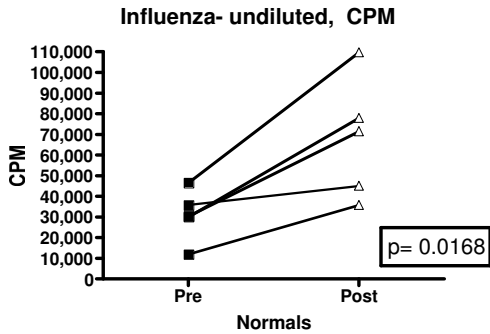
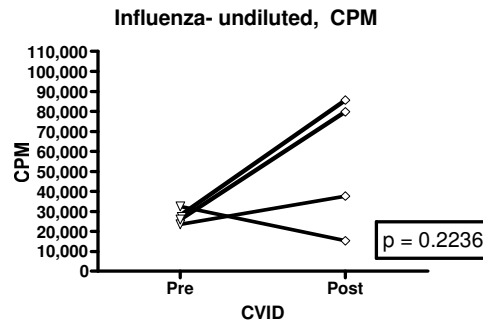


Figure 8



Figures 9-16 represent the change in Stimulation Index from pre to post Influenza vaccination expressed as $SI = \text{cpm of stimulated cells} / \text{cpm of non-stimulated cells}$. (SI post- SI pre). These results show no statistically significant difference from pre and post vaccination lymphocyte antigen stimulation index for Influenza or control antigen for all patients both normal and CVID.

Figure 9

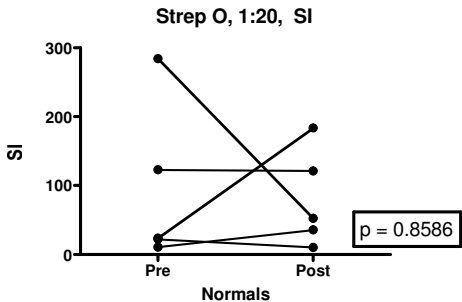


Figure 10

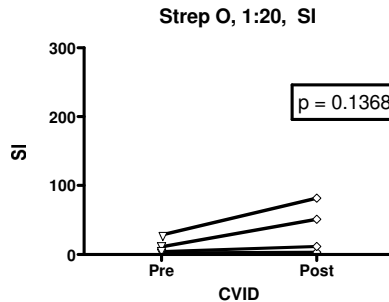


Figure 11

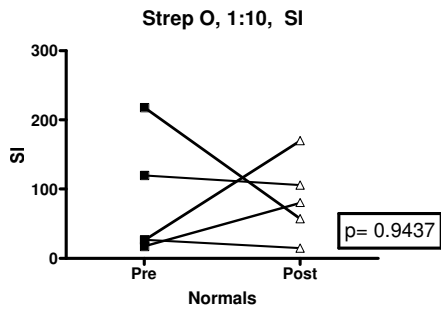


Figure 12

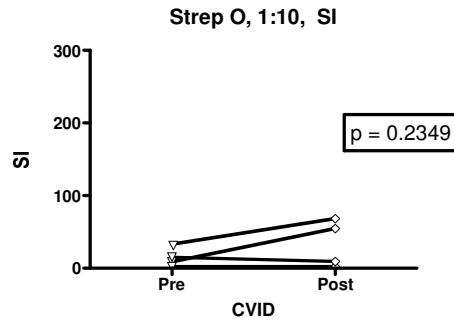


Figure 13

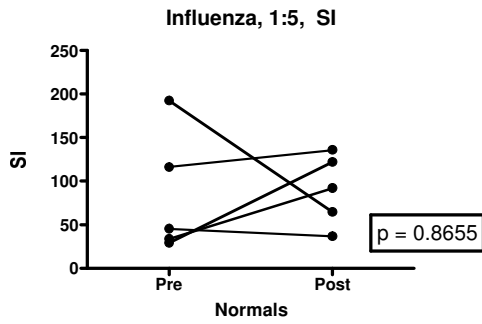


Figure 14

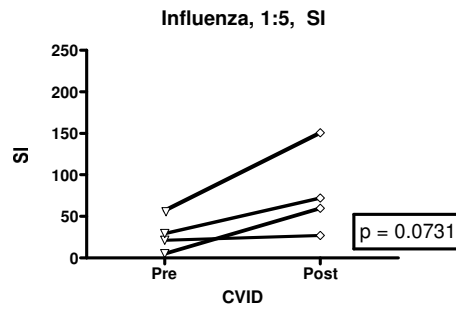


Figure 15

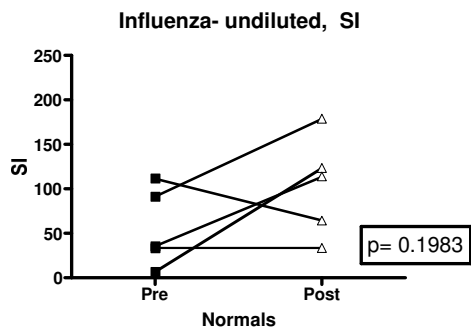
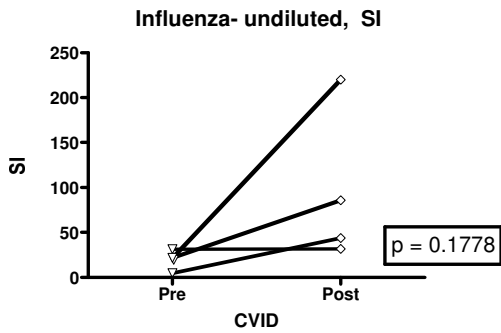


Figure 16



Figures 17 and 18 show no significant difference between the mean cpm (Flu-undiluted) of both normal and CVID patients pre and post vaccination

Figure 17

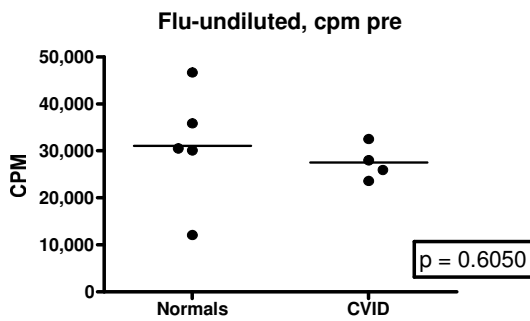
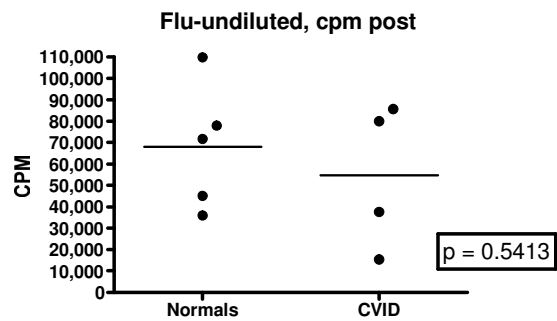


Figure 18



Figures 19 and 20 show no significant difference in mean SI with Streptolysin O 1:10 pre and post vaccination in both groups.

Figure 19 [p= 0.9437]

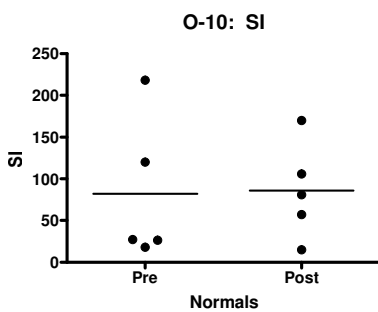
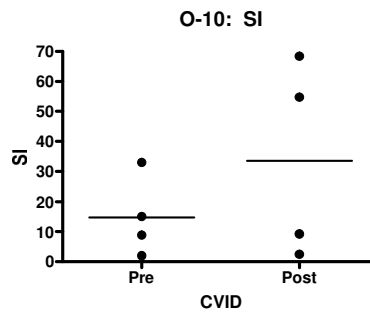


Figure 20 [p= 0.2349]



Figures 19 and 20 show no significant difference in mean SI with undiluted Influenza pre and post vaccination in both groups.

Figure 21 [p= 0.1983]

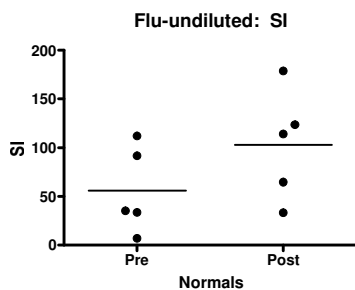
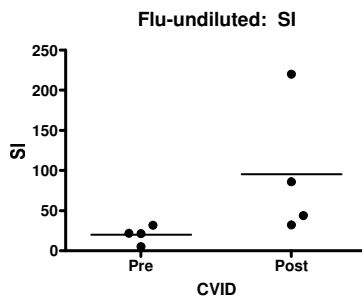


Figure 22 [p= 0.1778]



Figures 23 and 24 show no significant difference between the mean cpm of non stimulated cells, pre and post vaccination, in both groups.

Figure 23

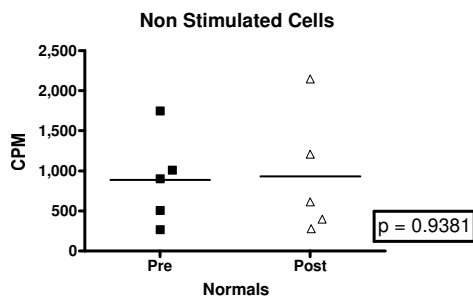
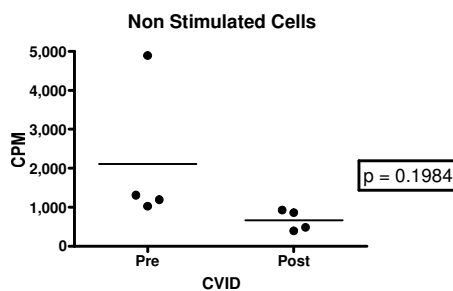


Figure 24



Discussion: This study revealed no significant change pre and post vaccination with Influenza vaccine in the CVID patients for both delta cpm and SI. However, a significant response was seen $p=(0.0168)$ for delta cpm in the normal patients for undiluted Influenza vaccine. None of the stimulation indices were significant. 2 of the CVID patients did have a robust response post vaccination but the CVID group as whole did not make a significant response.

Some patients with CVID have been found to have abnormal T cell responses. As noted earlier, T cell abnormalities were common in one study by Cunningham Rundells.(2) In that study, 40% of those 176 had sub-normal lymphocyte proliferative responses to one or more mitogens.(3) Our data supports this in part by the clear separation in response values in our CVID subjects. It is plausible that these data reflect the variable lymphocyte response in different patients who meet the diagnostic criteria for CVID.

None of the participants reported a history consistent with Influenza this year. Gate studied lymphocyte proliferation in adults 9 to 11 months after an influenza outbreak. He found a lymphocyte response only in patients with a history of both vaccination and Influenza infection. (6,7). The lack of statistically significant response to antigen post vaccination may be accounted for by a lack of clinical infection. However, a statistically significant difference in pre and post cpm in the normal group would discount that.

We looked at the mean cpm for pre and post cpm in both groups. This was done because the values were higher in the normal group. However, no significant difference was found. We did the same for the mean SI for streptolysin O 1:10 and undiluted influenza vaccine. Again there was no significant difference found. Lastly we looked at the mean values for non stimulated cells pre and post vaccine in both groups. No significance was found.

Although there are some suggestions made by these results, the study is too small to be powered. As some patients with CVID may benefit from vaccination to Influenza, a larger study that is adequately powered to do sub-group analysis should be performed.

References

1. Ballou M. Primary immunodeficiency disorders: antibody deficiency. *Journal of Allergy & Clinical Immunology*. 109(4):581-91, 2002 Apr.
2. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. *Clin Immunol* 1999;92:34-48
3. Hermans P, Diaz-Buxo J, Stobo J. Idiopathic late-onset immunoglobulin deficiency: clinical observations in 50 patients. *Am J Med* 1976;61:221-37.
4. Gerber MA, Rapacz P, Kalter SS and Ballou M: Cell-mediated Immunity in Cat-Scratch Disease. *J Allergy Clin Immunol* 78(5): 1887-890, 1986

5. Ballow M, McLean RH, Damjanov I, Lowenstein MB: Immunologic Reconstitution, Autoimmunity, and T-cell Immune Deficiencies. *Cell Immunology* 45: 364-376, 1979.

6. Cate, T. R and J. R. Kelly. 1970. Hong Kong influenza antigen sensitivity and decreased interferon response of peripheral lymphocytes. *Antimicrob. Ag. Chemother.* 1969, p156-160.

7. Briggs, W A. Influence of Prior Cellular Immunity on the In Vitro Lymphocyte Response to Virus Antigens After Influenza Vaccination. *Infection and Immunity*, May 1981, p. 503-507.