

Levels of IL-17A in Sera From Patients with Autoimmune Complications of Group A Streptococcal Infections

Youngjae Park, Edward Kaplan, Dwight Johnson, T. Dileepan, and Patrick Cleary
 Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota, USA

Introduction

- Th17 T cells are differentiated helper T cells which release a cytokine IL-17A that attracts neutrophils to infections. Despite their crucial role in the adaptive immunity, they are also associated with autoimmune disease.
- Group A Streptococcal (GAS) infections commonly induce pharyngitis. Sore throat, fever, and large lymph nodes are the typical symptoms (Fig. 1).

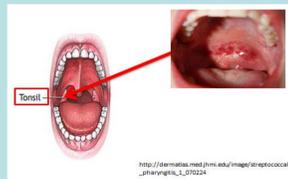


Fig. 1. Illustration of a tonsil when the mouth is open (left) and a photo of patient's tonsil with sore throat (right).

- Also, GAS infections are linked to autoimmune disease such as Rheumatic Fever and Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus (PANDAS). Obsessive Compulsive Disorder (OCD) is the main symptom of PANDAS (1).
- Studies from the Cleary laboratory showed that Th17 cells dominate responses to GAS intranasal infection in a mouse model (2). Unpublished studies from the laboratory showed that children's tonsils from recurrent tonsillitis contain T cells that are activated by GAS to express IL-17A (Fig. 2).

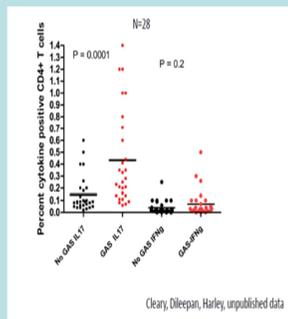


Fig. 2. Percent of cytokine positive CD4+ T cells responding to IL-17A.

- Because IL-17A is known to be associated with multiple sclerosis (MS), which is another autoimmune disease, we hypothesized that the concentration of IL-17A in sera from Obsessive Compulsive Disorder (OCD) and PANDAS patients is higher than a healthy control. In this study we investigated the level of IL-17A in OCD and PANDAS patients.

Method Development

- Sandwich Human IL-17A ELISA was developed to estimate levels of IL-17A in serum from OCD patients and a PANDAS patient. The serum samples were obtained from a serum collection from a previous study (3).

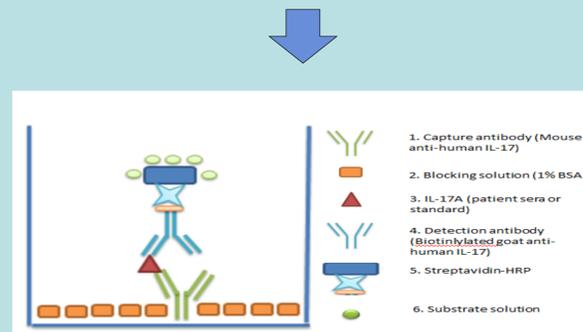


Fig. 3. Sandwich Human IL-17A ELISA: human IL-17A DuoSet ELISA was purchased from R&D System: mouse anti-human IL-17A (4.0 µg/mL in PBS, pH 7.2-7.4), Reagent Diluent (1% BSA in PBS, 0.2 µm filtered), Wash buffer (0.05% Tween® 20 in PBS), Streptavidin conjugated to horseradish-peroxidase (1:200), Substrate solution (1:1 mixture of color reagent A (H₂O₂) and color reagent B (tetramethylbenzidine)), Stop solution (H₂SO₄), recombinant human IL-17A (400 pg/mL in Reagent diluent).

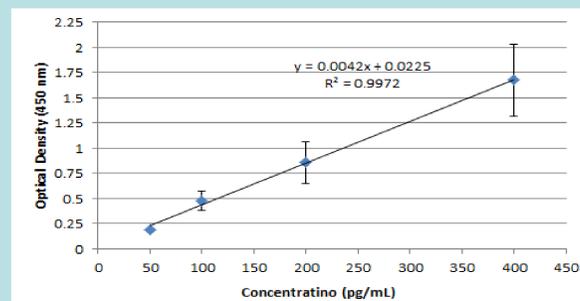


Fig. 4. Standard curve of IL-17A. Points represent the mean (±SEM) corresponding to known concentrations (50 pg/mL, 100 pg/mL, 200 pg/mL, and 400 pg/mL).

- It was observed that unknown substances in sera suppressed binding of IL-17A to the capture antibody. In addition, a healthy serum from each individual showed different levels of suppression.

Results

- One serum sample from a PANDAS patient had elevated levels of IL-17A relative to sera from controls who were not known to have had a recent GAS infection. However, it was not possible to know whether elevated IL-17A was associated with OCD symptoms or due to inflammation from the tonsillectomy.

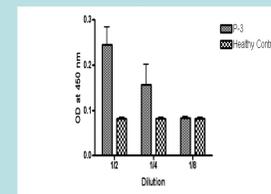


Fig. 5. Mean (+SEM) OD of IL-17A in serum (P-3) from a child who has been diagnosed with PANDAS

- YALE011 and YALE032 showed increase in levels of IL-17A between time before and after exacerbation onset. However, YALE032 also exhibited decrease in levels of IL-17A before and after time of the second exacerbation onset. These small changes may not be significant.

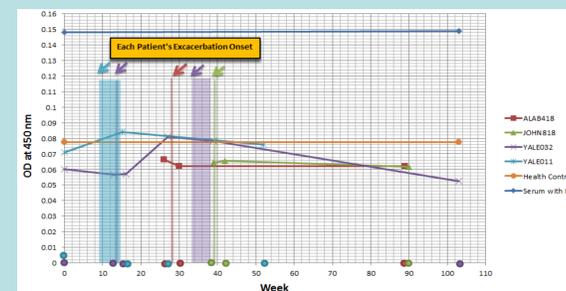


Fig. 6. ELISA measurement of IL-17A in sera from OCD patients at different time points surrounding recurrence of exacerbation onsets. Points indicate weeks patients were tested. The levels of IL-17A in sample sera, which were obtained before and after onset of exacerbation were tested. A known concentration of IL-17A (400 pg/mL) was added to a serum from a healthy person as a positive control.

Table 1. Description of the patients whose serum specimens were obtained for the study.

Patient ID	Study week	Week of exacerbation	Symptoms by GAS infection/week	Treatment/week
ALAB418	26, 30, 89	28	No symptom	No information
JOHN818	39, 42, 90	39	Sore throat / 15.8 Fever / 26.4 Sore throat / 46.7	Amoxicillin / on or before 50.9
YALE032	0, 13, 16, 27, 39, 103	13, exacerbation reoccurred during time period 33 through 39	Fever / 3.8, 4, 7.3 Sore throat, redness / 38 Sore throat / 61.3 Fever / 66.1	No information
YALE011	0, 15, 52	Exacerbation occurred during time period 8 through 12.1, exact date unknown	Fever, sore throat / 84.3, 102 Sore throat / 38.9	Zithromax / on or before 42.7

Conclusions

- Reproducible assay for IL-17A in human serum was developed and it was recognized that serum suppressed sensitivity of the assay.
- One sample serum from a patient undergoing tonsillectomy showed elevation of IL-17A but the significance of that increase is unknown.
- Although increasing levels of IL-17A were observed after onset of OCD exacerbation in sera from two patients, those increases were not large enough to be significant.

Future Work

- It is possible that IL-17A was denatured by multiple rounds of freezing and thawing. Therefore, fresh patient samples will be tested in the future.
- More serum specimens from PANDAS patients and controls have been acquired and will be assayed for IL-17A in order to further test whether onset of symptoms is associated with higher serum levels of IL-17A or not.
- In order to determine if the elevation of IL-17A in serum samples from OCD patients is significant, the levels of IL-17A in the serum samples will be re-estimated using Sandwich Human IL-17A ELISA and the data will be statistically analyzed.

Acknowledgment

Archived sera were originally obtained following the Human Experimentation Guidelines of the US Department of Public Health and reviewed by local Institutional Review Boards (3). The use of these archived sera by this study was determined to be exempt from further review by University of Minnesota Human Subjects Review Committee.

Bibliography

1. Murphy T K, Kurlan R, Leckman J. The immunobiology of Tourette. Journal of child and adolescent psychopharmacology 2010;20(4):317-331.
2. Dileepan T, Linehan J L, Moon J, Pepper M, Jenkins M K, Cleary P. Robust antigen specific th17 T cell response to group A Streptococcus is dependent on IL-6 and intranasal route of infection. PLOS pathogens 2011;7(9):e1002252-e1002252.
3. Johnson D R, Kurlan R, Leckman J, Kaplan E L. The human immune response to streptococcal extracellular antigens: clinical, diagnostic, and potential pathogenetic implications. Clinical infectious diseases 2010;50(4):481-490