

PHI's Fourth Research Award Final Report Regulatory T Cells as a Biomarker of Post-Polio Syndrome

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There are large numbers of polio survivors today who contracted acute paralytic poliomyelitis prior to the advent of widespread vaccination programs against poliovirus (PV). What is underappreciated is the fact that polio survivors are many years later at risk of developing new neurological deterioration, a condition recognized in the late '80s as post-poliomyelitis syndrome (PPS). PPS is a slowly progressive disease in individuals previously affected by paralytic poliomyelitis that is characterized primarily by new muscle weakness and atrophy. Diagnosis of PPS is time-consuming and complicated by the need to rule out other diseases that could explain the new symptoms. The ability of a physician to diagnose a specific disease can be significantly aided by the availability of one or several disease biological markers (termed biomarkers). No biomarker(s) has (have) been identified for PPS as yet that could assist in providing a definitive, easy and rapid diagnosis.



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Disease biomarkers are measures of biological parameters indicative of a disease process. They are useful because they can assist in diagnosis or provide a means of monitoring the disease as it progresses and the effectiveness of different therapies. The study conducted at the University of Arkansas for Medical Sciences (UAMS) was, therefore, designed to answer a simple question: Are there signs or changes in the immune system (that is an immunological signature) *that can be linked to post-polio syndrome (PPS)*? If so, these changes in the immune system could be potentially used as a biomarker to diagnose PPS

quickly and efficiently and to possibly provide clues as to the causes of PPS.

In this study we had three groups of individuals – (1) healthy individuals who never had disease associated with poliovirus and were vaccinated with the polio vaccine, (2) stable polio survivors, who suffered from poliomyelitis and may have had the vaccine but currently do not have symptoms associated with PPS, and (3) individuals with PPS. We recruited 28 individuals during the one-year period of this study, which included 12 healthy and 12 PPS individuals.

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However, the number of stable polio individuals interested in participating in the study was much lower than we had anticipated. We were only able to recruit four stable polio survivors.

Our strategy was to study the cells present in the blood. We wanted to see whether the types of immune cells seen in individuals with PPS were different from those from healthy individuals or individuals with stable polio. Blood contains B cells and T cells. These cells are major components of the immune system in humans. B cells produce antibodies that protect from diseases caused by viruses or bacteria.

The polio vaccine, for example, causes the body's B cells to make antibodies that specifically recognize and kill poliovirus and thus, can protect the vaccinated individual from being infected by the virus. The T cells can recognize cells that are infected by a specific virus and kill these sick cells. Poliovirus-specific T cells will kill poliovirus infected cells of the body to protect the rest of the cells in the body from getting infected.

T cells which kill infected cells are known as effector T cells. The functions of effector T cells are controlled by another group of T cells that are called regulatory T cells. The regulatory T cells 'manage' the effector T cells and provide restrictions on the effector T cells, so that they do not indiscriminately continue to kill cells.

The regulatory cells are usually present in very low numbers in the blood. However, during infection or other situations where there is inflammation/injury in the body, the number of the regulatory T cells can increase dramatically in the blood. Thus, the

number of regulatory T cells present in the blood can provide clues about whether something is different or unusual in the individual.

Each of the individuals who participated in the study donated blood. To determine whether there is evidence of an immune biomarker in polio survivors, we examined blood donated by each of the individuals to determine whether:

(i) unusual levels of PV-specific antibodies are present in individuals with PPS;

(ii) higher numbers of the regulatory T cell type are only present in the blood of individuals with PPS; and

(iii) increased regulatory T cells are a signature of past poliomyelitis (that is present in stable polio survivors as well as PPS).

To date no one has used this approach to study PPS. Therefore, it was important first to just measure the levels of poliovirus antibodies and regulatory T cells in the blood of PPS and healthy individuals. When the results are grouped according to whether the person has PPS or is healthy, the type of data that one might expect is shown in Figure 1. Each circle represents the number of regulatory T cells or level of antibody measured in a person.

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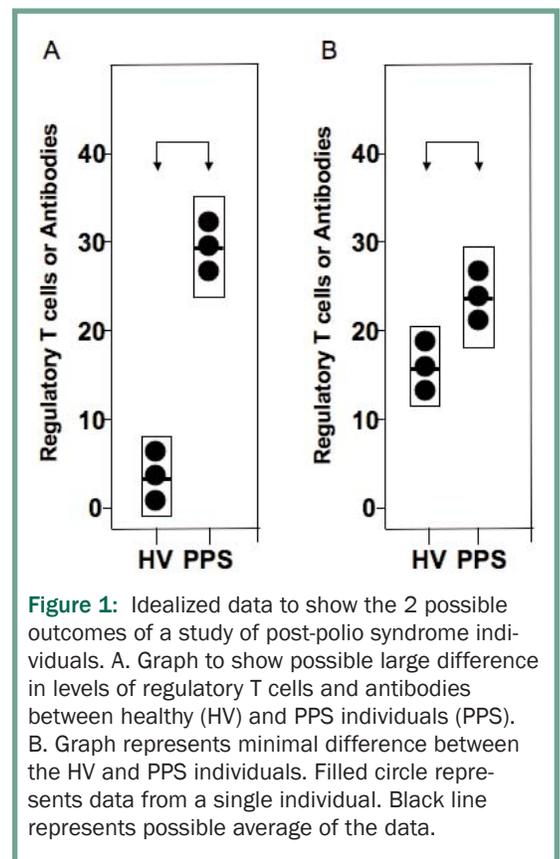


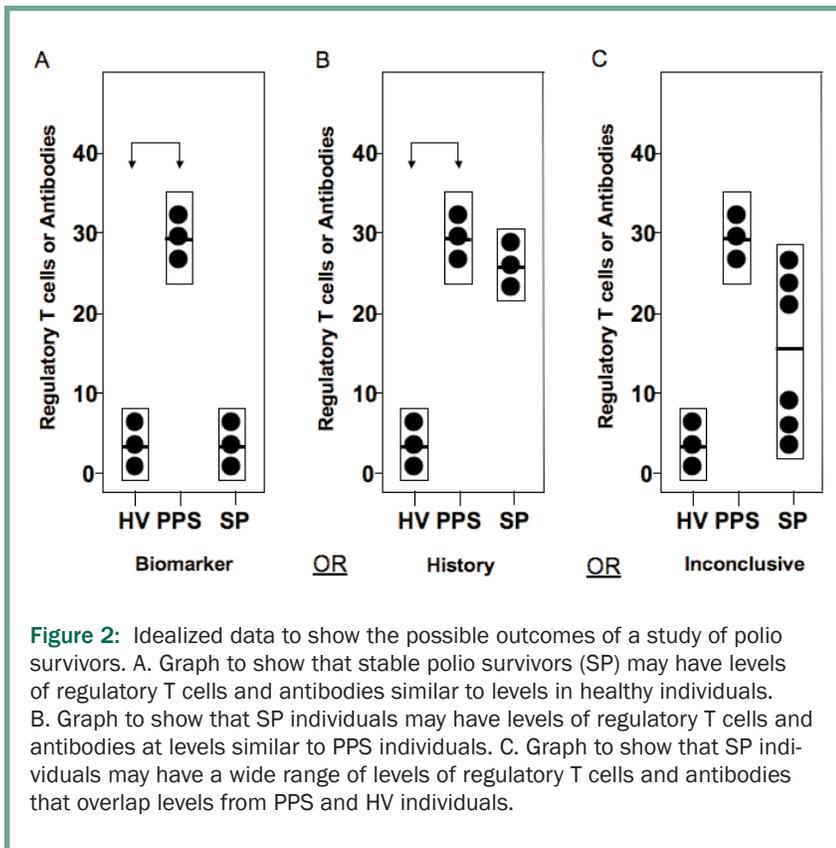
Figure 1: Idealized data to show the 2 possible outcomes of a study of post-polio syndrome individuals. A. Graph to show possible large difference in levels of regulatory T cells and antibodies between healthy (HV) and PPS individuals (PPS). B. Graph represents minimal difference between the HV and PPS individuals. Filled circle represents data from a single individual. Black line represents possible average of the data.

The healthy individuals would have similar but low levels of antibodies and regulatory T cells, while the PPS individuals would have higher levels of these markers. Ideally, the levels

have been tested, then it would be possible to say that high levels of antibodies and regulatory T cells are linked with PPS.

But, there is still a potential problem with using these immune properties to help diagnose PPS. This is because the blood from polio survivors who are stable needs to be studied as well (see Figure 2). If the stable polio survivors have levels of antibodies and regulatory T cells similar to that of healthy individuals (as drawn in Figure 2A), then we would be more certain that high levels of these biological markers are specifically associated with PPS and these markers could potentially be used as tests for PPS. But if the data from the stable polio survivors are similar to that of the PPS subjects (Figure 2B), then it would suggest that the levels of antibodies and regulatory T cells are indicators of a person who has a medical history of poliomyelitis, as both groups of polio survivors have high levels of antibodies and regulatory T cells. In this case, polio-specific antibodies and regulatory T cells cannot be used as biomarkers of PPS.

Figure 2C shows another possible outcome of the results. If a large number of stable polio survivors could be studied, it is entirely possible that some individuals would have high levels of antibodies and regulatory T cells in their blood, while some would have low levels. Such a range of results could mean that people with high levels of these biological components are at risk of developing PPS, while those who have low levels are not at risk of developing PPS.



of polio-recognizing antibodies or regulatory T cells could be potentially used to help diagnose PPS, if we could see a large difference in the antibody and regulatory T cell levels when groups of PPS individuals are compared with healthy individuals. That is, the differences between groups of PPS and healthy individuals were more like what is seen in A rather than B (Figure 1). If such differences were consistently seen after many healthy and PPS individuals

Figure 3 shows the range of results obtained from the study at UAMS of 12 healthy and PPS individuals and the 4 stable polio individuals. The top of the box indicated for each of the three groups shows the highest level of regulatory T cells obtained from the individuals and the bottom of the box indicates the lowest. The line in the center marks the average number for each group.

Our data suggests that PPS individuals have higher levels of antibodies and regulatory T cells circulating in their blood than healthy age-matched individuals, while SP individuals have variable levels of these immune components, which overlap with both the PPS and the healthy individuals.

The cause for the increased levels of these immune components is currently unknown. Importantly, however, the lack of sufficient data from SP donors makes it difficult to make definitive conclusions regarding the role of regulatory T cells (and/or antibodies) as an easily available diagnostic marker for PPS.

It is important to remember that this is a very small study. We do not have enough SP donors, to make a final conclusion about whether the regulatory T cells can be used to diagnose only PPS. However, in a larger study: if there are sufficient numbers of SP donors, and they do not have high levels of this cell type but PPS individuals do have high levels, then:

- The target may be used as biomarker of PPS for faster, definitive diagnosis.
- The target T cells may provide clues about what leads to the development of PPS.

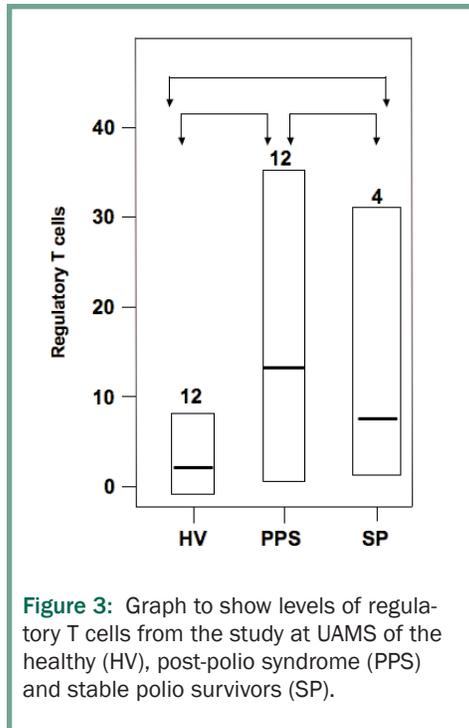


Figure 3: Graph to show levels of regulatory T cells from the study at UAMS of the healthy (HV), post-polio syndrome (PPS) and stable polio survivors (SP).

The observations from this study suggest that there is some kind of problem with the immune responses in polio survivors, which could contribute to PPS. Whether immune components have a direct role in the initiation and progression of PPS or merely indicate the presence of a problem with the immune system in these individuals is unclear at this time. ▲

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Update on the Fifth Award

PHI's Review Panel is currently evaluating eight proposals submitted to vie for \$25,000 to be given for Year 2009.

Contributions may be made specifically to The Research Fund. Individuals wanting to remember The Research Fund in their wills should contact PHI's Board of Director's representative Lawrence Becker, PhD (lbecker@bookwork.net) or Joan L. Headley at director@post-polio.org or 314-534-0475.