Role of protein TRIM21 in the antiviral response to influenza

CRC Proposal

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Study Purpose and Rationale

Influenza is a common acute respiratory illness that occurs in outbreaks and epidemics worldwide nearly every year. Yearly vaccination with a trivalent inactivated intramuscular vaccine has been recommended in older adults, those with chronic medical conditions, as well health care workers. With new concern about pandemic strains of influenza, universal vaccination for those ≥ 6months of age has now been recommended.[i] In healthy adults, the inactivated intramuscular vaccine has been shown to be 88-89% effective in reducing serologically confirmed influenza in healthy adults. Those people also had decreased influenza-like-illnesses, reduced physician visits and lost workdays.[ii]

90% of influenza-related deaths occur among people over 60 years of age and these same people also have increased morbidity from the disease. Yearly vaccination of this group is recommended with the hopes that it would reduce complications from the disease. However, the data from a few randomized controlled trials and multiple large observational studies of patients in long term care facilities and in the community have conflicting results. A 2005 Cochrane review of these prior studies showed that vaccination resulted in a significant reduction in pneumonia, hospital admission and death from influenza or pneumonia.[iii] However, a more recent case control study in 2008 of community dwellers showed that influenza vaccination did not reduce the risk of pneumonia, after adjustment for the presence and severity of comorbidities.[iv]

It has been hypothesized this lack of an effect is due to a weaker immune response in the elderly. Mechanisms cited include: generating insufficient antibody during vaccination, early waning of antibody titers, and inadequate cytokine generation. Some of these theories have been called into question with recent data that show elderly people do maintain their antibody titers just as well as younger people.[v]

These observations prompt us to better elucidate the factors that control the immune response to viruses in general. In the past few years, a protein called TRIM21 has been implicated in playing such a role. It is a protein predominantly expressed in immune cells including T-cells, B-cells, and dendritic cells but is also expressed at lower levels in other tissues.[vi] Originally identified as one of the antigens in autoimmune diseases such as Sjorgen's , this protein has now been shown be induced during RNA viral infection and to play a role in regulating IRF3, a protein important for cytokine regulation during the anti-viral response, though the exact molecular mechanism as to how this occurs is under debate.[vii] [viii]Mice deficient in TRIM21 demonstrate an autoimmune phenotype.[ix] Studies to evaluate whether these mice are at lower risk for viral infection still need to be done. In the past year, a separate laboratory has identified TRIM21 as an avid binder to the effector arm of the antibody molecule, with higher affinity than any other protein. It is intracellularly located and postulated to carry out trafficking of antibody-viral complexes to the proteasome for degradation.[x] Levels of TRIM21 have been measured in human peripheral mononuclear blood cells (PBMCs) in one study using commercially available reagents[xi] though levels pre and post-infection have not been established in young people or the elderly.

My hypothesis is that lower levels of TRIM21 are correlated with those older adult patients who have more severe viral disease and are in the hospital. The mechanism is that the lack of TRIM21 makes these people less able to clear their viral infection (even though they received vaccination) leading to hospitalization, as compared to those who are managed in the outpatient setting with presumably less severe disease. A better understanding of the determinants of the anti-viral response in older adult patients may help the medical community improve vaccine designs or provide therapeutic adjuncts to reduce complications from influenza, such as hospitalization and pneumonia.

Study Design and Statistical Procedures

This will be a single-center, cross-sectional, observational study of older adult patients vaccinated for influenza. It will involve collecting single venous blood samples from previously vaccinated but now influenza positive older adult patients (defined as age ≥ 60) (1) admitted to the Allen hospital for influenza or influenza-like illness, or (2) seen in the office setting by outpatient providers for influenza-like illness, and found to be influenza positive. The primary endpoint for this study is TRIM21 protein level, as analyzed by western blot analysis.

Demographic data will be collected on each patient (age, gender), as well as their medical co-morbidities, whether they come from the community or a long term care facility, and confirmation that they received the influenza vaccine for this particular season, as well as which type (intramuscular or intranasal), and whether they received the vaccine in the prior season.

Finally, the clinical outcome of each patient should also be collected via electronic chart review or through communication with the outpatient physician. For the inpatient group – we would focus on whether patients also developed bacterial pneumonia, their length of hospital stay, ICU necessity. For the outpatient group, we would also want to know if they later developed bacterial pneumonia, how many days of work they missed. We would also want to know if any ultimately were admitted to the hospital, and if so, those patients will have to be reassigned to the inpatient group.

Each blood sample will be given an identification code which will also be associated with the above collected information about the patient. However, the samples will only be known by this identification code during the laboratory processing and quantitative analysis. Once the data has been collected, they will be grouped according to inpatient vs. outpatient.

An unpaired student's t-test will be used to compare the protein levels of TRIM21 between the inpatient and outpatient group. There is no published data available regarding what are clinically adequate levels of TRIM21. The study will be powered for 80% with P=0.05. Based on an informal survey of my own and my colleagues' experiences at the Allen Hospital of patients admitted for influenza who were known to be vaccinated in the current season, I estimate that we could collect 50 patients over the course of the entire influenza season at the Allen Hospital for the inpatient group. In the outpatient group, we could collect a similar number of patients utilizing both community providers and AIM clinic providers. Assuming a sample size of 50 per group, we could detect with 80% power and p=0.05 an effect size that is 0.57 of the standard deviation of the TRIM21 levels.

Study Procedures

A single venous blood draw of approximately 6mL would be required from each patient for this study. In the inpatient setting, this could be performed in conjunction with other blood draws for clinical care, but would represent an extra vial of blood obtained from the patient. In the outpatient setting, this would also represent an extra vial of blood obtained from the patient, but not all of these patients would usually receive blood draws.

Peripheral blood mononuclear cells (PBMCs) will be isolated from whole blood using standard methods and cell lysates prepared and snap frozen in liquid nitrogen. Equivalent protein lysates from each patient will be loaded onto separate lanes of standard protein gels, electrophoresed and then immunoblotted according to standard procedure with anti-TRIM21 antibodies, to be obtained from (Yang et al. or Chemicon rabbit anti-Ro52 polyclonal ab) Known amounts of recombinant TRIM21 expressed in bacterial cells will be used to create a standard curve for calculating the level of TRIM21 in each sample. Western analysis with anti-Beta-actin or anti-GAPDH will be used to ensure equal loading between lanes.

In addition, in the outpatient setting, flu swabs are usually done at the discretion of the outpatient provider but in this study it will be done on each patient who enrolls.

Study Drugs or Devices

NA

Study Questionnaires

NA

Study Subjects

Older adult patients vaccinated against influenza during the study year season who later develop influenza and come to medical attention either through admission to the hospital or in the outpatient setting. Inclusion criteria are age ≥ 60, laboratory confirmation of positive influenza, and confirmation of influenza vaccination for the current influenza season.

Recruitment

The study will take place in the Allen Hospital, the community hospital of the Washington heights area and various outpatient clinics in the surrounding community, including AIM, with the presumption these reflect similar populations. NYC-CUMC medicine and family medicine residents and Allen hospitalists working at the Allen Hospital during the season will be asked to contact the study manager whenever a flu+ patient over the age of 60 is admitted to the hospital, so that those patients can be enrolled and appropriate blood samples be drawn. Outpatient providers in the community (ILI-Net[1] and AIM) will be

[1]. The ILI-Net is a network of outpatient providers that have agreed to provide surveillance statistics to the state department of health (DOH) regarding numbers of influenza-like illness on a weekly basis, categorized by age. They

asked to enroll patients whom they have high suspicion of influenza and have been previously vaccinated. Upon agreement, those patients will be flu swabbed and have their blood drawn. If their flu swab comes back negative for influenza, those patients will be removed from the study. Based on recent ILI-Net survey data, approximately half of flu swabs sent in the outpatient setting return positive for influenza.[xii]

Confidentiality of Study Data

Patients' medical records will be kept confidential as per hospital and HIPAA policy.

Potential Risks

There is minimal risk posed to the patients as the study only involves one extra blood draw.

Potential Benefits

There is no direct benefit to current patients in either inpatient or outpatient setting. However, the knowledge gleaned from this study may become useful in designing future vaccines to improve efficacy for older adult patients.

Alternati ves

There are no clinical interventions. Instead of TRIM21 protein levels, mRNA levels can be measured but usually RNA is less stable than protein. Instead of utilizing laboratory confirmed influenza as one of the criteria, one could theoretically use influenza-like symptoms. However, many other viruses cause these symptoms (which are often subjective) and thus having objective data in the form of a positive flu swab would be important.

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can also submit to DOH a set number of influenza swabs for analysis free of charge. In 2009, 9913 outpatient visits were for ILI in this network, of which 5% were for people with age >= 50.

References

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