Impaired CD4+ and T-helper 17 cell memory response to *Streptococcus pneumoniae* is associated with elevated glucose and percent glycated hemoglobin A1c in Mexican Americans with type 2 diabetes mellitus

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Individuals with type 2 diabetes are significantly more susceptible to pneumococcal infections than healthy individuals of the same age. Increased susceptibility is the result of impairments in both innate and adaptive immune systems. Given the central role of T-helper 17 (Th17) and T-regulatory (Treg) cells in pneumococcal infection and their altered phenotype in diabetes, this study was designed to analyze the Th17 and Treg cell responses to a whole heat-killed capsular type 2 strain of *Streptococcus pneumoniae*. Patients with diabetes demonstrated a lower frequency of total CD+T-cells, which showed a significant inverse association with elevated fasting blood glucose. Measurement of specific subsets indicated that those with diabetes had, low intracellular levels of interleukin (IL)-17, and lower pathogen-specific memory CD4+ and IL-17+ cell numbers. No significant difference was observed in the frequency of CD4+ and Th17 cells between those with and without diabetes. However, stratification of data by obesity indicated a significant increase in frequency of CD4+ and Th17 cells in obese individuals with diabetes compared with nonobese individual with diabetes. The memory CD+T-cell response was associated inversely with both fasting blood glucose and percent glycated hemoglobin A1c. This study demonstrated that those with type 2 diabetes have a diminished pathogen-specific memory CD4+ and Th17 response, and low percentages of CD+T-cells in response to *S. pneumoniae* stimulation. (Translational Research 2014;163:53–63)

Abbreviations: %A1c = percent glycated hemoglobin A1c; BMI = body mass index; CCHC = Cameron County Hispanic Cohort; CM = central memory; c-RPMI = fetal calf serum; FBG = fasting blood glucose; FBS = fetal bovine serum; IFN-γ = interferon γ; IL = interleukin; MFBG = mean

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fasting blood glucose; PBMC = peripheral blood mononuclear cell; RAGE = receptors for advanced glycation end products; RPMI = Roswell Park Memorial Institute medium; Th = T helper; Th17 = T-helper 17; Treg = T regulatory

AT A GLANCE COMMENTARY

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Background

Type 2-diabetes and its associated inflammation results in alteration of immune system and increase is susceptibility to infections by pathogens such as Streptococcus pneumoniae. The study therefore investigated the CD4+ and T-helper 17 response in those with and without diabetes.

Translational Significance

These studies were performed using specimen from human subjects and therefore the results can be directly correlated with the disease status in human. Results from these studies will be used to further explore these pathways in animals models of diabetes to determine specific defects which results in increase susceptibility to pneumococcal infections in those with diabetes.

Streptococcus pneumoniae is the most frequently identified pathogen in community-acquired pneumonia in the United States in the elderly. It is also an important cause of infection in individuals with underlying medical conditions such as type 2 diabetes and heart disease.[1] Epidemiologic studies show that pneumococcal infections are more severe and associated with more complications in individuals with diabetes than in healthy adults.[2,3] Diabetes increases the risk of pneumococcal bacteremia and mortality 1.5-fold and 3- to 4-fold respectively.[3,4] Hospitalization rates for individuals with diabetes and pneumococcal pneumonia are significantly higher than for healthy individuals of similar age (19% vs 15%).[5] The incidence of invasive pneumococcal disease is also higher in those with type 2 diabetes (25.2–39.39 cases/100,000/y) compared with individuals without diabetes (7.5–9.3cases/100,000/y).[3] These clinical observations suggest that individuals with diabetes have an immune dysfunction that limits control of *S. pneumoniae* infection.

Elimination of pneumococci requires effective innate and adaptive immune responses. Innate responses require an influx of neutrophils at the site of infection, deposition of complement factor C3 on pneumococci, and subsequent opsonophagocytic killing of complement-coated pneumococci by neutrophils and macrophages.[6-9] Recently, the importance of T-helper 17 cells (Th17) cells in prevention of carriage or early pneumonia has been reported.[10] Interleukin (IL)-17, the effector cytokine secreted by Th17 cells has pro-inflammatory functions that enhance pneumococcal clearance by recruiting and priming neutrophils for secretion of antibacterial proteins and peptides such as beta defensins, and by promoting interferon γ (IFN-γ) production to enhance macrophage function for enhanced phagocytosis and intracellular pneumococcal killing.[11,12]

Diabetes was recently shown to be associated with an imbalance in the ratio of T-regulatory (Treg)/Th1/Th17 cells, with the preferential differentiation of CD4+ and Th17 cells as opposed to Th1 or Treg cell populations.[13-15] It was further shown that an increase in the number of Th17 cells and its signature cytokine IL-17 exacerbated inflammation. Given the importance of CD4+ and Th17 cells in an effective immune response to pneumococcal infections, it is likely that alterations in CD4+Th-cell response may, in part, play a role in the observed increase in the susceptibility of patients with diabetes to pneumococcal infection and disease.

MATERIALS AND METHODS

Subjects. The study was conducted using participants from Cameron County Hispanic Cohort (CCHC). The CCHC is a community-based cohort with more than 2000 participants with high rates of obesity and diabetes.[16,17] The rates of diabetes among participants of the CCHC were found to be twice the national rates of diabetes among all Americans and nearly twice as high as previously established rates among Mexican Americans.[16,17] For this study, individuals with diabetes were defined based on American Diabetes Association 2006 criteria, which include a diagnosis of diabetes and on medication for diabetes, or those with a fasting blood glucose (FBG) level of more than 126 mg/dL or an hemoglobin A1c level of more than 6.5. Those with FBG values of less than or equal to 126 mg/dL and no history of diabetes or diabetes medication were classified as patients without diabetes. In the study samples, 20 individuals were identified with diabetes compared with 16 without diabetes. For analysis, the American Diabetes Association 2010 and 2006 criteria for defining diabetes were used. However, observations did not find any differences in analysis based on the two criteria; therefore, results presented are based on the 2003 diagnosis criteria for diabetes.