ORIGINAL RESEARCH

Innate Cellular Immunity in Newly Diagnosed Pulmonary Tuberculosis Patients and During Chemotherapy

Victory Fabian Edem, MSc, Ganiyu Olatunbosun Arinola, PhD

Ibadan, Nigeria

Abstract

BACKGROUND Leukocyte migration (LM) and intracellular killing aspects of the innate immune response play important roles in protection against and containment and cure of *Mycobacterium tuberculosis* infection, and thus may be exploited as immunotherapeutic targets to improve the management and treatment outcomes of patients with tuberculosis (TB).

OBJECTIVES The aim of this study was to assess LM and mediators of intracellular killing in patients with TB at the time of diagnosis and during anti-TB chemotherapy and compare them with apparently healthy controls.

METHODS We recruited 24 patients who were newly diagnosed with pulmonary TB and 20 apparently healthy individuals. Blood was drawn from patients with TB at the time of diagnosis, and after 2, 4, and 6 months of anti-TB chemotherapy and control. In vitro percentage LM (%LM) upon stimulation with Bacillus Calmette-Guérin vaccine, percentage nitroblue tetrazolium (%NBT) reduction, plasma concentrations of hydrogen peroxide (H_2O_2), and nitric oxide (NO) were assessed in both groups.

FINDINGS Percentage NBT was significantly reduced in patients with TB at 2 months of anti-TB chemotherapy compared with patients at diagnosis and in healthy controls, whereas %LM was significantly increased in patients at 4 months of anti-TB chemotherapy compared with patients at diagnosis and controls. Mean plasma H_2O_2 and NO were significantly reduced in patients at diagnosis and throughout the period of anti-TB chemotherapy compared with the control group. Significant decreases were demonstrated in mean plasma H_2O_2 and NO in patients at 2 and 4 months of anti-TB chemotherapy, respectively, compared with patients at diagnosis. There was significant positive correlation between %NBT with plasma H_2O_2 and NO, but %LM was negatively correlated with plasma H_2O_2 in this group.

CONCLUSION The intracellular killing aspect of innate cellular immunity is deficient in patients with TB, especially 2 to 4 months after commencement of treatment. Therefore, measures (eg, arginine supplementation) to improve intracellular killing in these patients is advocated. Moreover, %LM assay with Bacillus Calmette-Guérin vaccine as an antigen may be used to differentiate those newly diagnosed patients from those on anti-TB chemotherapy.

KEY WORDS anti-TB chemotherapy, intracellular killing, leukocyte migration, nitroblue tretrazolium, pulmonary tuberculosis

INTRODUCTION

Death from tuberculosis (TB) is increasing as a result of poverty, malnutrition, synergy with the human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) pandemic, and the emergence of multidrug- and extensively drug-resistant strains of *Mycobacterium tuberculosis*.[1] The host immune system is a critical factor for containment and cure of *M. tuberculosis* (Mtb) infection, whereas immunotherapy as an adjunct to drug treatment has the potential to improve treatment outcome.[2,3] Hence, there is a need for a clear understanding of immune response to Mtb infection to propose an efficient adjuvant immunotherapeutic agent or mechanism.

The innate immune response plays an important role in protection against Mtb. After MtB enters the alveolar space via inhalation, it is phagocytosed by alveolar resident macrophages and dendritic cells as an innate immune response to contain and eradicate the bacilli.[4] This leads to a rapid inflammatory response and recruitment of other immune cells to the lungs. However, the bacteria use different mechanisms to evade elimination, thereby exploiting the macrophage as a niche for growth and expansion.[4]

During infection, polymorphonuclear phagocytes use oxygen-dependent and oxygen-independent mechanisms in an attempt to eliminate invading pathogens.[5] The oxygen-dependent mechanism involves the production of reactive oxygen intermediates (ROI) such as superoxide radical, hydroxyl radical, hydrogen peroxide (H_2O_2), and hypochlorous acid in the respiratory burst pathway as well as production of reactive nitrogen intermediates such as nitric oxide (NO) and peroxynitrite via the inducible nitric oxide synthase. Although phagocytes have been implicated in the control of mycobacterial infections,[6,7] the mechanisms by which they are attracted to the site of infection as well as the mechanisms by which they exert protective function are not clearly resolved.

This study, therefore, assessed 2 aspects of phagocytosis (leukocyte migration [LM] and mechanisms of intracellular killing) in Nigerian patients with TB at the time of diagnosis and throughout anti-tuberculosis (anti-TB) chemotherapy. In vitro percent migration of leukocytes was determined after stimulation with Bacillus Calmette-Guérin (BCG) vaccine, whereas intracellular killing mechanism was determined by evaluating plasma levels of NO, H_2O_2, and leukocyte nitroblue tetrazolium (NBT) dye reduction tests. The aim of the present study was to identify which aspect of cellular innate immune response should be used as immunotherapeutic target.

PARTICIPANTS AND METHODS

We recruited 44 participants for the study, which comprised 24 patients with newly diagnosed pulmonary tuberculosis and 20 apparently healthy individuals without pulmonary tuberculosis after obtaining written informed consent. Participants with HIV and helminthes infection were excluded, as were patients on any medication or those who were pregnant. The study protocol was reviewed and approved by the University of Ibadan/University College Hospital Joint Institutional Research Ethics Committee. All patients were recruited from the Medicine Outpatient Clinics, University College Hospital, Ibadan, Nigeria, by a consultant chest physician after laboratory tests, chest x-rays, and clinical history.

At diagnosis of TB, 5 mL of blood was withdrawn from the antecubital fosa vein into lithium heparin tubes at diagnosis; blood was drawn again at 2, 4, and 6 months (completion of duration) of anti-TB therapy.

Percentage Leukocyte Migration. Percentage leukocyte migration (%LM) was determined as previously described.[8] Leukocytes were isolated from whole blood using 6% dextran. After separation of plasma by centrifugation, 6% dextran was mixed with packed cells (1:1) and incubated for 45 minutes at 37°C. Leukocyte-rich supernatant was obtained and washed 3 times in Kreb-Ringers solution, filled into capillary tubes, and anchored into a migration chamber filled with either Kreb-Ringers solution or antigen (BCG) and Kreb-Ringers solution (1:50). This was incubated for 18 hours at 37°C and the area of LM in the chamber containing antigen was compared with the area of migration in the chamber without antigen. The %LM was calculated as follows:

%LM = (area of migration in antigen solution/area of migration in medium alone) × 100.

Percentage LM value of 80% or less was considered positive.[8]

Percentage Nitroblue Tetrazolium Dye Reduction. Percentage nitroblue tetrazolium (%NBT) dye reduction was based on a previously described method.[8] For stimulated NBT procedure, 50 μL of NBT solution (0.2% NBT), 25 μL heparinized blood, and 25 μL of stimulant solution (nonviable bacterial extract) were mixed gently, incubated at 37°C for 10 minutes, and then incubated at 25°C