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Increased Severity of Tuberculosis in Guinea Pigs with Type 2 Diabetes

A Model of Diabetes-Tuberculosis Comorbidity

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Impaired glucose tolerance and type 2 diabetes were induced in guinea pigs to model the emerging comorbidity of *Mycobacterium tuberculosis* infection in diabetic patients. Type 2 diabetes mellitus was induced by low-dose streptozotocin in guinea pigs rendered glucose intolerant by first feeding a high-fat, high-carbohydrate diet before *M. tuberculosis* exposure. *M. tuberculosis* infection of diabetic guinea pigs resulted in severe and rapidly progressive tuberculosis (TB) with a shortened survival interval, more severe pulmonary and extrapulmonary pathology, and a higher bacterial burden compared with glucose-intolerant and nondiabetic controls. Compared with nondiabetics, diabetic guinea pigs with TB had an exacerbated proinflammatory response with more severe granulocytic inflammation and higher gene expression for the cytokines/chemokines interferon-γ, IL-17A, IL-8, and IL-10 in the lung and for interferon-γ, tumor necrosis factor-α, IL-8, and monocyte chemoattractant protein-1 in the spleen. TB disease progression in guinea pigs with impaired glucose tolerance was similar to that of nondiabetic controls in the early stages of infection but was more severe by day 90. The guinea pig model of type 2 diabetes—TB comorbidity mimics important features of the naturally occurring disease in humans. This model will be beneficial in understanding the complex pathogenesis of TB in diabetic patients and to test new strategies to improve TB and diabetes control when the two diseases occur together. *(Am J Pathol 2014, 184: 1104—1118; http://dx.doi.org/10.1016/j.ajpath.2013.12.015)*

Host susceptibility to *Mycobacterium tuberculosis* is influenced by a variety of chronic communicable and noncommunicable diseases that increase the risk of infection and the development of active tuberculosis (TB) disease. Moreover, *M. tuberculosis*—infected patients with concurrent diseases often have more severe TB, which further complicates treatment responses to conventional antimicrobial drugs. The risk factors most frequently linked to *M. tuberculosis* susceptibility are HIV infection, malnutrition, tobacco use, air pollution, alcoholism, extremes in age, chronic kidney disease, and diabetes. The highest relative risk for TB is associated with profound immunosuppression associated with HIV infection. However, recent epidemiologic evidence suggests that in the face of a growing diabetes epidemic, the population-attributable risk of diabetes may be equivalent to or exceed that of HIV/AIDS. In countries with the highest TB and diabetes incidences, type 2 diabetes mellitus in particular may account for up to 20% of active TB cases, whereas <5% may be attributable to HIV.[1,2]

Type 2 diabetes accounts for approximately 95% of diabetes cases and is associated with obesity, poor diet, and a sedentary lifestyle, all of which are often linked to urbanization. An estimated 371 million people were diagnosed as having type 2 diabetes in 2012, with most residing in low- and middle-income countries. Moreover, an additional 280 million people are prediabetic, many of whom have undiagnosed insulin resistance with nondiabetic hyperglycemia

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and impaired glucose tolerance (IGT).[3] Furthermore, the global incidence of type 2 diabetes is projected to rise to approximately 552 million by 2030.[4] Evidence in human studies suggests that glucose control, more than any other feature of altered metabolism in diabetic patients, influences the susceptibility to *M. tuberculosis* infection, highlighting the importance of uncontrolled hyperglycemia in TB risk.[5] The increased difficulty in controlling blood glucose levels in diabetic patients with TB, combined with poor responses to antimicrobial drug treatment, has the potential to further hamper current TB control efforts worldwide.[6] An animal model that more closely mimics the pathogenesis of this comorbidity in humans is essential for identifying more effective strategies for antimicrobial drug treatment and for control of blood glucose levels in diabetic patients with TB. In addition, the influence that IGT and insulin resistance have on TB susceptibility and pathogenesis has not been adequately investigated.

Currently, the animal models most often used to study the pathogenesis of TB concurrent with diabetes are inbred strains of mice treated with high doses of the cytotoxic drug streptozotocin (STZ).[7,8] These models mimic chronic hyperglycemia resulting from total insulin deficiency, as in human type 1 diabetes mellitus. Although this strategy has provided valuable information on how absolute insulin deficiency and persistent hyperglycemia affect active TB disease, it fails to take into account a variety of other metabolic defects associated with IGT and type 2 diabetes through dietary manipulation. Unlike murine models, lipid metabolism in the guinea pig more closely resembles that of humans, making it ideal for studying cardiovascular disease risks and other consequences of altered glucose and lipid metabolism associated with type 2 diabetes.[9] Moreover, most mouse strains used to model TB, including those previously used in diabetes-TB comorbidity studies, fail to respond to *M. tuberculosis* infection with the development of pulmonary and extrapulmonary granulomata with caseous necrosis, as is typical in humans, as well as guinea pigs, nonhuman primates, and rabbits.[10] The value of the guinea pig TB model has been further validated recently in studies of natural transmission of *M. tuberculosis* from human patients with TB to guinea pigs. A subpopulation of guinea pigs exposed to aerosols from patients with TB developed active TB disease and an array of clinical and pathologic responses, which more accurately reflects the clinical variation of naturally occurring *M. tuberculosis* infection in humans.[11]

Clinical studies have shown that the increased susceptibility of diabetic patients to *M. tuberculosis* is accompanied by an altered host response to infection. Diabetic patients with active TB have higher bacterial burdens based on sputum culture and are refractory to first-line antimicrobial combination therapy, with longer time to sputum conversion and higher mortality rates during therapy.[12–14] Diabetic patients often have atypical radiographic findings, with more frequent involvement of lower lung lobes, and some studies indicate a higher rate of cavitary disease, consistent with more severe pulmonary pathology.[15,16] However, the impact that diabetes has on the development of extrapulmonary TB in humans is conflicting with some studies, indicating a relative risk similar to or less than that of nondiabetic patients, whereas others show a predisposition for extrapulmonary and even miliary disease patterns in diabetic patients.[5,17,18] In patients with TB and poorly controlled type 2 diabetes, an exaggerated innate and type 1 cytokine response has been demonstrated clinically.[19] However, the impact that insulin resistance alone has on the response to *M. tuberculosis* infection is unknown. The goal of this study was to develop an animal model that more closely mimics the clinical and immunologic manifestations of diabetes-TB comorbidity in humans.

Materials and Methods

Induction and Confirmation of Glucose Intolerance and Type 2 Diabetes

Sixty outbred Dunkin-Hartley guinea pigs, weighing 300 to 400 g, were obtained from Charles River Laboratories (Wilmington, MA) and were maintained in individual housing. Guinea pigs were divided into three groups: nondiabetic, IGT, and type 2 diabetic ($n = 20$ per group). Eleven weeks before infection with *M. tuberculosis,* guinea pigs were fed a custom-formulated high-fat, high-carbohydrate (HFHC) diet (Dyets Inc., Bethlehem, PA) *ad libitum* to induce IGT. The diet consisted of 18% protein, 30% fat, and 52% carbohydrate, with the carbohydrate portion consisting of 45% sucrose and 55% fructose. The dietary fat composition consisted of an equivalent kilocalorie per kilogram of beef tallow and Primex vegetable shortening (Stratas Foods, Memphis, TN), creating a fatty acid composition of 42% saturated, 50% monounsaturated, and 8% polyunsaturated fatty acids. After 8 weeks of the HFHC diet, type 2 diabetes was induced in half of the guinea pigs with IGT using a single s.c. injection of STZ to induce subtotal β-cell cytotoxicity.[20] STZ treatment consisted of an optimized single dose of 200 mg/kg in citrate buffer (pH 4.5) after anomer equilibration at 4°C for 2 hours[21] and 20 minutes after pretreatment with an intramuscular injection of 0.5 mg/kg of the α2-adrenergic receptor antagonist yohimbine.[22] A standardized oral glucose challenge [oral glucose tolerance test (OGTT)] was used to assess the severity of glucose intolerance by administering a 2-g/kg dose of D-glucose (0.5 g/mL) after a 12-hour fasting period and measuring glucose levels 0, 60, 90, 120, and 150 minutes after administration using the FreeStyle Lite glucometer (Abbott Diabetes Care, Alameda, CA), which has been validated for accuracy in the guinea pig against the glucose oxidase method, as previously described.[23]

Infection with *M. tuberculosis* and Euthanasia

Low-dose aerosol exposure of guinea pigs to *M. tuberculosis* was performed using the Madison chamber aerosol