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Mycobacterium Oral Immunogenicity Tuberculosis Infection

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Abstract

Orientation and considerations, the long-term success of kidney transplant recipients is hampered by post-transplant tuberculosis (TB). In order to demonstrate the pattern, risk factors, and prognosis of TB infection in our transplant patients, it was important for us to provide specific examples. Patients and techniques this study involved a retrospective analysis of the medical records of 491 people who received kidney transplants at our hospital between January 1986 and December 2009. The demographic information, transplant characteristics, clinical symptoms, diagnostic standards, treatment approach, and long-term prognosis of this patient cohort were examined. 16 patients (32%), with a mean age of 32,5 12,7 (range: 13-60) years and a mean post-transplant time of 36,6 months, acquired post-transplant TB (range: 12,3 months–15,9 years). 10/16 (62,6%) of the illnesses had pulmonary manifestations. The ESAT6 and CFP10 lines of transgenic carrot plants have been developed to produce Mycobacterium tuberculosis proteins. For ESAT6 and CFP10, the target proteins are present in carrot storage roots at concentrations that are not less than 0.056% and 0.002% of the total storage protein (TSP), respectively. Oral vaccination of mice outcome in the induction of both humoral and cell-mediated immunity, as has been demonstrated. These outcomes indicate that the proteins under consideration are suitable as a potential edible tuberculosis vaccine.

Keywords: Tuberculosis; Vaccine; Mycobacterium

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Introduction

Robert Koch, a German, discovered Mycobacterium tuberculosis in 1882, which is the source of the moniker "bacillus of Koch" (abbreviated BK). Tuberculosis (TB) is an infectious illness that strikes at random and requires mandatory declaration. In many impoverished nations, including Tunisia, where the prevalence was reported to be 27, 07/100 000 people in 1995, TB is the most significant infectious illness affecting humans [1]. Rapid identification is the key to treating TB, which is a big issue in conditions when the immune system becomes compromised, like acquired human deficiency syndrome (AIDS), chronic renal failure, or organ transplant recipients receiving immunosuppressive therapy. Compared to the general population, kidney transplant patients had a 20–74 times higher risk of tuberculosis (TB). The development of BK, an intracellular germ, was made possible by iatrogenic immunosuppression in transplant recipients, which accounts for a progressive reduction in cellular immunological function [2]. Posttransplant TB, a potentially fatal infection, affects the long-term success of kidney transplant recipients. Its diagnosis is frequently postponed, though.

One should expect TB among kidney transplant recipients with the introduction of more strong immunosuppressive regimens and a rise in TB incidence in the general population [3]. Because of the microorganism, TB In many nations around the world, Mycobacterium tuberculosis is currently a pressing issue. The incidence of tuberculosis cases, including fatal outcome, has been rising steadily in recent years. HIV and hepatitis transmission, which the WHO considers to be socially relevant diseases, alter the observed dynamics of tuberculosis morbidity [4]. The Bacillus Calmette-Guerin (BCG) vaccine, a live attenuated M. bovis strain, was developed in the 1920s to combat tuberculosis; by the turn of the 20th century, about 3 billion people had received the shot globally. The RD1 region, which is distinctive to M. tuberculosis and contains vital virulence components including ESAT6 and

CFP10, is absent from the genome of all M. bovis BCG strains. Despite the fact that BCG is ineffective against adults who have pulmonary tuberculosis, the most common form of tuberculosis, and that BCG is primarily used as a preventative vaccination for the protection of uninfected children, BCG is still the only vaccine against tuberculosis that is currently available [5].

Numerous research teams are now working to discover and develop the next generation of antituberculosis vaccines in an effort to avoid the disease. At various phases of clinical trials, there are now more than ten candidate vaccines and one therapeutic vaccine against tuberculosis [6]. In general, they can be split into two groups: primary prevention vaccines (designed to replace BCG) and booster vaccines. The majority of them are subunit vaccines (used for revaccination). To stop the pathogen from reactivating during latent tuberculosis, booster vaccinations are required. The proteins that interfere with the essential elements in the protection against tuberculosis, i.e., those able to induce a potent T-cell response and the secretion of -interferon, are among the secreted M. tuberculosis proteins that are used in the design of booster vaccines and are among the most significant [7]. Among M. tuberculosis's most important cell virulence factors are the ESAT6 and CFP10 proteins. The host cell membrane or the entire host cell is probably lysed as a outcome of their ability to elicit a potent T-cell response. The ESX-1 system, which is made up of at least ten genes, produces these proteins in secretion. Purified recombinant ESAT6 and CFP10 assemble into heterodimeric complexes in an in vitro setting. The formation of a homodimeric complex between recombinant ESAT6 and CFP10 has been demonstrated in yeast [8].

Due to the fact that the tuberculosis pathogen is an airborne bacterium, the warm-blooded hosts' mucous membranes can operate as a platform for inducing the organism's immune response, both mucosal and systemic. Determining how to construct a vaccine so that it triggers an immune response at the mucosal level is thus undoubtedly a challenging but exciting task for potential tuberculosis vaccines. The transgenic plants that produce protective antigens, or so-called edible vaccines, are one of the promising strategies used in this case. There aren't many studies on plant expression systems and transferring the M. TB gene esat6 sequence to plants as putative antituberculosis vaccines [9]. It has been tried to combine the ESAT6 antigen with other tuberculosis antigens like Ag85B or Mtb72F and to employ other adjuvants like CTB, LTB, LipY, or ELP with additional expression in different plant species including Arabidopsis thaliana, tobacco, and lettuce. Agroinfiltration, nuclear, and chloroplast transformations, as well as other plant expression systems, have all been employed for this aim [10].

Materials and Method

Patients and Methods

Patients

In this retrospective investigation, we examined the medical records of 491 kidney transplant recipients who received their care at our institution between June 1986—the first kidney transplant—and December 2009. The exclusion criteria were the beginning of tuberculosis 3 months or earlier after the return to dialysis or prior to kidney transplantation. For TB, sixteen patients underwent therapy. The presence of clinical, biological, and/or radiological indicators of suspicion was combined with bacteriological, histological, and/or therapeutic evidence to make the diagnosis of tuberculosis (TB).

Methods

The microbial contamination examination included the use of direct light microscopy to identify acid-fast bacteria (AFB) in at least one Ziehl-Neelsen-stained sample of urine, respiratory tract secretions, or other biological liquids, or positive cultures for the pathogen responsible for the cause on a particular Lowenstein medium or one of its many substitutes (Jensen, Coletsos, etc.). On the liquid from the puncture or a piece from an organ biopsy, the histological investigation revealed the presence of a massive-cellular granuloma with necrosis caseous.

There were five categories of radiographic patterns: normal findings, miliary pattern, pleural effusion, parenchymal cavitation, nodules, pulmonary infiltration, and hilar or mediastinal lymphadenopathy. This makes the total frequency of radiographic patterns more than 100% because it is possible for radiographic patterns to be associated. The following microorganisms were tested for in sweets: Candida albicans, Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter haemolyticus, Cytomegalovirus, and aspergillus.

The time between each patient's TB diagnosis and their kidney transplantation date as well as the circumstances surrounding their TB discovery was noted. Mendel-Mantoux skin testing was done by injecting tuberculin, a pure protein derived from the BCG vaccine, into the volar surface of the forearm. If the induration is less than 10 millimetres, the test is positive and read after 72 hours. Using Statview 5.0 software, a disseminated TB was identified when two organs were affected. Standard deviation was used to express values as mean. The 29 controls, which were transplanted at the same time as our 16 patients and were matched for age, sex, and type of dialysis, were compared to our 16 patients. Allograft malfunction, time on dialysis, and the number of patients were compared between the groups.

Isolation of M. tuberculosis Genomic DNA

Using a DNeasy Blood and Tissue Kit in accordance with the manufacturer's instructions, the genomic DNA of an M. tuberculosis clinical isolate collected from a patient was separated from the biomass of the organism.

Construction of Transgenic Carrot Plants

Agrobacterial transfer was used to change the carrot callus tissues that were produced from carrot zygotic embryos. Utilizing selective media enhanced with the antibiotic kanamycin, the transformants were chosen. The kanamycin-resistant plants were raised hydroponically in a greenhouse so that their storage roots could develop.

Plant DNA Isolation

Using an AxyGen (United States) DNA isolation kit and following

the manufacturer's instructions, the genomic DNA of carrot plants was extracted. By employing the relevant primers listed above and DNA extracted from the transformed carrot plants' leaves (1 g) as a template for PCR, the presence of target genes (esat6 or cfp10) in the genomes of the created transgenic carrot plants was confirmed. The following ingredients were used in the PCR: 20 L of the buffer (67 mM Tris-HCl, pH 8.9, 16 mM (NH4)2SO4, 1.5 mM MgCl2, 0.01% Tween 20, 200 M of each dNTP, 1 AU of Taq polymerase (SibEnzim, Novosibirsk, Russia), and 100 M of each primer. 34 cycles of PCR were carried out after a preliminary 94°C for 2 minutes heating step.

Producing Carrot Storage Root Extracts

Carrot storage root samples (6 g) from at least six different plants were minced, pulverised in a mortar in liquid nitrogen, and then homogenised in 20 mL of a buffer containing 0.1 M Tris-HCl (pH 7.5), 12 mM 2-mercaptoethanol, 1% SDS, 10 mM EDTA, and 3 mM PMSF in the presence of insoluble PVP. The homogenate was centrifuged at 10,000 rpm for 20 minutes. PVP was added to the supernatant in the range of 2.5–10.0% of the volume of the extraction buffer and centrifuged for 20 min. at 10,000 rpm. The proteins were precipitated using a 10% solution of trichloroacetic acid. The TSP and pure rESAT6 and rCFP10 recombinant protein concentrations in carrot storage roots were calculated based on Bradford assay.

Results

There were two women and 14 guys. The average age was 32,5, 12,7 years (13 to 60). 62% of patients were older than 30 years old, and the median age was 34. One instance had a history of urogenital TB, while the other two had had direct contact with TB carriers. In two cases, blood type was A, in one, it was B, in three, it was AB, and in ten, it was O. End-stage renal disease was brought on by glomerulonephritis in 5 instances, diabetic nephropathy in 1, lupus nephritis in 1, interstitial nephritis in 4, hypertension in 1, and undetermined causes in 4 cases. 38,6 months were spent on dialysis (10,3 months–21,1 years). It exceeds controls by a wide margin. In the department of biology, sterile leukocyturia was observed in 2 cases, graft malfunction in 5, biological inflammatory syndrome in 12, and pancytopenia in 1. In nine cases, bacterial investigation provided conclusive evidence of tuberculosis (AFB at direct light microscopy in 7 cases, positive culture in 9 cases). A confection containing Aspergillus, Cytomegalovirus, and Candida albicans was discovered in three different instances. The outcomes of two of the five cases' tuberculin skin tests were positive. Radiographic patterns revealed abnormalities in every instance, including mediastinal lymphadenopathy in two cases, spondylodiscitis L5, cavitation, pleural effusion in five cases, nodules in two cases, and pulmonary infiltration in six cases.

Due to drug interactions, the dose of the calcineurin inhibitor and the steroid was increased in two cases and the dose of the steroids alone in one. All patients were monitored. Recovery from TB was achieved in 8 cases, graft malfunction in 7 cases (43,7%), tissue-proof acute rejection in 3 cases, and graft loss in 4 cases after a mean follow-up of 291,3 months (88-755 months). After stopping the medication, the hepatotoxicity shown in 3 cases and the hyperuricemia seen in 4 cases may be reversed. Two patients (12.5%) passed away, and in one case it was due to TB meningitis, while in the other it was due to severe sepsis. Regarding transplant and patient survival, B patients did not differ significantly from controls.

Each storage root was tested by PCR for the presence of DNA fragments with the expected sizes of the target esat6 and cfp10 pieces in the carrot genomic DNA in order to confirm the transgenic nature of the developed carrot transformants. Given that the length of the amplified fragments in lanes 2–6 matches the expected PCR fragment of the plasmid (288 bp), it is likely that the target esat6 gene has incorporated into the nuclear genome of the investigated plants. Because the amplified fragments in lanes 1–5 match the expected PCR fragment of the plasmid (303 bp), it is likely that the target cfp10 gene has been incorporated into the nuclear genome of the investigated plants.

Discussion

The patients in our department who had kidney transplants who had TB showed the following signs. With 50% of patients being identified within the first 2 years following transplantation and a high coinfection rate of 18.7%, the incidence is significant within a short period of time. The clinical symptom that was most frequently present (93, 7%) was fever. The primary negative outcomes of anti-TB treatment were graft dysfunction (43, 7%), liver function damage (18, 7%), and hyperuricemia (25%). Patients could have a 12, 5% mortality rate. In contrast to the prevalence seen in developing nations (11, 8 to 13, 3%), which was lower than what we discovered, the prevalence of TB was 3, 2%. Even more people are at risk of developing latent TB.

The peculiar clinical characteristics of TB may go unnoticed due to the infection's muted response. The following clinical anomalies are frequently seen: pyrexia, pulmonary infiltrates, exudative pleural effusion, and exudative ascites. 93, 7% of cases in our analysis had a moderate, persistent fever of unknown aetiology, compared to 71% to 82, 9% in the literature. In contrast to the 40% reported in the literature, 31, 2% of patients in our study had general state impairment. In 62, 6% of our patients, pulmonary TB were found. The majority of kidney transplant recipients still have this condition. Compared to 56.1% in the literature, pulmonary symptoms were seen in 37.5% of the cases, with coughing being the most common symptom (12.5% of patients), and spittle being present in 39% of the cases. Alternative delivery methods for vaccines that target the mucosa, which is where Mycobacterium tuberculosis invades, are required. Subunit vaccinations containing recombinant TB antigens are being researched as a safer method of treating the TB disease. The cost of purification, cold storage, transportation, and sterile injections is greatly reduced when antigens are delivered orally in plant cells that have been bio encapsulated. The ability of transgenic plants to elicit a particular immunological response even when the target antigen is produced at low levels is one of its appealing qualities. The synthesis of M. tuberculosis antigens in plant expression systems has been shown to be feasible by a number of research teams. There has been evidence that the ESAT6 antigen accumulates in the heat-labile toxin B component of E. coli. The ability of the hybrid protein to form pen tamers and the preservation of

both components' antigenic structures has both been proven. It has been demonstrated through analysis of the immunological reactions to oral administration of these antigens in mice that the delivered antigens can elicit Th1 and Th2 immune responses. Lactuca sativa L. plants and Cichorium intybus L. var. foliosum Hegi's hairy root culture have received the genes encoding the M. tuberculosis ESAT6 and Ag85B antigens, both individually and in a fused form (ESAT6-Ag85B). The accumulation level and immunogenicity of the target proteins in plant tissues, however, have not been examined by the scientists.

Transgenic tobacco plant tissues have produced the fusion protein, which consists of the M. tuberculosis antigens Ag85B and ESAT6 linked with elastin-like peptide. The authors' studies on mice and pigs have proven the recombinant fusion protein's immunogenicity (T cell-mediated immune response). It has been demonstrated that the Ag85B and ESAT6 antigens' B- and T-cell epitopes found in complex fusion proteins made in plant expression systems are not vulnerable to changes and maintain their capacity to bind the relevant antigens. These studies have focused largely on model plant species that are not fit for human eating and are hence unpromising candidates for future use as edible vaccines. These outcome show that M. tuberculosis antigens can be successfully expressed in plant cells while retaining their antigenic structure and can accumulate in plant tissues at the level necessary for the establishment of the immune response, even though the immunogenicity of produced recombinant proteins has only been tested in experiments with laboratory animals in two works.

Proteins can be modified post-translationally in a plant expression system through glycosylation, hydrolysis, cleavage, or polymerization. In the lane containing the entire protein extract of the transgenic carrot storage root, several bands were seen when the ESAT6 protein was visualised by Western blot hybridization. The configurations of the relevant lanes indicate that the ESAT6 protein produced in the storage roots of carrots is likely to form homodimeric complexes and is present in various forms. Given that rESAT6, a recombinant protein, was used to produce the polyclonal serum needed to bind the target protein in the Western blot assay, it stands to reason that when the total protein extract is examined, all of the molecules containing the ESAT6 polypeptide will be visible. According to research on the immunogenic qualities of transgenic carrot storage roots, recombinant ESAT6 or CFP10 antigens generated in plants have the ability to elicit both cell-mediated and humoral immune responses when administered orally to a warm-blooded organism. ESAT6's toxicity for the test animals has been established, and we've also discovered that this protein might be harmful to transformed plants' in vitro regeneration processes. Since CFP10 is a chaperone for ESAT6, these two proteins work together. The creation of a recombinant fusion protein, which can, in our judgement, lessen the harmful effects of the ESAT6-antigen, is another potential path in the research involving these antigens.

Conclusion

Due to their immunosuppressed condition and the epidemiological prevalence of the disease, Tunisian kidney transplant recipients have a high risk of contracting tuberculosis (TB). A high prevalence of extra pulmonary and disseminated localizations, which are seen in a third of cases in our patients, characterise its distinctive clinical presentation. Therefore, in practical practise, this differential diagnosis needs to be taken into consideration. Long-term anti-TB treatment for at least 9 months is advised to prevent TB recurrence, which was common (18,7% of cases). The data presented in this study for the first time show how transgenic carrots, whose storage roots may be ingested without any heat processing, may serve as a platform for generating the immunogenic mycobacterial proteins ESAT6 and CFP10 and developing an edible antituberculosis vaccine.

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Conflict of Interest

The author has no known conflict of interest associated with this paper.

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