Use of an Immunotherapeutic Vaccine to Treat a Life-Threatening Human Arteritic Infection Caused by *Pythium insidiosum*

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A 14-year-old Thai boy presented because of a history of headache, mandibular swelling, and facial nerve palsy. A microorganism identified as *Pythium insidiosum* was cultured from the mandibular abscesses. Despite treatment with amphotericin B, iodides, ketoconazole, and surgery, the infection progressed. Magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) of the neck revealed an aneurysm in the external carotid artery. The aneurysm was removed. MRA performed later showed stenosis of the internal carotid artery. Immunotherapy was recommended as a last resort. One hundred microliters of the *P. insidiosum* vaccine was subcutaneously injected into the patient’s left shoulder, and 14 days later a similar dose was administered. Four weeks following the first vaccination, the patient’s headache had disappeared, the facial swellings had dramatically diminished, the cervical lymph node had shrunk, and the proximal left internal carotid artery stenosis had significantly improved. One year after the vaccinations, the boy was considered clinically cured.

Infections caused by fungal and parafungal organisms are occurring with increasing frequency in patients with debilitating illnesses such as leukemia and AIDS, as well as those undergoing immunosuppressive therapy [1–4]. Within this group of organisms are the traditional pathogenic fungi and a long list of newly recognized emerging opportunistic fungal and parafungal organisms. Among the emerging pathogens is the pathogenic oomycete *Pythium insidiosum*, a fungus-like organism classified in the kingdom Stramenopila, phylum Oomycota [5] (organisms in the kingdom Stramenopila were formerly classified in the kingdoms Prototista and Chromista [6]). Pythiosis insidiosi occurs in humans and lower animals in the tropical, subtropical, and temperate areas of the world [6].

The finding that some of the immunogens of *P. insidiosum* possessed curative properties when injected into equines with pythiosis insidiosi was unexpected [19, 20]. Since then, the vaccine has been used as a therapeutic weapon against infections caused by *P. insidiosum* in equines [21]; at least two different immunogens were reported to have cured equines in Australia [20], Costa Rica [19], and the United States [21]. These two vaccines used hyphal elements and cultured filtrate antigens (CFAs) of *P. insidiosum*, respectively [22]. Recently, a modified formulation of the CFA vaccine, with a property absent in the original vaccine, was found to cure some cases of chronic equine pythiosis insidiosi [23]. We now report that this modified therapeutic vaccine saved the life of a Thai boy with a life-threatening arteritic infection due to *P. insidiosum*.

**Case Report**

A 14-year-old boy was admitted to the Ramathibodi Hospital (Bangkok, Thailand) because of 10 days of progressive head-
ache. The illness had begun 16 days before admission, in November 1995. Prior to the onset of symptoms, he had sustained a small skin injury on the posterior portion of his neck while swimming in a flooded area near a rice field. Four days following the skin injury, he developed three acne-like nodules at the injured site. He then was admitted to a local hospital because of a severe headache and soft-tissue swelling at the occiput. The swollen mass returned to normal after 2 days of dexamethasone treatment. However, the patient continued to have severe headaches and developed a left-facial-nerve palsy before admission to the Ramathibodi Hospital.

The boy had a history of postsplenectomy β-thalassemia hemoglobin E disease, of 4 years’ duration. He had received at least three blood transfusions per year after his operation. Headache, bilateral facial-nerve palsy, and progressively extensive facial cellulitis were recorded on admission. Empirical antibiotic treatment with cefotaxime (100 mg/[kg·d]) and chloramphenicol (75 mg/[kg·d]) were prescribed without success. A CT scan of the head and neck showed diffuse cellulitis. Abscesses in the bilateral retromolar fossa and in both ears were also observed.

Pain and headache were relieved and the soft-tissue swelling subsided after surgical drainage of the abscesses. A nonsporulating fungus-like organism was isolated in pure culture of tissue taken from the left and right pinna. Because of the possibility of a fungal infection, amphotericin B (0.5 mg/[kg·d]), increasing to 1 mg/[kg·d]) was administered. The isolate was later identified as Pythium insidiosum.

Although the abscess and cellulitis subsided, 1 week later the pain and headache reappeared. Swelling of the left side of the tongue was also noticed. Saturated potassium iodide (1 g/mL) was prescribed at a dosage of 3 mL/d, gradually increasing to 9 mL/d. Despite this treatment, no clinical improvement was observed. MRI of the head and neck demonstrated soft-tissue involvement and regional lymph node enlargement (figure 1). Surgical exploration of the left parapharynx and masticator space was performed. During this procedure, the left abnormal cervical lymph nodes and the abnormal left great auricular nerve were removed.

Histopathologic examination of the material showed follicular hyperplasia with sinus histiocytosis and granulomatous inflammation. Aseptate hyphal elements of Pythium insidiosum were observed on silver-stained affected tissue. After failure of treatment with amphotericin B and iodides, chemotherapy with ketoconazole (300 mg/d) was initiated. In addition, granulocyte-macrophage colony stimulating factor (GM-CSF) was given 5 days immediately after surgical exploration.

The headache and swelling of the tongue diminished after surgical intervention. Although treatment with ketoconazole and iodides continued, pain and headache reappeared 3 weeks later. A CT angiogram revealed an aneurysm in the left external carotid artery, 1.0 cm above the bifurcation, and stenosis (with irregular walls) of the internal carotid artery (figure 2). A third surgical intervention was performed on 1 February 1996 to remove the aneurysm. The excised tissue was oval (2.5–4 cm in diameter), with necrosis-like material within its lumen. Histopathologically, eosinophils, macrophages, CD3+ T-cells, and plasma cells were observed in the hematoxylin and eosin-stained sections. Hyphal elements of Pythium insidiosum were detected within the lumen and the vessel’s wall in silver-stained tissue sections (figure 3).

Pain and headache disappeared immediately after the surgical intervention. Five weeks after surgery, the headache and swelling of tissue recurred. MRI and magnetic resonance angiography (MRA) of the neck revealed the persistence of cervical and paracervical lymph node enlargement and persistent stenosis of the left internal carotid artery (figure 4). These findings suggested that Pythium insidiosum had invaded that artery as well. Surgical removal of the left internal carotid artery was not recommended. Since amphotericin B, ketoconazole, and iodides had not proved effective over a 3-month period, chemotherapy was discontinued 1 week before vaccination. Surgery and two courses of GM-CSF alone had also been ineffective in controlling the infection; thus, the Pythium insidiosum vaccine (PIV) was suggested as a last-resort treatment.

Methods

Vaccine Preparation

The PIV had been used only for experimental purposes in equines and dogs with the disease; thus, consents from the
horses, an inflammatory reaction always developed at the site of vaccination. This inflammatory response not only indicated that the host’s immune system was functioning but also predicted that the equine probably would be cured by the vaccine. Anergic horses with proven pythiosis insidiosi never developed such a reaction to the vaccine and did not respond to immunotherapy [19–22].

To avoid an excessive immunoresponse in the young boy with P. insidiosum arteritis, several dilutions of the original PIV were tested before the trial started. One hundred microliters of each PIV dilution (1:100 to 1:100,000) was injected as a skin test on his right forearm. A mild inflammatory reaction was observed only with the 1:100 dilution of the PIV. Thus, the undiluted batch of PIV was selected. One hundred microliters of the PIV was subcutaneously injected in the patient’s left shoulder.

Results

Clinical Course

Twenty hours after vaccination, a wheal and flare reaction had developed at the injection site. Forty-eight hours post-vaccination, the wheal attained its maximum size of 11 cm in diameter. No other side effects occurred except for itching at the vaccination site. The skin reaction disappeared 10 days post-vaccination. Fourteen days after the first dose, the facial and tongue swellings had diminished. The same day, a second dose of vaccine was administered in the patient’s right shoulder. Forty-eight hours later, the wheal at the vaccination site had attained a diameter of 8 cm.

Two weeks after the second vaccination, the patient’s headache had disappeared, his facial and left tongue swellings had

Figure 2. CT angiogram of the young boy whose MRI is shown in figure 1. This image of the left common carotid artery, including its bifurcation (Electron Beam Computed Tomography; Imatron, San Francisco), demonstrates an aneurysm of the left external carotid artery (arrow) and focal narrowing of the adjacent proximal internal carotid artery caused by Pythium insidiosum (arrowhead).

Ramathibodi Hospital Committee, the patient, and his parents were obtained before the vaccination trial was begun. The vaccine used in this case was a modification of the original Men-doa vaccine (patent pending) [23]. In brief, inocula of P. insidiosum strain American Type Culture Collection (ATCC) 558643 were grown in Sabouraud dextrose broth (Difco, Detroit). The hyphal mass was washed twice with sterile distilled water and disrupted by sonication. The resulting supernatant was separated from the pellet and then mixed with the liquid phase containing the exoantigens of P. insidiosum. The mixture was then precipitated. The vaccine was stored at 4°C until used.

Vaccine Administration

A dose of 100 μL of the 2-mg/mL PIV had been utilized to vaccinate horses with the disease. In successfully treated

Figure 3. Aseptate longitudinal sections and cross-sections of the hyphal elements of Pythium insidiosum from the wall of the external carotid aneurysm (Gomori’s methenamine silver stain; original magnification, ×400).
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This was not surprising since a similar phenomenon had been recently observed in other pythiosis insidiosi cases [9]. The immunodiffusion test is reliable in equine pythiosis insidiosi, but some negative results have been reported for humans and dogs with the disease [7, 9]. When a serum specimen taken before vaccination was tested in a new ELISA for *P. insidiosum* [24], positive titers of 1:6,400 were recorded. To monitor the vaccination’s progress, sera collected 1, 2, 6, and 12 months post-vaccination were also evaluated with the ELISA. No changes in the ELISA titers were recorded after 1 and 2 months post-vaccination. A decrease in titers from 1:6,400 to 1:800 6 months after vaccination, however, indicated that *P. insidiosum* may have been eliminated from the infected tissues, a finding that substantiated the clinical data. The titer of antibody to *P. insidiosum* continued to decrease. Low titers, however, may persist for years, as has been previously reported with regard to equines cured by immunotherapy [25].

**Discussion**

The response of our patient to the PIV was remarkable. Aside from the wheal and flare reactions at the site of vaccination, also observed after successful vaccination of equines with the disease, no deleterious side effects developed. Within 4 weeks after immunotherapy, the patient’s headaches had disappeared, tissue swelling decreased, and he had gained 4.0 kg. Although we used the full-strength vaccine (2 mg/mL), the patient tolerated the PIV very well. The spectacular response to the PIV in this case is also supported by the hundreds of equine pythiosis insidiosi cases cured by this remarkable therapeutic vaccine. Thus, the PIV may be used as an alternative therapy for humans with the disease. More cases, however, need to be evaluated to validate the PIV’s curative properties in humans with the disease. In spite of this, the report presented herein is of importance because the available antifungal drugs have had little or no effect on this emerging disease. Thus, the PIV appears to be an attractive therapeutic choice. To our knowledge, this is the first case of pythiosis insidiosi arteritis in a human treated and cured by an immunotherapeutic PIV. It is also the first case cured by a vaccine prepared with cellular components derived from a eukaryotic pathogen.

Traditionally, vaccines have been used only for prophylactic purposes. The use of vaccines for the treatment of diseases, even though an old idea, has only recently received attention [26, 27]. The long-held medical dogma that vaccines are only for prevention has been challenged by scientists working toward the development of immunotherapeutic vaccines against viruses [28], parasites [29], bacteria [30], and fungal [31] and parafungal pathogens [32]. Despite the impressive data regarding the PIV and other curative vaccines, however, strong skepticism exists about the use of therapeutic vaccines as weapons for the treatment of infectious diseases.

The skeptics have argued that when a host is invaded by an organism its immune system will mount an immune response...
that eventually will eliminate the invader. If the immune system fails, the use of drugs is the only avenue to pursue in efforts to save a patient’s life. However, the findings generated in studies of therapeutic vaccines have indicated that a new line of research is necessary to investigate the mechanism by which these therapeutic antigens elicit an immunologic reaction that kills the pathogens in infected tissues.

The specificity of the PIV to cure cases of equine pythiosis insidiosi has been well established. For instance, when Miller [20] and later Mendoza et al. [32] vaccinated equines that had pythiosis insidiosi with Conidiobolus coronatus protein extracts, the horses neither responded to the vaccination nor developed inflammatory reactions at the injection sites. Moreover, when horses infected with C. coronatus were vaccinated with the PIV, they did not respond at all to the vaccine. By contrast, when some of the above horses infected with P. insidiosum were injected with the PIV, 57% were cured. A control group of 24 horses with proven equine pythiosis insidiosi that had been injected only with a placebo (extract of culture medium used to prepare the PIV) later all died of the disease. These findings suggested that the immunogens of the PIV are specific against P. insidiosum and that upon challenge an immunoresponse is mounted against the hyphal elements of P. insidiosum.

The mechanisms underlying the response to the PIV are not well understood. However, histopathologic and immunologic studies of cured equines suggested that cellular immunity could play a major role in the clearance of P. insidiosum from infected tissues [19, 20, 22, 32]. Those studies have shown that after successful immunotherapy, the eosinophilic inflammatory reaction, typical of this disease, gradually changed to a mono-nuclear immunoresponse. Numerous macrophages, cytotoxic T lymphocytes (CTLs), and plasma cells had replaced the eosinophilic granuloma. The role of humoral immunity in the clearance of the hyphae of P. insidiosum from the infected tissues is not clear. However, the data obtained in this case and those

Figure 5. These photographs show the patient before vaccination (A) and 12 months after the first of two injections of vaccine against Pythium insidiosum (B).
mania major can induce a switch from a nonprotective immunoresponse (mediated by Th2) to protective Th1 immunity [34]. In that study the expression of a single protein entirely modified the functional immunoresponse to the intact organism. The investigators further hypothesized that immune intervention, by tolerization protocols, "may be considered for downregulation of deleterious immune responses and for long protection against infectious diseases." The results obtained after vaccination of equines and in the case presented here tend to validate their assumption. It is interesting that both L. major and P. insidiosum are eukaryotic organisms, and they elicited a similar immunoresponse in infected hosts.

The dramatic events leading to the cure in this case indicate that manipulation of the immune system with immunogens obtained from pathogens, such as P. insidiosum, is possible and that use of PIV in immunotherapy for pythiosis insidiosi should be considered for humans who have not responded well to the available chemotherapy. It is important to mention that the PIV has been tested in another Thai with arteritic pythiosis insidiosi. Details of this new trial will be published elsewhere.

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