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

Arunee Thitithyanont, Leonel Mendoza, Ampaiwan Chuansumrit, Roongnapa Pracharktam, Jiraporn Laothamatas, Boonmee Sathapatayavongs, Somsak Lolekha, and Libero Ajello

From the Faculty of Medicine (Departments of Medicine, Pathology, Pediatrics, and Radiology), Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; Medical Technology Program, Department of Microbiology, Michigan State University, East Lansing, Michigan; and Emory University School of Medicine, Department of Ophthalmology, Atlanta, Georgia

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 No-
Vember 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 follow-
ing 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 head-
aches 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 exten-
sive facial cellulitis were recorded on admission. Empirical
antibiotic treatment with cefotaxime (100 mg/[kg·d]) and
chloramphenicol (75 mg/[kg·d]) were prescribed without suc-
cess. 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 nonsporu-
lating fungus-like organism was isolated in pure culture of
tissue taken from the left and right pinna. Because of the possi-
bility 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 *P. insidiosum*.

Although the abscess and cellulitis subsided, 1 week later
the pain and headache recurred. 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 increas-
ing 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 (fig-
ure 1). Surgical exploration of the left parapharynx and masse-
teric space was performed. During this procedure, the left
abnormal cervical lymph nodes and the abnormal left auricular
nerve were removed.

Histopathologic examination of the material showed follicu-
lar hyperplasia with sinus histiocytosis and granulomatous in-
flammation. Aseptate hyphal elements of *P. insidiosum*
were observed on silver-stained affected tissue. Hyphal elements of *P. insidiosum* were
observed within the lumen and the vessel’s wall in silver-stained
sections (figure 3). After failure of treat-
ment with amphotericin B and iodides, chemotherapy with
ketoconazole (300 mg/d) was initiated. In addition, granulo-
cyte-macrophage colony stimulating factor (GM-CSF) was
given 5 days immediately after surgical intervention.

The headache and swelling of the tongue diminished after
surgical intervention. Although treatment with ketoconazole
and iodides continued, pain and headache recurred 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

Figure 1. Coronal FSE T₂-weighted (TR/TE/NEX/4100/112ef/3)
MRI of a young boy with headaches and soft-tissue swelling demon-
strated extensive deep and superficial soft-tissue infection by *Pythium
insidiosum* involving the left temporal fossa, left pinna, infratemporal
masticator space, and left cervical carotid space (arrow), with multiple
pus pockets in the left pinna (arrowheads).

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 *P. 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 surgi-
cultural intervention. Five weeks after surgery, the headache and
swelling of tissue recurred. MRI and magnetic resonance angio-
graphy (MRA) of the neck revealed the persistence of cervical
and paracervical lymph node enlargement and persistent steno-
sis of the left internal carotid artery (figure 4). These findings
suggested that *P. insidiosum* had invaded that artery as well.
Surgical removal of the left internal carotid artery was not
recommended. Since amphotericin B, ketoconazole, and io-
dides had not proved effective over a 3-month period, cho-
motherapy was discontinued 1 week before vaccination. Surgery
and two courses of GM-CSF alone had also been ineffective
in controlling the infection; thus, the *P. 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...
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 \textit{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 \textit{P. insidiosum} may have been eliminated from the infected tissues, a finding that substantiated the clinical data. The titer of antibody to \textit{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...
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).

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 mononuclear 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
mania major can induce a switch from a nonprotective immune response (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.

Acknowledgments

The authors are grateful to Drs. Phongjian Hathirat, Suthus Sripoja, Supawadee Prakunhwrgsit, Paisan Leelachaikul, and Prawat Nitiyanant and to the residents, nurses, and paramedical personnel involved in this case.

References


Figure 6. Two-dimensional TOF MR angiorgram of the neck (TR/TE/NEX40/8.7/1) demonstrating normal appearance of the left cervical internal carotid artery (arrowhead) and complete occlusion of the left external carotid artery (arrow), 12 months after the first vaccination against Pythium insidiosum. Recorded with regard to equines cured by the PIV have shown that the titers of antibody to P. insidiosum remain unchanged after vaccination, suggesting that the role of humoral immunity in the activity of the PIV is small [9, 20].

It is well known that the T helper 1 (Th1) subset produces IL-2 and IFN-γ and is involved in clonal expansion of CTLs, macrophage activation, and IgG isotypes that mediate complement activation on sensitized pathogens. The Th2 subset produces IL-4 and IL-5, which activate neutralizing antibodies and IgE, the initiator of hypersensitivity and eosinophilia [33]. On the basis of cellular events before and after successful immunotherapy in humans and animals with pythiosis insidiosi, one can speculate that a switch from a Th2 immunoresponse before vaccination (eosinophilic granuloma and the presence of IgE) to Th1 immunity (macrophages and CTLs; mononuclear infiltrate) may be the driving factor in the curative properties of the PIV.

This is not an isolated phenomenon. It has been reported that the transgenic expression of different proteins of Leish-