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(54) **IMMUNOTHERAPY OF CANINE LEISHMANIASIS**

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None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides a method for treating canine leishmaniasis by immunotherapy.

8 Claims, No Drawings

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IMMUNOTHERAPY OF CANINE LEISHMANIASIS

RELATED APPLICATIONS

The present invention claims the benefit of U.S. Provisional Patent Application No. 62/502,214 filed May 5, 2017, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention provides an immunotherapy of canine leishmaniasis by photodynamic vaccination.

BACKGROUND OF THE INVENTION

Photodynamic therapy (PT) eliminates diseased cells/pathogens by using photosensitizers (PS) that are excitable by light to produce cytotoxic reactive oxygen species (ROS) in the presence of oxygen. Since the ROS simultaneously attack multiple molecules of very different properties, PT is considered to have the potential to circumvent the problem of drug-resistance common to both infectious and non-infectious diseases. By their innate ability to dwell in the endosome/phagolysosomes of antigen-presenting cells, *Leishmania* are a suitable carrier for vaccine delivery.

A novel cell-mediated immunotherapy is being developed according to the *Leishmania* strategy of vaccine delivery (Chang et al., 2016 Parasit Vectors. 9:396) against difficult-to-cure diseases, e. g., canine leishmaniasis. The current clinical management of this disease entails prolonged treatments of sick dogs for 30 days with heavy daily dosage of very toxic drugs (antimonials/miltefosine) followed by a daily maintenance dose of allopurinol for life. Still, relapses of the disease are frequent (up to 50%) within the first year (Manna et al., 2015 Parasit Vectors. 8: 289).

Commonly assigned U.S. Pat. Nos. 7,261,887; 7,238,347, 9,327,017, and U.S. Patent Publication No. 2017/0042989 disclose using *leishmania* as a carrier for vaccine delivery or for the delivery of peptides and proteins. All of these documents are incorporated herein by reference.

SUMMARY OF THE INVENTION

The present invention provides a method of treating leishmaniasis in canines using a combination of chemotherapy and immunotherapy by photodynamic vaccination. The method includes the steps of administering an effective amount of a chemotherapeutic agent to a canine diagnosed with leishmaniasis and a solution containing a photo-inactivated *Leishmania* at 10^7/0.1 ml.

DETAILED DESCRIPTION OF THE INVENTION

While this invention is susceptible of embodiments in many different forms, and will be described herein in detail, specific embodiments thereof with the understanding that the present disclosure is to be considered as an exemplification of the principles of the invention and is not intended to limit the invention to the specific embodiments illustrated.

Method of Treating Canine Leishmaniasis

With institutional IRB-approval and dog owner's consent, a direct observational open label trial was initiated for immunotherapy of 20 diseased dogs, of which 9 were each immunized with frozen photoinactivated *Leishmania* at 10^7/0.1 ml and 11 similarly immunized after s.c. chemotherapy with meglumine antimoniate at 100 mg/kg/day for 30 days followed by allopurinol at a maintenance dose of 10 mg/kg/day. All dogs were assessed clinically for disease signs, blood-biochemical profiles, anti-*Leishmania* antibodies by IFAT, and parasite loads of lymphnode aspirates by quantitative real-time RT-PCR of *Leishmania* DNA every 3 months for >3 years.

Results. Prognosis was improved for the group with immunotherapy after the initial 30 day-chemotherapy based on clinical scores and parasite loads assessed. The immunotherapy was found to stop relapse of the disease completely when used together with allopurinol and worked better than using allopurinol alone.

Suitable chemotherapeutic agents include antimony-containing compounds, for example, meglumine antimoniate or glucantime®, sodium stibogluconate or Pentostam®.

In one form of the invention the *Leishmania* is exposed to a photosensitizer. The photosensitizer is taken up by endocytosis or via plasma membrane penetration. The photosensitizer resides in an organelle or cytosol of the *Leishmania*.

Suitable photosensitizers include those set forth in U.S. Patent Publication No. 2017/0042989 and U.S. Pat. No. 9,327,017. In one form of the invention the photosensitizer is selected from phthalocyanines, naphthalocyanines, porphyrins, chlorins and bacteriochlorins.

Conclusions/Significance: When applied appropriately, the immunotherapy appears to boost the feeble immunity expected to develop after chemotherapy. Work is on-going to see if it is robust enough to clear the infection completely from immunized dogs, and to enroll additional dogs for both prophylactic and therapeutic trials.

The appended claims should be construed broadly and in a manner consistent with the spirit and the scope of the invention herein.

We claim:

1. A method of treating canine leishmaniasis comprising:

administering a chemotherapeutic agent to a canine diagnosed with canine leishmaniasis, an antimony containing compound meglumine antimoniate s.c. at 100 mg/kg/day for a 30 day period followed by s.c. allopurinol at a daily maintenance dose of 10 mg/kg/day; and

periodically administering immunotherapy to the canine with a vaccine solution containing a photo-inactivated *Leishmania* at 10^7/0.1 ml after the chemotherapeutic agent meglumine antimoniate administration is completed.

2. The method of claim **1** wherein the Leishmania contains a photosensitizer selected from the group consisting of phthalocyanines, naphthalocyanines, porphyrins, chlorins and bacteriochlorins.

3. The method of claim **2** wherein the photosensitizer is a phthalocyanine or a porphyrin derivative.

4. The method of claim **3** wherein the photosensitizer is cationic and soluble.

5. The method of claim **3** wherein the phthalocyanine is selected from those with amino groups, anilinium types and pyridyloxy types.

6. The method of claim **2** wherein the photosensitizer resides in an organelle of the Leishmania or its cytosol.

7. The method of claim **2** wherein the photosensitizer is taken up into the *Leishmania* by endocytosis or via plasma membrane penetration.

8. The method of claim 1 wherein the step of periodically administering a solution to the canine containing a photo-inactivated Leishmania occurs 1-3 times during a second 30 day period.

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