20 *Leishmania* spp.

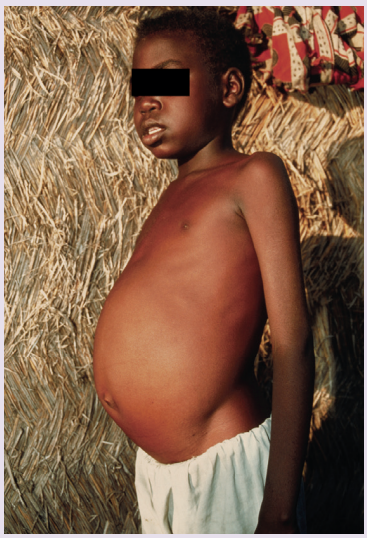

Figure 20.1 A child with visceral leishmaniasis. As in the patient described in the case history, the liver and spleen are enlarged, causing distension of the abdomen. *Courtesy of World Health Organization, Special Programme for Research and Training in Tropical Diseases, http://www.who.int/tdr/index.html. Image #9706290. Additional photographic credit given to Andy Crump who took the photo in Sudan, 1997.*

A 72-year-old man retired to the south of Spain but returned to the UK for the summer months. He began to develop fever, malaise, loss of appetite, and weight loss. He was admitted to hospital and had temperatures reaching 39°C. Both his liver and spleen were palpable. No lymph nodes could be felt. Blood tests showed a **pancytopenia**. Routine investigations for an infection were negative and he did not improve with broad-spectrum antibiotics. His condition deteriorated and the size of the liver and spleen increased (**Figure 20.1**). A bone marrow examination did not show any sign of hematologic malignancy. No organisms were seen on staining. His history was explored again. Four months before his illness he had been on a camping break in Spain to a coastal area. He recalled seeing many thin dogs in the vicinity. Part of his bone marrow sample was sent to a reference laboratory for *Leishmania* **polymerase chain reaction** (**PCR**). This returned positive. He was successfully treated with a course of liposomal amphotericin B and over the ensuing 3 months his liver and spleen became impalpable and his blood tests returned to normal. His diagnosis was visceral leishmaniasis probably due to *Leishmania infantum*.

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

CAUSATIVE AGENT

Leishmania are protozoan parasites. They have an intracellular form called an amastigote (**Figure 20.2**) and an extracellular, flagellated form called a promastigote (**Figure 20.3**).

There is variety in the clinical diseases caused, geographic distribution, and animal reservoirs. The genus *Leishmania* is divided into groups, complexes and species. Classification was classically determined by **isoenzyme typing**; molecular methods (using DNA sequencing) are now more common. **Table 20.1** lists species and the diseases they cause.

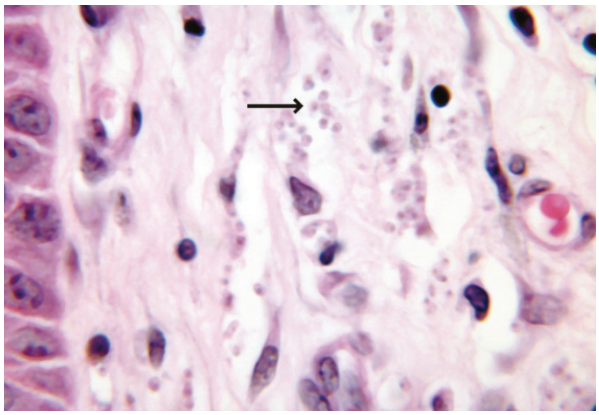

Figure 20.2 A skin biopsy showing *Leishmania* amastigotes (arrowed). *Courtesy of the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #331. Additional photographic credit is given to Dr Martin D. Hicklin who created the image in 1964.*

ENTRY AND SPREAD WITHIN THE BODY

People are infected after the bite of a sandfly laden with *Leishmania* promastigotes. Under the skin, the promastigotes are rapidly phagocytosed by macrophages. For cutaneous disease, lesions are confined to the locality of the sandfly bite. For *L. braziliensis* and *L. panamensis* cutaneous spread can occur and later this can involve mucous membranes of the mouth or nose. *L. donovani* and *L. infantum* are capable of deeper spread within macrophages to the rest of the **mononuclear phagocytic system**, mainly present in organs such as the liver, spleen, and bone marrow. They are responsible for visceral leishmaniasis. In India, visceral leishmaniasis is called **kala-azar**. Relapse of infection after an interval may be manifest as a widespread cutaneous form of disease, called post kala-azar dermal leishmaniasis (PKDL). This occurs in India and East Africa. The life cycle of *Leishmania* is shown in **Figure 20.4**.

PERSON-TO-PERSON SPREAD

In areas with visceral leishmaniasis, sandflies can ingest protozoa when they feed from the skin. Numbers of *Leishmania* in the skin are even higher in PKDL. However, leishmaniasis is largely a zoonosis. Different animal reservoirs occur in different regions. They include rodents, gerbils, hyraxes, sloths, and the domestic dog.

The sandfly vector is a *Phlebotomus* species in the Old World and *Lutzomyia* species in the New World. Sandflies are small, less than 5 mm in size, and bite at dusk or during the night (**Figure 20.5**). They are not capable of flying great heights above the ground and usually bite individuals sleeping close to the ground. In the case described above, the patient was probably infected through sandflies when he was lying near the ground on his camp bed. He normally lived in a flat. The sandflies will have carried infection from the local dog population.

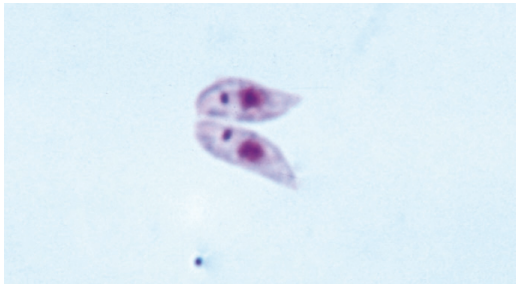

Figure 20.3 Elongated *Leishmania* promastigotes. *Courtesy of the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #544.*

Female sandflies bite and take blood from their target host. Any amastigotes ingested from the skin change into promastigotes. These pass into the sandfly midgut, proliferate, cause damage to the digestive valve system, and are regurgitated to the biting mouthparts and then onto the skin of the next host to be bitten.

Another form of transmission for visceral leishmaniasis has been described among intravenous (IV) drug users in Southern Europe. Infection can be passed on with shared needles and equipment. In one study, about half of discarded needles in Madrid were positive by PCR for *Leishmania*.

EPIDEMIOLOGY

Notification of cases of leishmaniasis is not universal. Numbers of people afflicted by the disease are therefore estimates. There are about 50 000 to 90 000 new cases of visceral leishmaniasis annually, and 95% of these are seen in Brazil, China, Ethiopia, India, Iraq, Kenya, Nepal, Somalia, South Sudan and Sudan. The annual incidence of cutaneous leishmaniasis is 0.6 to 1 million new cases. Ninety-five percent of cutaneous leishmaniasis occurs in the Americas,

Table 20.1 Species of Leishmania and the diseases they cause

Complex	Species	Disease
	L. tropica	Cutaneous leishmaniasis
	L. major	Cutaneous leishmaniasis
	L. aethiopica	Cutaneous leishmaniasis
L. mexicani complex	*L. mexicani*	Cutaneous leishmaniasis
	L. amazonensis	Cutaneous leishmaniasis
	L. venezuelensis	Cutaneous leishmaniasis
	Subgenus Viannia	
	L. [V.] guyanensis	Cutaneous leishmaniasis
	L. [V.] peruviana	Cutaneous leishmaniasis
	L. [V.] panamensis	Muco/cutaneous leishmaniasis
	L. [V.] braziliensis	Muco/cutaneous leishmaniasis
L. donovani complex	*L. donovani*	Visceral leishmaniasis
	L. infantum (also known as *L. chagasi* in New World)	

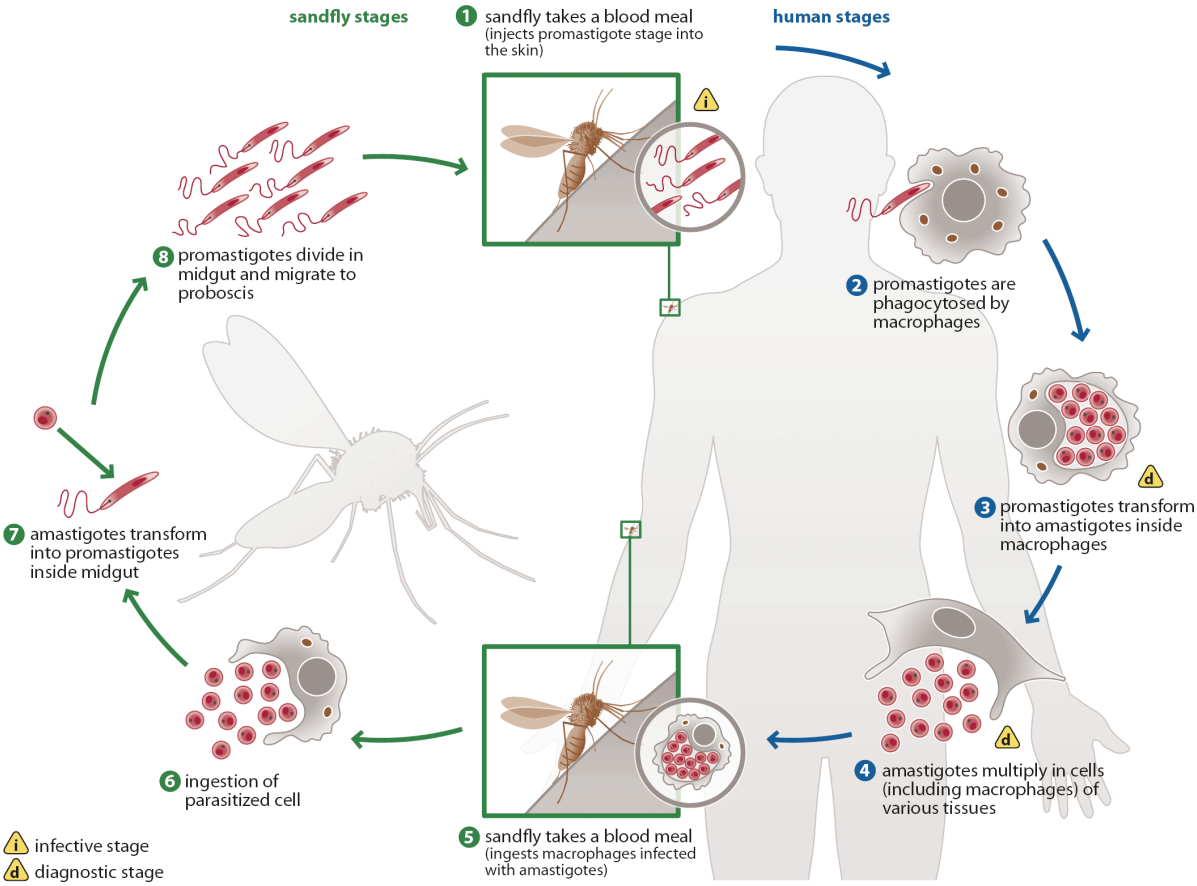

Figure 20.4 Life cycle of *Leishmania* spp. *Leishmania* promastigotes are inoculated by sandflies into human and other animal hosts at the time of taking a blood meal (1) Promastigotes are phagocytosed by macrophages (2) Within macrophages, promastigotes transform into amastigotes (3) Amastigotes can multiply in various cell types (4) Macrophages containing amastigotes are ingested by sandflies taking a blood meal (5) and the life cycle continues within the sandfly vector (6–8). *Courtesy of the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #3400. Additional photographic credit is given to Alexander J da Silva, PhD, and Melanie Moser who created the image in 2002.*

Figure 20.5 Phlebotomus sandfly. *Courtesy of the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #6274. Additional photographic credit is given as follows: World Health Organization (WHO), Geneva, Switzerland.*

the Mediterranean basin, the Middle East, and Central Asia. About 90% of mucocutaneous disease occurs in Bolivia, Brazil, Ethiopia, and Peru.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

As promastigotes enter the skin they are phagocytosed by macrophages and neutrophils. They change into amastigotes. Classically, any pathogen engulfed by a phagocyte is wrapped within host-cell plasma membrane. This forms a phagosome. Various membrane molecules are imported and exported as cytoplasmic vesicles fuse with or erupt from the phagosome. Eventually, **lysosomes** fuse with the phagosome and discharge their contents. Lysosomal enzymes lyse susceptible pathogens. On engulfment, phagocytes are activated to produce **reactive oxygen species** and reactive nitrogen intermediates. They secrete tumor necrosis factor-α (**TNF-α**), which contributes to their activation state. Macrophages are activated further

by T-helper 1 lymphocytes (**Th-1**) through interferon-γ (**IFN-γ**). These are stimulated by antigen-presenting cells, most efficiently by **dendritic cells**. They secrete **interleukin**-12 (IL-12). When it takes time to deal with a pathogen, the combination of Th-1 cells and macrophages organize into **granulomas**.

To survive, *Leishmania* need to subvert the above process. Various effects have been described but the mechanisms by which these occur are not altogether clear. Some leishmanial molecules that have been shown experimentally to play a part are lipophosphoglycan, a surface membrane metalloprotease (gp63), cysteine proteases, and a *Leishmania* homolog of activated C kinase receptor (LACK). The outcome is that macrophage activation and the generation of reactive oxygen and nitrogen intermediates are suppressed, *Leishmania* resist lysosomal attack, dendritic function is compromised, and LACK induces a **Th-2** response. Variations in this interplay occur between different *Leishmania* species. Furthermore, this may be affected by simultaneous co-infections such as viruses infecting *Leishmania* parasites, viruses co-inoculated by sandflies and, importantly, by HIV infection in hosts.

Some *Leishmania* species may use neutrophils as a "Trojan horse". The appearance of neutrophils at the site of the sandfly bite is promoted by the pro-inflammatory effect of sandfly saliva. Cell entry is favored by complement-mediated opsonization, provided that there is no complement-mediated lysis of *Leishmania*. Leishmanial lipophosphoglycan promotes the former and inhibits the latter. Neutrophils fail to kill *Leishmania* after **phagocytosis** and undergo **apoptosis**. Apoptotic fragments may contain *Leishmania*. The neutrophils release **MIP-1β**, a **chemokine** that attracts macrophages. When macrophages phagocytose the apoptotic neutrophil fragments, *Leishmania* enter "silently" and continue to multiply. The macrophages release **transforming growth factor-β** (**TGF-β**), which is anti-inflammatory.

In mice experimentally infected with *L. major*, there is a clear polarization of Th-1 and Th-2 responses. Some strains mount a Th-1 response and control infection, unlike others (BALB/c) which mount a Th-2 response and experience fatal disseminated infection. Humans infected with *L. major* experience localized cutaneous disease. Conversely, *L. donovani*, which causes visceral leishmaniasis in humans, can be controlled by BALB/c mice. *L. donovani* does not lead to a polarized Th-1 and Th-2 response between mouse strains. While there are differences between mice and humans in *Leishmania* infection, the experimental experience with mice indicates the important role of host genetics.

In humans, markers of a Th-1 (IFN-γ) and Th-2 (interleukin (IL)-4) response are both present at the same time. IL-4 down-regulates Th-1 responses and so do other cytokines such as IL-10, IL-13, and TGF-β. These cytokines are more prominent in forms of infection that are not self-limiting like visceral leishmaniasis and PKDL. IL-10 seems to play a greater role in susceptibility to these infections than IL-4. Otherwise, a Th-1 response tends to heal other forms of infection. An exuberant inflammatory response mediated by Th-1 cells may paradoxically enable spread of cutaneous forms of disease.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

Infection may be asymptomatic. *Leishmania* may reside in the body for years and only cause clinical disease if the host becomes immunocompromised.

Cutaneous leishmaniasis is seen on exposed parts of the body where the sandflies are likely to bite (**Figure 20.6**). Sandflies are unable to bite through clothing. Thus, lesions may be found on the face, arms, and lower legs. Lesions may be single or multiple. They are usually apparent 2–6 weeks after the bite. Initially there is a red **papule**. This gradually enlarges over a few weeks. The lesion may take on a raised painless, ulcerated form or is **papulo-nodular**. Secondary infection is possible and then lesions are more likely to be painful. Without specific treatment, lesions will usually self-heal, but over prolonged periods. This may take 6 months to a few years. Over this period, lesions may seem to regress and then relapse. All *Leishmania* species are capable of causing cutaneous disease, but the host immune response may alter the clinical picture. A weakened immune response with a high parasite burden causes diffuse cutaneous leishmaniasis (DCL) with multiple, spreading **papular** lesions. There may also be lymphatic spread with localized nodules along the track of lymphatics. A strong immune response with a low parasite burden causes a condition called leishmania recidivans (LR). The immune response effectively clears the initial site of infection. A series of small papules surround this central clearing and these in turn are cleared.

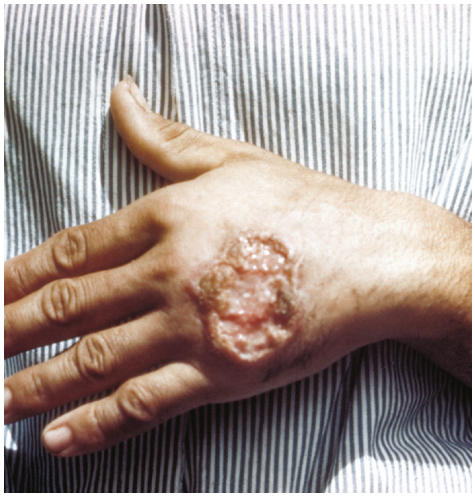

Figure 20.6 Cutaneous leishmaniasis of an ulcerating form. *Courtesy of the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #352. Additional photographic credit is given to Dr DS Martin.*

Mucocutaneous leishmaniasis is associated with *L. braziliensis* and *L. panamensis*. As these species' names suggest, this form of leishmaniasis is restricted to South America. Cutaneous lesions occur first as in purely cutaneous leishmaniasis. These can self-heal, but the parasite does not disappear from the body. After an interval, sometimes of several years, the parasite re-emerges in the mucous membranes of nose or mouth. Local inflammation results in nasal stuffiness. There is then progressive destruction of the anatomy of the nose or mouth and infection can progress backward toward the throat and larynx (**Figure 20.7**). Eating and drinking become difficult and secondary infections in the upper and lower respiratory tract often occur. These latter effects can prove fatal unless the infection is treated. There can be considerable scarring and disfigurement if treatment is delayed.

Visceral leishmaniasis is associated with *L. donovani* in India and East Africa and with *L. infantum* around the Mediterranean and South America. A cutaneous lesion may not be apparent. After an incubation period of a few months, illness is heralded by fevers. These may continue for about one month before abating. The spleen progressively enlarges first and then the liver (**Figure 20.1**). Both may become massively enlarged. The enlarged spleen causes **hypersplenism** and consumption of blood cells, but infection within the bone marrow also causes a pancytopenia with **anemia**, **leukopenia**, and **thrombocytopenia**. In dark skins, the anemia plus hormonal effects of chronic infection cause an altered appearance. In India, the graying of the complexion is called kala-azar. Leukopenia predisposes to secondary infections, which themselves may be life-threatening. Thrombocytopenia can predispose to bleeding and there may be life-threatening hemorrhage. On blood tests, there is also a fall in albumin levels. A drop in oncotic pressure can result in **edema**. This may be peripheral in the legs or ascites within the abdomen. There is also a polyclonal stimulation of **IgG** antibodies. The polyclonal stimulation of B lymphocytes can compromise their ability to respond to other infections. Visceral leishmaniasis runs a chronic and progressive course. Patients become wasted. It is invariably fatal unless treated. If treated, some parasites may escape killing and return later to cause post-kala-azar dermal leishmaniasis (PKDL). In India, this interval may be 2–3 years, but shorter intervals have been observed in Sudan. The host now has some immunity from the first spell of visceral leishmaniasis. The parasite is largely confined to the skin, with extensive papulo-nodular lesions starting on the face and peripheries and then spreading to most of the body surface. This may self-cure, only to relapse and remit at a later date.

Leishmaniasis and HIV co-exist in many areas. The immunocompromised nature of HIV has caused more florid manifestations of leishmaniasis. Parasite burdens are higher. Species that may only cause cutaneous disease may become visceral.

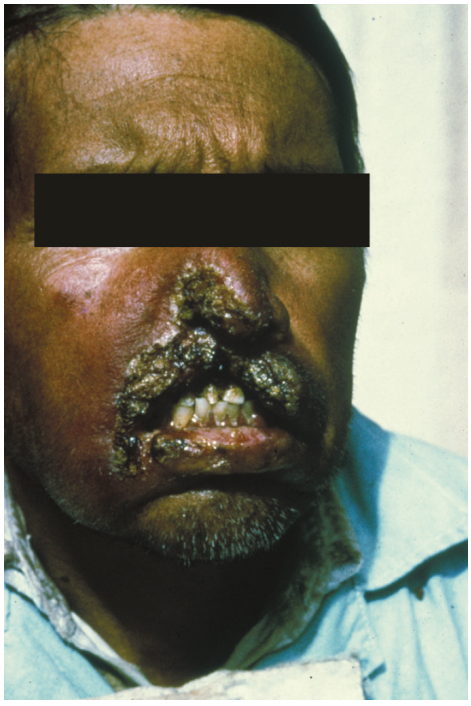

Figure 20.7 Mucocutaneous leishmaniasis in a patient with progressive destruction of tissues around the lips and nose. *Courtesy of World Health Organization, Special Programme for Research and Training in Tropical Diseases, http://www.who.int/tdr/index.html. Image #9106015. Additional photographic credit given to Andy Crump who took the photo in Sudan, 1997.*

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

In endemic areas, cutaneous and mucocutaneous leishmaniasis may be diagnosed on purely clinical grounds. The clinical picture of fever, **splenomegaly**, and anemia due to visceral leishmaniasis may also be caused by other diseases. Investigations are required to confirm the diagnosis. These could entail direct visualization of the parasite in a tissue sample, culture of samples, detection of antigen, detection of nucleic acid by PCR, or immunodiagnosis. The latter includes **serology** or, historically, a leishmanial skin test. Determining the exact species by culture or PCR can be important in choosing between treatment options.

Deep-tissue samples may be obtained by bone-marrow aspirate, a splenic aspirate, lymph-node aspirate, or sometimes liver biopsy. Splenic sampling runs the risk of serious splenic hemorrhage. Cutaneous lesions may be squeezed firmly with fingers to exclude blood, superficially incised with a scalpel at their edge, and then tissue-fluid expressed and impressed onto a glass slide. On staining of tissue samples, intracellular amastigotes are sought. Their appearance is characteristic with a small kinetoplast body adjacent to the nucleus. This is called a **Donovan body** (**Figure 20.8**). The sensitivity of tissue sampling varies with the sample – >90% for splenic aspirate, 55–97% for bone marrow, and 60% for lymph

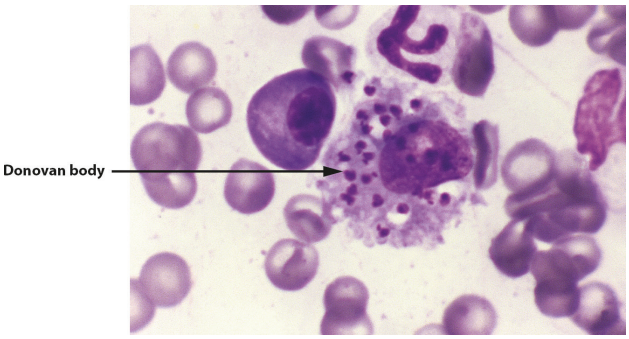

Figure 20.8 Characteristic *Leishmania* amastigote forms (Donovan bodies, arrowed) on an impression smear. Darkly staining small kinetoplast adjacent to nuclei. *Courtesy of the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #30. Additional photographic credit is given to Dr Francis W Chandler who created the image in 1979.*

nodes. If facilities are available, culture can help but now in resource-rich settings PCR is applied with a sensitivity of >95%. Leishmanial antigen can be detected in urine. A latex agglutination technique called KATEX has shown sensitivities of 68–100% for visceral leishmaniasis. After successful treatment, antigen disappears from the urine.

Serology is usually negative in cutaneous leishmaniasis and should be reserved for visceral disease. Serologic tests are unable to distinguish current from past infection. A commonly used test for anti-leishmanial antibody is the direct agglutination test (DAT). Promastigotes are formalin-fixed onto slides and serum is placed on top. Agglutination is observed after 24 hours. DAT has a sensitivity of about 95% and its specificity is about 86%. A dipstick test has been developed with the K39 antigen impregnated on a reagent strip. Blood is added to the strip and a reaction noted after 20 minutes. The K39 dipstick has a sensitivity of about 94% and specificity of about 90%. The K39 antigen is also used in ELISAs.

Historically, a test similar to the tuberculin skin test for tuberculosis was used for leishmaniasis. This was the Montenegro skin test. Leishmanial antigen was implanted in the forearm and the induration after 48–72 hours was measured. Now standardized antigen preparations are no longer available.

DIFFERENTIAL DIAGNOSIS

In endemic areas, cutaneous and mucocutaneous leishmaniasis may have a characteristic appearance. Cutaneous lesions may have to be differentiated from other infected insect bites, tuberculosis, fungal infection, **myiasis**, and skin cancers. Mucosal sites may also be affected by syphilis, histoplasmosis, paracoccidioidomycosis, and leprosy. Visceral leishmaniasis may have to be differentiated from other causes of fever, splenomegaly, and anemia. The differential diagnosis includes malaria, schistosomiasis, typhoid fever, brucellosis, tuberculosis, rickettsial infection, sarcoidosis, **Still's disease**, and hematologic malignancy.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

MANAGEMENT

Small, single lesions of cutaneous leishmaniasis in immunocompetent hosts may be left to self-heal in geographic areas with *L. major*. Other cutaneous lesions, mucocutaneous disease, and visceral leishmaniasis require treatment. There are a number of treatment permutations dependent on form of disease, geographic location, species, and availability of agents. Originally, the mainstay of treatment was pentavalent antimony compounds. These include sodium stibogluconate and meglumine antimonate. They are administered by intramuscular (IM) injection on a daily basis for durations up to 28 days. The IM injections can be painful and there can be systemic toxicity. Alternative treatments are very welcome, as about 60% of visceral leishmaniasis infections in Bihar, India are resistant to treatment with pentavalent antimonials. Amphotericin B and the formulation of liposomal amphotericin B represent advances in treatment. However, they require IV administration, are also toxic, and are much more expensive, especially liposomal amphotericin B. An oral, tolerable agent is now available for visceral leishmaniasis. Oral miltefosine for 28 days was shown to be equally effective with amphotericin B for visceral leishmaniasis in India. Depending on geographic location, agents may be used singly or in combination. In certain situations, other options for cutaneous lesions include intra-lesional injections of pentavalent antimonials, topical paromomycin, and oral azoles.

PREVENTION

Vaccines have been trialed for leishmaniasis but have not been encouraging to date. Prevention has therefore focused on sandflies and efforts directed at human and animal reservoirs. Targeting animal reservoirs is difficult, but it is important to identify and treat PKDL patients who are a key human reservoir for visceral leishmaniasis. As sandflies mainly bite at night, sleeping under a bed-net might afford some protection. But the sandflies are small and can get through the mesh of the nets. However, if the nets are impregnated with a pyrethroid insecticide the sandflies are killed. Insecticide-treated bed-nets are also a key component of malaria control programmes. Furthermore, indoor insecticide spraying on walls has been used both for malaria and leishmania control.

SUMMARY

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY, AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

- *Leishmania* are protozoan parasites.
- There is a large number of species.
- The extracellular stage is called the promastigote and the intracellular stage is the amastigote.
- Spread is by sandflies either from animal reservoirs or humans with heavy skin loads of parasite. The latter occurs in a condition called post-kala-azar dermal leishmaniasis.
- The ability to spread within the body is a function of both the species of *Leishmania* and the host immune response.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

- *Leishmania* are phagocytosed by neutrophils and macrophages.
- T-helper 1 lymphocytes help macrophages to kill *Leishmania*.
- Some species may be more successful at subverting the immune response and causing disseminated infection.
- Subversion of the immune response involves suppression of macrophage activation and diversion toward a T-helper 2 type of response.
- In mice infected with *L. major*, there is a clear polarization of T-helper 1 and 2 responses, depending on the genetic background of the mice.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

- Cutaneous leishmaniasis is caused by a large number of species with infection confined to one locality.
- Lesions enlarge gradually over a few weeks, become papulo-nodular or ulcerate.
- Lesions may self-heal.
- Mucocutaneous leishmaniasis is caused by *L. braziliensis* and *L. panamensis*.
- After an initial cutaneous lesion, there is a later mucosal lesion, which is progressively destructive of nose or mouth.
- Visceral leishmaniasis is due to *L. donovani* or *L. infantum*.
- Infection spreads through the mononuclear phagocytic system with enlargement of spleen and liver, and bone-marrow infiltration.
- Skin complexion changes giving rise to the Indian term, kala-azar.
- After treatment of visceral leishmaniasis relapse may be confined to the skin with post-kala-azar dermal leishmaniasis.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

- Diagnosis of cutaneous or mucocutaneous leishmaniasis may be purely clinical.
- Stained-tissue samples may show characteristic Donovan bodies.
- Species-specific PCR has a high sensitivity.
- Assays exist for leishmanial antigen or antibody.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

- Simple cutaneous lesions may self-heal.
- **Parenteral** pentavalent antimonial drugs have been traditional treatment for all forms of disease.
- Parenteral amphotericin B, either conventional or liposomal, can be used for visceral leishmaniasis.
- Oral miltefosine is easier to administer.
- Combinations of agents may be required for treatment
- Vector control and treatment of the human reservoir of PKDL patients are important preventive measures.

FURTHER READING

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Students can test their knowledge of this case study by visiting the Instructor and Student Resources: [www.routledge.com/cw/lydyard] where several multiple choice questions can be found.