

## Cutaneous Leishmaniasis

### Introduction

Cutaneous leishmaniasis is caused by *L. tropica*, *L. major*, *L. viannia* and *L. aethiopica* in the old world and *L. viannia* and *L. mexicana* complex in the new world. *L. tropica* is widely distributed around the Mediterranean basin, Afghanistan, Kenya, Armenia, Azerbaijan, Turkmenistan and Uzbekistan. *L. aethiopica* is seen in the highlands of Ethiopia and *L. major* occurs in the Middle East, West Africa, North Africa and Kenya. *L. mexicana* complex is found in Central America and the Amazon Basin.

### Life cycle

Infection starts when a sandfly takes a blood meal from an infected host. Small amounts of blood, lymph and macrophages infected with *Leishmania amastigotes* are ingested. Once ingested the **amastigotes** transform to **promastigotes** in the sandfly. The non infective **promastigotes** divide and develop into infective **metacyclic promastigotes**. These are formed in the midgut of the sandfly and migrate to the proboscis. When the sandfly bites the host, promastigotes inoculated at the site of the bite are phagocytosed by macrophages. After phagocytosis, transformation to dividing **amastigotes** occurs within 24 hours. Reproduction at all stages of the lifecycle is believed to occur by binary fission. No sexual stage has been identified.

### Morphology

Leishmania parasites exist as flagellated extracellular **promastigotes** in the sandfly vector and as aflagellar obligate intracellular **amastigotes** within mononuclear phagocytes of their vertebrate hosts. The various species are not distinguishable morphologically from one another. When stained with Romanowsky stains such as Giemsa, **amastigotes** appear as round or oval bodies ranging from 2 - 3 $\mu$  in diameter with a well defined nucleus and kinetoplast, a rod shaped specialised mitochondrial structure that contains extranuclear DNA. The flagellated **promastigote** form is spindle shaped, measuring 10 - 20 $\mu$ m in length, not including the length of the flagellum. As in the amastigote form a nucleus and kinetoplast are clearly visible.

### Clinical Disease

Following a bite from an infected sandfly, a small red papule appears at the site of the bite about 2 – 8 weeks later. The papule increases in size centrifugally. The patient then mounts either a

hypersensitive response or an anergic response. In a hypersensitive response, the papule eventually ulcerates, becomes depressed and then eventually heals through scarring. The patient is now immune from subsequent bites. In an anergic response, the nodule grows and spreads over large areas of skin. This resembles lepromatous leprosy.

## Laboratory Diagnosis

### 1. Microscopy.

The margin of the lesion contains amastigotes whereas the centre contains debris and dead skin material. Thus the margin of the lesion is punctured aseptically with a hypodermic needle and syringe containing a small amount of saline. The aspirate which is drawn up into the needle is prepared as follows:

- a) Air dry smears.
- b) Fix in methanol for 1 minute
- c) Stain with Giemsa 1 in 10 in buffered distilled water pH 6.8 for 30 minutes (or use rapid Field's stain)
- d) Wash the slide in buffered water and drain dry

Amastigotes of leishmania should be seen in positive smears. They are frequently seen within the cytoplasm of the macrophage. In many samples a very small number of parasites are present and culture can be a more sensitive method. Extensive searching of the film is necessary.

### 2. Culture

The aspirates can be cultured in Novy-Nicolle-MacNeal (NNN) or Schneider's Drosophila medium. In culture the **amastigote** stage converts to the **promastigote** stage. However, this is not a rapid technique, as the parasites may take anything from 10 - 21 days to grow.

### 3. Polymerase chain reaction

Gene amplification techniques are powerful and sensitive methods and are useful in diagnosis of cutaneous leishmaniasis particularly when organisms cannot be detected microscopically. It is also very useful for the speciation of *Leishmania* parasites which is required to determine the appropriate drug treatment.

## Mucocutaneous leishmaniasis

Mucocutaneous leishmaniasis or espundia initially develops like cutaneous leishmaniasis but develops into lesions in the mucocutaneous junction of the pharynx resulting in the break down of the palate of the mouth and nose or more rarely the genitalia or anus. This occurs from a few weeks

to several years after the cutaneous lesion has healed. These lesions result in disfiguring deformities of the nose and mouth.

### **Laboratory Diagnosis of Muco-cutaneous leishmaniasis**

#### **1. Microscopy**

Finding the organisms in a histological section of the lesion provides definitive diagnosis of mucocutaneous leishmaniasis. However, the organisms are very rare in this form of the disease and culture can be a more sensitive method (see visceral leishmaniasis).

#### **2. Polymerase chain reaction**

The PCR method has the advantage of not only low numbers of parasites in aspirates but also histological sections. This makes this a very sensitive method in diagnosing mucocutaneous leishmaniasis when parasites are difficult to detect.

