All members of the genus Trypanosoma (trypanes-to bore, soma-body) exist at sometime in their life cycle, as the trypomastigote (trypanosomal) stage with an elongated spindle-shaped body, a central nucleus, a posterior kinetoplast and a long undulating

FIGURE 4.4: Morphological stages of haemoflagellates

membrane. Volutin granules are found in the cytoplasm. In addition to the typical forms, cells with atypical features are frequently found, a condition known as .polymorphism

A blood sucking insect constitutes the intermediate host and vector. The vector becomes infective to the vertebrae host only after an extrinsic incubation period ,during which the parasite undergoes development and multiplication. In the vector the trypanosomes follow one of two modes of development and are accordingly classified into 2 groups—Salivariaand Stercoraria. In salivaria, the trypanosomes migrate to the mouth parts of the vectors (anterior station) so that infection is transmitted by their bite (inoculative transmission). Examples are T. gambienseand T. rhodesiensecausing African trypanosomiasis, which are transmitted by the bite of (tsetse flies. In stercoraria, the trypanosomes migrate to the hindgut (posterior station and are passed in faeces (stercorarian transmission). Examples are T.cruzi causing Chagas' disease which is acquired by rubbing the feces of the vector bug into the wound caused by its bite, and T.lewisi, the rat trypanosome which is transmitted .by ingestion of the faeces of infected rat fleas

Classification

.The trypanosomes infecting humans are classified into the following groups

i. T. brucei subspecies(human strains) causing African trypanosomiasis or sleeping .sickness

T. brucei gambiense

T. brucei rhodesiense

The third subspecies T. brucei bruceiis not infective for humans, but causes) nagana, an important disease of animals in Africa. This is believed to be the ancestral type of trypanosome from which the other two subspecies have been derived by adaptation to the human host. T.b.gambienseappears to be better adapted to human and produces a milder chronic infection while the adaptation .(of T.b.rhodesienseto human is more recent so that it causes a more acute infection

.ii. T. cruzicausing South American trypanosomiasis or Chagas' disease

iii. T.rangelia nonpathogenic trypanosome causing harmless human infection in .South America

.Trypanosomes infect several animal species, sometimes causing important diseases :Some examples are

i. T.brucei(animal strains) causing the economically important disease nagana in .African cattle

.ii. T. evansicausing the disease 'surra' in horses, mules, camels and also in elephants lt is transmitted mechanically by biting flies (Tabanidae, Stomoxys) and also by .vampire bats. The infection is found in India

iii. T. equiperdumcausing 'stallion's disease' in horses and mules. It is transmitted .by sexual contact, without the need for an insect vector

iv. T. lewisi causing a common harmless infection in rats all over the world. The vector is the rat flea, which passes the infective metacyclic trypomastigotes in .feces, which when ingested by a rat, infect it

A trypanosome resembling T. lewisiwas reported from Madhya Pradesh, India in the peripheral blood of two persons with short-term fever. Three human trypanosome infections have been reported from Malaysia, but their identity and .vectors are not known

—Human trypanosomiasis is strictly restricted to certain geographical regions the African and South American trypanosomiases being seen only in the respective

.continents. This is due to the vector species being confined to these places alone

(AFRICAN TRYPANOSOMIASIS (SLEEPING SICKNESS

.Trypanosomiasis is believed to have been extant in tropical Africa from antiquity

Tsetse flies and trypanosomes had kept man and cattle away effectively from a quarter

,of the area of the African continent. In the tsetse belt of tropical Central Africa

where several species of tsetse flies (Glossinaspecies) breed, most of them feed on

the blood of wild game animals, in which they transmit enzootic trypanosomiasis

which causes mild or harmless infection. However, in domestic cattle they cause

.nagana

Causative Agents

Trypanosomes which cause nagana in cattle and sleeping sickness in humans are

,morphologically indistinguishable. Based on host specificity, clinical manifestations

geographic distribution and epidemiological features they were originally classified

:into three species

;T. brucei, infecting cattle and wild game animals, causing nagana

T.gambiensecausing the West African sleeping sickness in humans; and

.T.rhodesiensecausing the East African sleeping sickness in humans

Subsequently it was accepted that all the above should be considered as belonging

to a single species called T. brucei, consisting of three subspecies designated T. brucei

.brucei, T. b.gambienseand T.b.rhodesiense

The suffix '-deme' has been employed to refer to populations of trypanosomes

that differ from others belonging to the same species or subspecies in regard to

specified properties. For example, the term nosodeme refers to trypanosome

populations causing similar clinical patterns of disease, serodemes to those possessing

.similar antigens, zymodemes to those showing similar isoenzyme pattern, etc

.For differentiation between the 'human strains' and 'animal strains' of T. brucei

the blood incubation infectivity test (BIIT) had been widely used. The strain is

incubated with oxalated human blood and then inoculated into the multimammate

rat or other susceptible rodents. The infectivity of ‘animal strains’ will be neutralised by human blood, while ‘human strains’ retain infectivity after incubation with human blood. In vitro culture systems are now employed instead of rodents for testing infectivity.More recently their differentiation is based on isoenzymes, DNA and RNA .characteristics

Morphology and Life Cycle

The morphology and life cycle of the human strains of T. brucei(T. gambienseand .(T. rhodesiense) are identical (Fig. 4.5

Human infection is acquired by the bite of the vector tsetse fly. The infective ,form of the parasite is the metacyclic trypomastigote. On introduction into the dermis ,this proliferates initially at. the site of inoculation and then through the lymphatics .enters the bloodstream

In the blood, three forms of trypanosomes are found, the long slender trypomastigote, a short broad form with the flagellum attenuated or absent and an intermediate form. The trypomastigotes are about 15 to 40 µm long and 1.5 to µm broad. In fresh blood films, they may be seen as colourless spindle-shaped ٣,٥ bodies that move rapidly, spinning the red cells around. In smears stained with Giemsa or other Romanowsky stains, the cytoplasm appears pale blue and the nucleus .red. The kinetoplast appears as a deep red dot and volutin granules stain deep blue .(The undulating membrane appears pale blue and the flagellum red (Fig. 4.6

When a vector tsetse fly feeds on a person with parasitaemia, it takes in the .trypomastigotes along with its blood meal, particularly the short broad forms

These become long slender forms in the midgut and hindgut of the fly, where they proliferate and ultimately reach the salivary glands. Here they become broad epimastigotes which multiply and fill the cavity of the gland. The fly becomes .infective when the epimastigotes become transformed into metacyclic trypomastigotes

It takes about 3 weeks from the time of the blood meal for the fly to become infective

FIGURE 4.5 Life cycle of T. brucei. (1) Elongated
,trypomastigote form in midgut of tsetse fly (2) Epimastigote
in salivary gland which develops into (3) Metacyclic
trypomastigote, the infective form for vertebrates. (4,5 and
are trypomastigote forms in vertebrate blood, — the (٦
slender form (4) the intermediate form (5) and the short
(stumpy form (6
Flagellates 47
extrinsic incubation period). Thereafter, the fly remains infective for life, about 6)
.months
The trypanosome remains extracellularly throughout its life cycle, both in the
vertebrate and in the vector. It was believed that the trypomastigote is the only
form present in vertebrates, but recently amastigote forms of the parasite have been
.found in the choroid plexus blocking blood vessels and obstructing CSF circulation
It is possible that this may have a role in the pathogenesis of cerebral manifestations
.of the condition
Trypanosomes exhibit antigenic variation of their surface glycoproteins. There
.is a cyclical fluctuation in the trypanosomes in the blood of infected vertebrates
Each successive wave represents a variant antigenic type (VAT) of trypomastigote
possessing variant surface specific antigens (VSSA) or variant surface glycoproteins
VSG). Besides this, trypanosomes have other mechanisms also helping them to evade)
.host immune responses
West African (Gambian) Sleeping Sickness
This infection caused by T.b.gambienseis endemic in scattered foci in West and Central
Africa between 15°N and 18°S latitudes.(The trypanosome was first isolated from
(.the blood of a steamboat captain on the Gambia river—hence the name gambiense
.The principal vectors are the riverine tsetse flies Glossina palpalisand G. tachinoides
Humans are the reservoir host and source of infection, though pigs and other domestic

animals can act as chronic asymptomatic carriers of the parasite. The disease may sometimes occur as epidemics. During epidemics, the vector fly has been found to transmit the infection mechanically through its soiled proboscis when it bites a susceptible person soon after biting an infected person. Congenital transmission also .has been recorded

The incubation period is about 1 to 2 weeks. The illness is chronic and can persist for many years. There is an initial period of parasitaemia, following which they are localised predominantly in the lymph nodes. Intermittent fever, chills and headache mark this stage. There is hepatosplenomegaly with lymphadenopathy particularly ,in the posterior cervical region. With the invasion of the central nervous system which occurs after several months, the sleeping sickness stage starts. This is marked by increasing headache, mental dullness, apathy and sleepiness. The patient falls .into profound coma followed by death from asthenia

Histopathology shows chronic meningoencephalitis. The meninges are heavily infiltrated with lymphocytes, plasma cells and morula cells which are atypical plasma cells containing mulberry shaped masses of IgA. Brain vessels show perivascular cuffing. This is followed by infiltration of the brain and spinal cord, neuronal .degeneration and microglial proliferation

East African (Rhodesian) Sleeping Sickness

This form of African trypanosomiasis is caused by T.b. rhodesiense. It is found in foci situated to the east of the area affected by T.b. gambiense. The principal vector is

.G. morsitans, G. palpalisand G. swynnertoniwhich live in the open savannah country

Though the infection is usually transmitted by the vector from man-to-man, the disease is actually a zoonosis, with the reservoir being game animals such as the bush buck .(Fig. 4.6)

East African trypanosomiasis is more acute than the Gambian form and may end fatally within a year of onset, before involvement of the central nervous system

develops. Fever, weakness, rapid loss of weight and myocarditis are the usual manifestations. Mania and delusion may occur, but the typical sleeping sickness picture .is seldom seen

Diagnosis

Diagnosis is established by the demonstration of the trypanosomes in peripheral blood, bone marrow, lymph nodes or cerebrospinal fluid. The methods available are direct microscopy of stained or unstained preparations, cultivation in Weinman's or Tobie's medium and inoculation into rats. Several serological tests have been developed for detecting antibodies. These include direct agglutination, indirect .haemagglutination, gel precipitation immunofluorescence and ELISA

Prophylaxis

.Preventive measures depend mainly on control of the vector

Treatment

Suramin and pentamidine are used for early cases. Melarsoprol is the only drug .effective in late cases with neurological involvement

FIGURE 4.6: Geographical distribution of trypanosomiasis in Africa. Lines indicate areas endemic for T.gambienseand dots T. rhodesiense

(SOUTH AMERICAN TRYPANOSOMIASIS (CHAGAS' DISEASE

History

.This condition, caused by Trypanosoma cruzi, is limited to South and Central America Carlos Chagas, investigating malaria in Brazil in 1909, accidentally found this trypanosome in the intestine of a triatomid bug and in the blood of a monkey bitten by the infected bugs. It was only later that Chagas found the trypanosome in the ,blood of a sick child and showed that it was responsible for an endemic disease which came to be named after him. In this instance, therefore, the parasite and the vector were discovered before the disease was identified. Chagas named the parasite .T. cruziafter his mentor Oswaldo Cruz

Vectors and Life Cycle

T. cruzipasses its life cycle in two hosts—vertebrate hosts including humans and the insect vector—the reduviid bug. The parasite occurs in three different but overlapping infection cycles, a sylvatic zoonosis in wild animals such as armadillos and opossums, a peridomestic cycle in dogs, cats and other domestic animals, and a domestic cycle in humans. Different vector species are active in these infection cycles. The vectors important in human infection are the reduviid bugs adapted to living in human habitations, mainly Triatoma infestans, Rhodnius prolixusand Panstrongylus megistus. These are large (up to 3 cm long) night biting bugs which typically defecate while feeding. The faeces of infected bugs contains the metacyclic trypomastigotes which are the infective forms. Infection is acquired when they are rubbed into the bite wound or enter through mucosal surfaces, particularly the conjunctiva, being transferred there by the person's fingers. The trypomastigotes may induce a local inflammatory reaction and swelling at the site of entry in the skin called 'Chagoma.' When they enter through the conjunctiva a unilateral .(oedematous swelling of the eyelids results (Romana's sign

The parasite spreads through the lymphatic system involving various tissues and cells throughout the reticuloendothelial system. Inside these cells, they get transformed into amastigote forms which divide by binary fission. After passing through promastigote and epimastigote forms, they again become tyrpomastigotes which are released into the blood stream. No multiplication occurs in the trypomastigote stage. Multiplication takes place only intracellularly in the amastigote form and to some extent as promastigotes or epimastigotes when about to be released from the .(cell (Fig. 4.7

When a reduviid bug bites a person with trypanosomes in peripheral blood they get into the midgut of the insect. Here, the trypomastigotes are transformed into .epimastigotes which migrate to the hindgut and proliferate

These in turn develop into metacyclic trypomastigotes which are excreted in faeces

.stercorarian transmission). The extrinsic incubation period is 8 to 10 days)

.FIGURE 4.7: Life cycle of T. cruzi

Trypomastigote form enters midgut of reduviid .١
bug and transforms into 2. Epimastigote form which
.migrates to hindgut, multiplies and becomes 3
Metacyclic trypomastigote which is shed in faeces
and infects vertebrates. 4. Trypomastigote in blood
.enters reticuloendothelial and other tissue cells 5
In which it passes through epimastigote and
promastigote stages to become amastigotes which
replicate and again through promastigote and
epimastigote stages become 6. Trypomastigotes
released into bloodstream. These are the infective
forms for the vector bug

Pathogenicity and Clinical Features

,The incubation period in man is 1 to 2 weeks. The disease manifests in two forms
acute and chronic. In the acute form, usually found in children, it presents with fever
and generalised nonpitting oedema of the body. The disease lasts for 3 to 4 weeks
and sometimes ends fatally with myocarditis or meningoencephalitis. The chronic
form found in adults presents as neurotropic, cardiotropic or viscerotropic forms
.and may last for several years

The pathogenesis depends on the intracellular multiplication of the amastigote
form in various locations causing damage to the cells and tissues. The sites commonly
affected are myocardium, skeletal muscles, neuroglial cells and cells of the reticuloendothelial system.
Damage to myocardium is often associated with conduction
'defects. Damage to autonomic nerve cells often leads to the so called 'megadisease
.consisting of megaoesophagus, megacolon and megaureter

Diagnosis

Diagnosis is by demonstration of T.cruziin blood or tissues, or by serology. In stained peripheral blood smears, the trypomastigote often appears in a C-shaped form (Fig. 4.8). T.cruzican be grown in NNN medium or its modifications. Guinea pig inoculation may be done with blood, CSF, lymph node aspirate or other tissue materials and the trypomastigote looked for in its blood smears. Xenodiagnosis may be attempted by allowing a parasite—free reduviid bug to bite the pateint .and by demonstrating the parasite in its intestinal contents

Serological tests employed for detection of antibodies include complement fixation Machado-Guerreiro test), indirect haemagglutination, immunofluorescence and) ELISA. Specific tests have been developed for demonstration of the parasite antigen in blood and urine.An intradermal test has been described for demonstration of .hypersensitivity. The antigen 'cruzin' is prepared from T. cruzicultures

Prophylaxis

Control and elimination of domestic and peridomestic vector bugs would help check the transmission of disease in endemic areas. Triatomine bugs are highly susceptible to chlorinated hydrocarbon insecticides, which form the major weapon for their control. Most human infections are transmitted by bugs living in cracks and crannies in the walls of ill kept tenement dwellings. Provision of better housing would prevent .such transmission

Treatment

No effective specific treatment is available. Nifurtimox and benznidazole have been used with some success in the acute cases. Allopurinol and ketoconazole have .also been found useful

LEISHMANIA

The genus Leishmania is named after Sir William Leishman who discovered the flagellate protozoon causing kala-azar, the Indian visceral leishmaniasis. All members of the

genus Leishmania are obligate intracellular parasites that pass their life cycle in two hosts, the mammalian host and the insect vector, female sandfly. In human and other mammalian hosts, they multiply within macrophages, in which they occur exclusively .in the amastigote form, having an ovoid body containing a nucleus and kinetoplast In the sandfly, they occur in the promastigote form, with a spindle shaped body .and a single flagellum arising from the anterior end

FIGURE 4.8

Classification

The taxonomy of leishmaniae is controversial because traditionally they have been .classified according to the clinical disease caused and the geographical prevalence This does not parallel the grouping based on genetic and biochemical features. For .medical purposes the old classification is still preferred

Leishmaniae produce two broad types of clinical disease, visceral and cutaneous ,including mucocutaneous) leishmaniases. Leishmaniae parasitic for humans have) .therefore been classified into two broad groups

(A. Causing Visceral Leishmaniasis (VL

,(The L.donovanicomplex infecting internal organs (liver, spleen, bone marrow .١ .causing visceral leishmaniasis

(B. Causing Cutaneous and/or Mucocutaneous Leishmaniasis (CL

(I. L. tropica, L.major, L. aethiopica—(Old world CL

II. L. mexicanacomplex; L. braziliensiscomplex and L. guyanensiscomplex (the 2 .(latter now regrouped under Viannia subgroup)—(New world or American CL

Each of these complexes contains a number of different varieties and subspecies which differ in several features such as antigenic structure, isoenzymes and other biochemical characteristics, growth properties, ecology and pathogenicity. Based on "geographical distribution, they have been classified as "Old World" or " New World .leishminases

LEISHMANIA DONOVANI

History

Sir William Leishman in 1900 observed the parasite in spleen smears of a soldier .who had died of 'Dum Dum fever' or kala-azar contracted at Dum Dum, Calcutta Leishman reported this finding from London in 1903, in which year Donovan also reported the same parasite in spleen smears of patients from Madras. The name Leishmania donovaniwas therefore given to this parasite. The amastigote forms of .the parasite as seen in smears from pateints, are called Leishman-Donovan(LD) bodies L. donovanicauses visceral leishmaniasis or kala-azar. It also causes the condition .(post-kala-azar dermal leishmaniasis (PKDL

.Leishmaniasis is a major public health problem in many parts of the world According to the WHO Report of 1990, 1.5 million cases of cutaneous leishmaniasis .and 500,000 cases of visceral leishmaniasis occur every year, spread over 82 countries About 350 million people are at risk of leishmaniasis, with 12 million people currently .infected

Morphology and Life Cycle

,The parasite exists in two forms, the amastigote form in humans and other mammals and the promastigote form in the sandfly and in artificial cultures (Figs 4.9A and .(B, and 4.10

.FIGURE 4.9A: Morphology of Leishmania donovani. a

.Amastigote (LD body). b. Promastigote. 1. Nucleus 2

Parabasal body 3. Blepharoplast 4. Vacuole 5. Axoneme

Flagellum .٦

FIGURE 4.9B: LD body in spleen smear of experimentally

(infected animal (Giemsa stain

The amastigote form (LD body) is an ovoid or rounded cell, about 2 to 4 µm ,in size. It is typically intracellular, being found inside macrophages, monocytes

.neutrophils or endothelial cells

Smears stained with Leishman, Giemsa or Wright stains show a pale blue cytoplasm

.enclosed by a limiting membrane. The large oval or round nucleus is stained red

Lying at right angles to the nucleus is the red or purple stained kinetoplast. In wellstained preparations, the kinetoplast can be seen to consist of the parabasal body

and a dot-like blepharoplast with a delicate thread connecting the two. The axoneme

arising from the blepharoplast extends to the anterior tip of the cell. Alongside the

.kinetoplast can be seen a clear unstained vacuole

FIGURE 4.10

The habitat of the amastigote LD body is the reticuloendothelial system. They

are found mostly within the macrophages in the spleen, liver and bone marrow and

less often in other locations such as the skin, intestinal mucosa and mesenteric lymph

nodes. They multiply by binary fission, producing numerous daughter cells that distend

the macrophage and rupture it. The liberated daughter cells are in turn phagocytosed

by other macrophages and histiocytes. Small numbers of LD bodies can be found

in peripheral blood inside polymorphonuclear leukocytes or monocytes. Rarely they

.may be seen in feces, urine and nasal secretions

When a vector sandfly feeds on an infected person, the amastigotes present in

peripheral blood and tissue fluids enter the insect along with its blood meal. In the

midgut (stomach) of the sandfly, the amastigote elongates and develops into the

.promastigote form

,The promastigotes, which are initially short oval or pear-shaped forms

subsequently become long spindle-shaped cells, 15 to 25 µm long, carrying a single

flagellum 15 to 30 µm in length. Stained films show pale blue cytoplasm with a red

nucleus in the centre. The kinetoplast lies transversely near the anterior end. Near

the root of the flagellum is present a vacuole. As the flagellum extends anteriorly

without curving back on the body, there is no undulating membrane. Promastigote

forms which develop in artificial cultures have the same morphology as those in .the sandfly

The promastigotes multiply by longitudinal binary fission and reach enormous numbers. They may be seen as large rosettes with their flagella entangled. In the

sandfly, they migrate from the midgut to the pharynx and hypostome, where they accumulate and block the passage. Such blocked sandflieshave difficulty in sucking blood. When they bite a person and attempt to suck blood, plugs of adherent parasites may get dislodged from the pharynx and deposited in the punctured wound. The promastigotes so deposited are phagocytosed by macrophages inside which they change into amastigotes and start multiplying. These, in turn enter the midgut of a sandfly when it bites the infected person. It takes about 6 to 10 days after ingestion of the amastigotes for the promastigotes to reach adequate numbers so as to block the buccal cavity and pharynx of the sandfly. This is, therefore, the duration of the extrinsic incubation period (Fig. 4.11). This is also synchronous with the gonadotropic cycle of the vector so that amastigotes ingested during one blood meal, are ready to be transmitted when the sandfly takes the next blood meal, after its eggs have .been laid

FIGURE 4.11: Life cycle of Leishmania donovani

Sandfly feeding on infected person ingests .١ amastigotes. 2. In the stomach of the sand-fly, the amastigote becomes promastigote, which multiplies by binary fission. 3. Promastigotes accumulate in pharynx and block the passage. 4. When the sandfly bites a person, 5. The promastigotes get deposited in the puncture wound. 6. They are phagocytosed by macrophage. 7. In which they multiply, distending the cell. 8. The macrophage

ruptures, releasing the amastigotes, some of

which are 9. Phagocytosed by other macrophages. 10. Amastigotes in peripheral blood and skin are ingested by sandflies while feeding, to

repeat the cycle

Ecological Types

The epidemiology and clinical features of visceral leishmaniasis and the ecology of the parasite are very different in different geographical areas. The different clinical syndromes have therefore been considered to be distinct entities and the parasites .causing them have been given separate species or subspecies status, as listed below

i. Indian visceral leishmaniasis caused by L.donovaniproducing the anthroponotic .(disease kala-azar, and its sequel 'post-kala-azar dermal leishmaniasis' (PKDL

This disease is not zoonotic, humans being the only host and reservoir. Vector is the sandfly Phlebotomus argentipes. (In India, classical kala-azar has rarely been .(seen caused by L.tropica

ii. Mediterranean—Middle Eastern leishmaniasis caused by L. donovani infantum or L.infantum) affecting mostly young children. It is a zoonotic disease, the) reservoir being dogs or wild canines such as foxes, jackals, and wolves. Vectors .are P. perniciosusand P. ariasi

iii. East African leishmaniasis caused by L.d.archibaldi. The disease is zoonotic, found mainly in rural areas. Reservoirs are dogs, mongoose and wild mammals. Vectors .are P. orientalisand P. martini

iv. South American leishmaniasis caused by L.d.chagasi(L.chagasi). The disease is zoonotic. Foxes and wild canines are the reservoirs. Dogs act as the link between the reservoir hosts and humans. The main vector is the sandfly Lutzomyia .longipalpis

v. In China, the disease resembles the Mediterranean type (L. infantum) in the .North West and the Indian type (L.donovani) in the East

vi. American (New World) visceral leishmaniasis is caused by L.chagasi. It is present .in most parts of LatinAmerica and resembles the disease caused byL.infantum

KALA-AZAR

The disease visceral leishmaniasis was first characterised in India, where it was known under the names, kala-azar (meaning black sickness), Dum Dum fever, Burdwan .fever or tropical splenomegaly

Clinical Features

The infection is transmitted by the bite of the sandfly P.argentipes. Instances of transmission of the disease by blood transfusion, sexual contact, inoculation and congenitally have been recorded, but these are extremely rare and of no epidemiological significance. Most infections are inapparent or subclinical and only about 3 per cent develop the typical kala-azar syndrome. The incubation period is usually from 2 to 6 months, though occasionally it may be as short as 10 days or as long as two years. Cutaneous lesion at the site of bite of the sandfly is not seen .in Indian patients, but is common in patients in Sudan and the Middle East

The onset is typically insidious. The clinical illness begins with fever, which may be continuous, remittant or irregular. Splenomegaly starts early and is progressive and massive. Hepatomegaly and lymphadenopathy also occur but are not so prominent. The disease progresses for several months, with periods of apyrexia ,followed again by fever. Emaciation and anaemia develop. The skin becomes dry rough arid darkly pigmented (hence the name kala-azar). The hair becomes thin and brittle. Epistaxis and bleeding gums are common. Most untreated patients die .in about 2 years due to some intercurrent disease such as dysentery or tuberculosis

About 10 to 20 per cent of pateints who recover develop post kala-azar dermal leishmaniasis (PKDL). The dermal lesions usually develop about a year or two after recovery from the systemic illness. The lesions are of 3 types—depigmented macules which appear commonly on the trunk and extremities, or erythematous patchesappearing on the face (butterfly patch), both of which develop into painless yellowish

pink non-ulcerating granulomatous nodules. The parasite can be demonstrated in the

lesions. PKDL is seen mainly in India. It is rare in East Africa and China and not

.seen elsewhere

Pathology

Kala-azar is a reticuloendotheliosis resulting from the invasion of the reticuloendothelial system by L.donovani.Parasitised macrophages disseminate the infection to

all parts of the body. In the spleen, liver and bone marrow particularly, the amastigotes

multiply enormously in the fixed macrophages to produce a 'blockade' of the reticuloendothelial system. This leads to a marked proliferation of the reticuloendothelial

.tissue in these organs

The spleen is the organ most affected. It is grossly enlarged and the capsule is

frequently thickened due to perisplenitis. It is soft and friable and cuts easily without

resistance, due to absence of fibrosis. The cut section is red or chocolate in colour

.due to the dilated and engorged vascular spaces. The trabeculae are thin and atrophic

Microscopically, the reticulum cells are greatly increased in numbers and are loaded

.with LD bodies. Lymphocytic infiltration is scanty , but plasma cells are numerous

The liver is enlarged. The Kupffer cells and vascular endothelial cells are heavily

parasitised, but hepatocytes are not affected. Liver function is therefore not seriously

affected, though prothrombin production is commonly decreased. The sinusoidal

.capillaries are dilated and engorged. Some degree of fatty degeneration is seen

.The cut surface may show a nutmeg appearance

The bone marrow is heavily infiltrated with parasitised macrophages which may

crowd out the haemopoietic tissues. Peripheral lymph nodes and lymphoid tissues

of the nasopharynx and intestine are hypertrophic due to infiltration with parasitised

.cells, though this is not frequently seen in Indian cases

Anaemia occurs as a result of infiltration of the bone marrow as well as by the increased destruction of erythrocytes due to hypersplenism. Autoantibodies to red cells may

contribute to haemolysis. Leucopenia, with marked neutorpenia, and thrombocytopenia

.are frequently seen. Polyclonal hypergammaglobulinaemia is a common finding

Immunity

The most important immunological feature in kala-azar is the marked suppression

of cell mediated immunity to leishmanial antigens. This makes possible the unrestricted

intracellular multiplication of the parasite. Cellular responses to tuberculin and other

antigens are also suppressed and may be regained some 6 weeks after recovery

.from the disease

In contrast, there is an overproduction of immunoglobulins, both specific antileishmania antibodies as well as polyclonal IgG and IgM immunoglobulins. Circulating

immune complexes are demonstrable in serum. Complement activation occurs, but

the antibodies do not appear to be relevant in defense against the parasites. Patients

who have recovered from the infection are considered immune to reinfection. HIV

.infection heightens susceptibility to visceral leishmaniasis

Laboratory Diagnosis

:Methods employed in laboratory diagnosis are as follows

:Demonstration of the parasite in materials obtained from patients, by .١

.a. microscopy

.b. culture

.c. animal inoculation

:Demonstration of antibodies or antigens by using .٢

a. specific leishmanial antigens; or

.b. non-specific antigens

.Non-specific serum tests .٣

.Absence of hypersensitivity to leishmanial antigen .٤

.Contributory findings in clinical laboratory tests .٥

Demonstration of Parasites in Material Obtained from Patients .١

:a. For microscopic demonstration of the parasite, the materials collected are

.i. peripheral blood

ii. bone marrow, and

.iii. splenic aspirate

i. Peripheral bloodcontains the amastigotes present inside circulating monocytes and less often in neutrophils, but the numbers are so scanty that a direct blood smear may not show them. Chances of detecting them are somewhat improved .by examination of a thick blood film or of the leucocytic edge in a blood smear It is best to examine buffy coat smears though even these are not often found positive. Buffy coat smears show a diurnal periodicity, more smears being .positive when collected during the day than at night

ii. Bone marrow aspirateis the most common diagnostic specimen collected. Generally the sternal marrow is aspirated by puncturing the sternum at the level of the nd or 3rd intercostal space, using a sternal puncture needle. This consists of٢ a short stout needle with a stylet. It has a movable guard which is fixed at to 2 cm from the tip, depending on the thickness of the chest wall over the ١ sternum. After disinfecting and anaesthetising the skin, the needle is introduced into the sternal marrow and about 0.5 ml of marrow fluid aspirated using a syringe. The puncture wound is sealed with celloidin or Tr. benzoin. Bone .marrow samples can be obtained also by puncturing the iliac crest

iii. Spleen aspiratesare richer in parasites and so more valuable for diagnosis. But the procedure can sometimes cause dangerous bleeding and so should be done carefully and only when a marrow examination is inconclusive. To guard against bleeding. prothrombin time and platelet count should be checked before the .procedure. The spleen should be palpable at least 3 cm below the costal margin The spleen is penetrated with a 21-gauge needle attached to a 5 ml syringe .and aspiration done by applying gentle suction

Lymph node aspiratesare not useful in the diagnosis of Indian kala-azar, though .it is employed in visceral leishmaniasis in some other countries

The materials collected, as described above can be tested by microscopy, culture .and animal inoculation

a. For microscopy,smears are stained by Leishman, Giemsa or Wright stains and (examined under the oil immersion objective. Amastigote parasites (LD bodies can be seen within macrophages, often in large numbers. A few extracellular forms .(can also be seen usually (see Fig. 4.10

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b.Culturesare made on Novy-McNeal-Nicolle (NNN) medium. This is a rabbit blood ,agar slope having an overlay of Locke's solution with added antibiotics (penicillin streptomycin, gentamicin) dispensed in screw capped bottles. The material is inoculated into the water of condensation and the culture incubated at 24°C for days. The parasite grows as promastigotes and can be demonstrated by ٧ examining a drop of the fluid under high power objective using reduced condenser aperture or preferably, phase contrast illumination. Stained smears can also be examined. If negative, the culture is reincubated and examined weekly for 4 to weeks. Schneider's liquid tissue culture medium with added foetal calf serum ٦ .is also used for culture

c. Animal inoculationis not used for routine diagnosis. When necessary, hamster is the animal employed. The materials are inoculated intraperitoneally, or intradermally into the skin of the nose and feet. The inoculated animals are kept at 23 to 26°C. In positive cases, the parasite can be demonstrated in smears taken from ulcers or nodules developing at the sites of cutaneous inoculation, or from the spleen. Animal inoculation is a very sensitive method, but takes several weeks .to become positive

Demonstration of Antibodies or Antigens.٢

a. Specific leishmanial antigens prepared from cultures have been used in a number .of tests to demonstrate specific antibodies. These tests include complement fixation

counter immunoelectrophoresis, immunofluorescence and ELISA tests. In kalaazar, the immunofluorescent antibody (IFA) titre usually rises to 64 or above

and declines slowly after treatment, eventually becoming negative. The direct

agglutination test for anti-leishmanial antibody has been found to be highly specific

and sensitive for diagnosis of kala-azar. A specific immunochromatographic

dipstick method for antibody has been developed using recombinant leishmanial

.antigens

.b. Specific antigen detection tests have been developed by immunoblotting and PCR

Nonspecific (nonleishmanial) antigens for serological tests have been used for

many decades. The antigen originally used was prepared from human tubercle

.(bacillus by Witebsky, Klingenstein and Kuhn (hence called the WKK antigen

,Complement fixation test with WKK antigen becomes positive early in the disease

,within weeks of infection. Positive reaction also occurs in some other conditions

including tuberculosis, leprosy and tropical eosinophilia. An antigen prepared

.from Kedrowsky's acid-fast bacillus is preferred

Nonspecific Serum Tests.٣

Some diagnostic tests for kala-azar are based on the greatly increased globulin content

of serum in the disease. The two tests widely used are Napier's aldehyde or Formol

.gel test and Chopra's antimony test

i. In Napier's aldehyde test 1 ml of clear serum from the patient is taken in a

small test tube, a drop of formalin (40% formaldehyde) is added, shaken and

kept in a rack at room temperature. A control tube with normal