LEISHMANIASES

- A complex of diseases that are caused by parasites of the *Leishmania* family
- The parasites are transmitted by sandflies (e.g. Phlebotomus)
- Leishmaniasis is a world-wide problem. About 350 million people in 88 countries are at risk. The number of cases is estimated at about 12 millions, with 1.5 - 2 million new cases every year
Early reports on leishmaniasis:

A report in the library of King Ashurbanipal of Assyria around 650 BC, based on Accadian texts that are still 2000 years older.

Abu Ali al-Husayn ibn Abd Allah ibn Sina (980-1037) ("Avicenna")

His book: Al Qanun fi al-Tibb (the Canon Medicinae) remained the standard medical reference book in Europe through the 17th century.
The LEISHMANIASES

1. Cutaneous leishmaniasis (oriental sore)

L. major (old world)
L. mexicana (new world)

Usually self-healing, live parasites persist for the rest of the life of the patient. Confers protection against re-infection
The LEISHMANIASES

2. Mucocutaneous leishmaniasis (espundia)

- L. braziliensis
- L. guayanensis
- L. panamensis

Pre-Incan pottery showing deformities due to mucocutaneous leishmaniasis

Ultimately fatal infection that destroys the mucosae of the nasopharyngeal cavity.
Death often through opportunistic bacterial infections
3. Visceral leishmaniasis (Kala Azar)

Second largest parasitic killer (after malaria).
The parasite-infected macrophages invade liver, spleen and bone marrow. Mostly fatal. Stray dogs as a reservoir. About 500’000 cases every year!
The Leishmania life cycle

Sandfly Stages
1. Sandfly takes a blood meal (injects promastigote stage into the skin)
2. Promastigotes are phagocytized by macrophages
3. Promastigotes transform into amastigotes inside macrophages
4. Amastigotes multiply in cells (including macrophages) of various tissues
6. Ingestion of parasitized cell
7. Amastigotes transform into promastigote stage in midgut
8. Divide in midgut and migrate to proboscis

Human Stages
1. Sandfly takes a blood meal (injects promastigote stage into the skin)
2. Promastigotes are phagocytized by macrophages
3. Promastigotes transform into amastigotes inside macrophages
4. Amastigotes multiply in cells (including macrophages) of various tissues
5. Sandfly takes a blood meal (ingests macrophages infected with amastigotes)

= Infective Stage
= Diagnostic Stage
Infected sandflies transmit their parasites via regurgitation of foregut contents during a blood meal.

A. Pump action sucks up blood in healthy fly (cardiac valve (Cv) is closed)

B. Pump action in infected fly with damaged cardiac valve

C. Contraction of food pump in infected fly pushes blood into the gut, and expels parasites into the host.

Cardiac valve is open and cibarial valve (Civ) is closed.
The initial Leishmania infection is a complex, multistep event and involves several cell types of the innate immune system.
The promastigotes injected by the sandfly eventually end up in the macrophages. In their phagolysosomes, they proliferate as amastigote, macrophage-adapted forms.
The phagolysosome of macrophages is the final site of Leishmania proliferation (as amastigote forms)

Arrows: amastigotes

Nutrient molecules provided by the phagolysosome
The surface of Leishmania is rich in lipophosphoglycans (LPGs), and the parasites secrete numerous peptidophosphoglycans and glycoproteins whose role is still incompletely understood.
After an infection, Leishmania procyclics first encounter neutrophils. They are taken up, but the LPG on their surface inhibits the fusion of lysosomes to the late endosomes containing the parasites. This gives them time to differentiate into amastigotes. Once the neutrophils perish (after a day or so), their remnants, together with the Leishmania amastigotes, are taken up by macrophages, where the amastigotes proliferate in the mature phagolysosomes.
The first steps of the interaction of the Leishmania parasite with the host’s immune system is complex. It is important for determining the outcome of the infection.
Leishmania infections are also influenced by host genetics!

<table>
<thead>
<tr>
<th>Leishmania species</th>
<th>Pathologya</th>
<th>Region and/or population</th>
<th>Type of study</th>
<th>Candidate genes/loci tested</th>
<th>Genetic influence detected</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. (V) guyanensis ♀</td>
<td>Cutaneous</td>
<td>French Guyana/ Fimang refugees</td>
<td>32 cases/35 controls; 16 families (n=211)</td>
<td>HLA, Gm, Kp</td>
<td>HLA-Cw7 in cases/controls* Not detected in families</td>
<td>35</td>
</tr>
<tr>
<td>L. (V) braziliensis ♀</td>
<td>Viscocutaneous</td>
<td>Brazil/74% Brazilian Caucasoids, 26% mulatos</td>
<td>43 cases/111 controls</td>
<td>HLA</td>
<td>HLA-DR**</td>
<td>36</td>
</tr>
<tr>
<td>L. (V) braziliensis ♀</td>
<td>Localized cutaneous</td>
<td>Venezuela/Andean background</td>
<td>24 cases/132 controls; 26 cases/150 controls; 24 families (n=191)</td>
<td>HLA</td>
<td>HLA-DOw3 in cases/controls* (first group) HLA-Aw22 in cases/controls* (second group) Not detected in families</td>
<td>37</td>
</tr>
<tr>
<td>L. (V) braziliensis ♀</td>
<td>Localized cutaneous (n=24), mucocutaneous (n=23)</td>
<td>Venezuela</td>
<td>46 cases/13 controls</td>
<td>HLA, IL-10, TNFA, TNFB</td>
<td>HLA-DR** TNFA* TNFB*</td>
<td>38</td>
</tr>
<tr>
<td>L. chagasi ♀</td>
<td>Visceral (VL)</td>
<td>Brazil/interfered Caucasoid, Negroid and Native Indian background</td>
<td>117 nuclear families (n=53)</td>
<td>HLA</td>
<td>Not detected</td>
<td>39</td>
</tr>
<tr>
<td>L. infantum ♀</td>
<td>Mediterranean visceral</td>
<td>Tunisia</td>
<td>156 cases/154 controls</td>
<td>HLA</td>
<td>Not detected</td>
<td>40</td>
</tr>
<tr>
<td>L. donovani ♀</td>
<td>VL post Kala-azar dermal (PKDL)</td>
<td>Sudan/Mashillit</td>
<td>67 nuclear families (n=312)</td>
<td>5q22-23 and 1q21.3 (IL-4, IL-9) 6q23-q24 (FNRGR1)</td>
<td>VL-IL-4** PKDL/FNRGR2*</td>
<td>41</td>
</tr>
<tr>
<td>L. donovani ♀</td>
<td>VL</td>
<td>Sudan/Mashillit</td>
<td>67 nuclear families (n=312)</td>
<td>NRAMP1</td>
<td>NRAMP1**</td>
<td>44</td>
</tr>
<tr>
<td>L. donovani ♀</td>
<td>VL</td>
<td>Sudan/Aringa</td>
<td>48 nuclear families (n=127)</td>
<td>2q35 (NRAMP1) 5q31-q33 (TFH) 6p12 (HLA/TNRA) 6q23-q24 (TNFA) 1q15 (FNRGR1)</td>
<td>NRAMP1**</td>
<td>42</td>
</tr>
<tr>
<td>L. donovani ♀</td>
<td>VL</td>
<td>Sudan/Aringa</td>
<td>63 nuclear families (n=280)</td>
<td>Genome-wide scan (350 markers)</td>
<td>22q12***</td>
<td>43</td>
</tr>
</tbody>
</table>

Lipoldova et al., Nat. Rev. Genetics, 2006, 294

ts 02/10
Host resistance against pathogens is not based on a unique set of genes, but is highly pathogen-specific

Example: the mouse genome

- **Leishmania major**
- **Listeria monocytogenes**
- **Plasmodium chabaudi**

Lipoldová et al., Nature Rev. Genetics 7, 2006, 294
A different immune response is induced by parasites that cause the cutaneous disease (CL) and those that cause the mucocutaneous disease (MCL).

<table>
<thead>
<tr>
<th>CL (e.g. L. major)</th>
<th>MCL (e.g. L. guayanensis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasites remain at the site of the bites where the lesion develops</td>
<td>After a primary lesion (CL), infected macrophages disseminate and formation of lesions in the nasopharyngal regions (destruction of soft tissues)</td>
</tr>
<tr>
<td><strong>Inhibition of the inflammatory response</strong></td>
<td></td>
</tr>
<tr>
<td>Inhibition of the production of NO and of the activation cytokines (IL-12, TNF-α)</td>
<td>In patients, a strong inflammatory response (TNF-a, IL-6) (MCL in 3-10% of patients with CL)</td>
</tr>
<tr>
<td>Increase of the secretion of immunosuppressive molecules (TGF-β)</td>
<td>Difficult to treat: poor response to antimony (induces NO)</td>
</tr>
</tbody>
</table>

Modified from N. Fasel, 02/10
The Leishmania RNA virus Genome (LRV-1)

dsRNA linear genome of 5.3 kb with two large, overlapping ORFs on the positive strand that encode a capsid protein and RNA dependent RNA polymerase (RdRp), and possibly a small 5’-proximal ORF.
An infection with virus-infected L. guayanensis induces an array of pro-inflammatory cytokines. This is most likely due to a stimulation of the host cell’s TLR3 (toll-like) receptors by the LRV virus RNA

↑ IL6
↑ CXCL10
↑ CCL5
↑ TNFa
↑ Costimulatory molecules (CD40, CD80, CD86)
↑ IFNβ

Similar results are obtained with L. guayanensis parasites isolated from patients with mucocutaneous leishmaniasis!

Modified from Nicolas Fasel, UNIL
The toll-like receptor 3 (TLR3) in the phagolysosome recognizes the double-stranded viral RNA liberated from virus-infected, decaying Leishmania amastigotes.

Activation of the TLR3 receptor by dsRNA triggers a complex signalling cascade that results in the production of proinflammatory cytokines and apoptosis.
Efficacy of killed whole-parasite vaccines in the prevention of leishmaniasis—A meta-analysis

Sassan Noazin,*, Ali Khamesipour, Lawrence H. Moulton, Marcel Tanner, Kiumarss Nasseri, Farrokh Modabber, Iraj Sharifi, E.A.G. Khalil, Ivan Dario Velez Bernal, Carlos M.F. Antunes, Peter G. Smith

a World Health Organization, 20 Avenue Appia, CH1211, Geneva 27, Switzerland
b Center for Research and Training in Skin Diseases & Leprosy, University of Tehran/Medical Sciences, P.O. Box 14155-6383, Tehran, Iran
c Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA
d Swiss Tropical Institute, Department of Public Health and Epidemiology, P.O. Box, 4002 Basel, Switzerland
e Public Health Institute, California Cancer Registry, 3944 State Street, Suite 330, Santa Barbara, CA 93105, USA
f Drugs for Neglected Diseases Initiative (DNDi), 1 Place St Gervais, CH1201, Geneva, Switzerland
g Leishmaniasis Research Center, Kerman University of Medical Sciences, P.O. Box 444, Kerman, Iran
h Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan
i Programa de Estudio y Control de Enfermedades Tropicales, PECET, Universidad de Antioquia, Apartado Aéreo 1226, Calle 62 # 52-59 Medellín, Colombia
j Departamento de Parasitologia, Instituto de Ciencias Biologicas, Universidade Federal de Minas Gerais, Caixa Postal 486, 31270-901 Belo Horizonte, MG, Brazil
k London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Noazin et al., Vaccine 27, 2009, 4747

“... However, our meta-analysis clearly demonstrates the overall inability of first-generation leishmaniasis vaccines evaluated to date in phase 3 clinical trials to protect vaccinated individuals against infection by the *Leishmania* parasite.”
“The results reported here suggest that the killed vaccines failed in people because, while generating some correlates of immunity that may provide adequate defense against a needle inoculum, they failed to generate and/or maintain the rapid, robust response at the site of secondary challenge induced by leishmanization that is required to prevent disease following delivery of parasites by sand fly bite.”
Vaccination does not protect from Leishmania infection by a sandfly, though it does protect from Leishmania infection via a needle.

Median number of parasites 28 days after infection (per ear)

**AMC**: control mice  
**ALM**: vaccinated mice  
**Healed**: mice infected and healed
Exposure to bites by uninfected sandflies provides a much better protection against infection by an infected fly than do all currently available vaccine candidates!

Locksley R, Nature Immunology 1, 2000, 457
Animals that were preexposed to bites by uninfected sandflies show fewer parasites at the bite site when infected (Figure A)

Kamhawi et al., Science 290, 2000, 1351

Animals that were preexposed to bites by uninfected sandflies are much less infective for sandflies that bite them once they are infected (Figure B)

median number of parasites
median number of infected flies