**IMMUNITY TO PARASITIC AND FUNGAL INFECTIONS**

- Chapter 20

### Overview of parasitic diseases

- Parasites live in or on a host and cause harm to the host while they derive benefits from the host

- Specifically the Protozoa and the Helminths
  - Protozoa are single celled eukaryotic organisms
    - pathogenic protists
  - Helminths are multicellular eukaryotic organisms (worms)
    - Nematodes or roundworms
    - Trematodes or flukes
    - Cestodes or tapeworms

### Topics Covered

- Overview of parasitic diseases
- Immune response to parasitic infections
- Immunity to malaria
- Immunity to schistosomiasis
- Immune effectors in parasitic infections
- How parasites evade the immune response
- General features of fungal pathogens
- Immunity to fungal infections

### Table 20.1 Major parasitic infections of humans

<table>
<thead>
<tr>
<th>Disease</th>
<th>Major species</th>
<th>Areas of endemcity</th>
<th>Infections (millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaria</td>
<td>Plasmodium falciparum, P. vivax, P. ovale, P. malaria</td>
<td>Worldwide in tropics and subtropics</td>
<td>300</td>
</tr>
<tr>
<td>Leishmaniasis, visceral</td>
<td>Leishmania donovani</td>
<td>India, China, Africa</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Leishmaniasis, cutaneous</td>
<td>Leishmania major, others</td>
<td>Worldwide in tropics and subtropics</td>
<td>12</td>
</tr>
<tr>
<td>Trypanosomiasis, African (sleeping sickness)</td>
<td>Trypanosoma brucei</td>
<td>Sub-Saharan Africa</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Trypanosomiasis, South American (Chagas' disease)</td>
<td>Trypanosoma cruzi</td>
<td>Latin America</td>
<td>20</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>Toxoplasma gondii</td>
<td>Worldwide</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>
Overview of parasitic diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mode of transmission</th>
<th>Site of parasitism</th>
<th>Duration of infection (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaria</td>
<td>Anopheles mosquito bite</td>
<td>Erythrocytes</td>
<td>1–2</td>
</tr>
<tr>
<td>Leishmaniasis, visceral</td>
<td>Sand fly bite</td>
<td>Macrophages</td>
<td>Lifelong</td>
</tr>
<tr>
<td>Leishmaniasis, cutaneous</td>
<td>Sand fly bite</td>
<td>Macrophages</td>
<td>Lifelong</td>
</tr>
<tr>
<td>Trypanosomiasis, African</td>
<td>Tsetse fly bite</td>
<td>Bloodstream</td>
<td>Months</td>
</tr>
<tr>
<td>(sleeping sickness)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypanosomiasis, South American</td>
<td>Reduviid bug bite</td>
<td>Blood, muscle</td>
<td>Lifelong</td>
</tr>
<tr>
<td>(Chagas' disease)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>Infective stages in cat feces,</td>
<td>Many cell types</td>
<td>Lifelong</td>
</tr>
<tr>
<td></td>
<td>undercooked meat</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Protozoa are single celled eukaryotic organisms

- The most serious and intensely studied protozoal infections include:
  - Malaria
    - *Plasmodium falciparum*
  - Leishmaniasis
    - *Leishmania donovani or L. major*
  - Trypanosomiasis
    - *Trypanosoma brucei or T. cruzi*
  - Toxoplasmosis
    - *Toxoplasma gondii*

Malaria *Plasmodium falciparum*

- Leading cause of death worldwide
- Protective immunity does not develop after first episode or exposure
- only after many years of repeated exposure does individual become more resistant to infection
  - Fewer parasites in bloodstream
  - Less fever and clinical signs of disease
- No strong immunity to malaria due to
  - the tremendous strain diversity
  - and remarkable level of antigenic drift or variation
- Disease symptoms are due to intra-erythrocytic cycles of infection that result in
  - High fever
  - Anemia
  - Cerebral disease
    - Acute infection of the central nervous system
    - Leads to disorientation, delirium, coma, and death

Malaria: Life Cycle of *Plasmodium falciparum*

- Sporozoites from anopheline mosquito bite
- Invade and replicate in the liver
- Emerge as merozoites and infect red blood cells (erythrocytes)
- Get sucked back up into another anopheline mosquito during another bite
**Leishmaniasis:** *Leishmania donovani* or *L. major*

- Parasitize Mφ of skin, liver, spleen, and bone marrow
- Causes skin ulcers and permanent scars
- Cutaneous leishmaniasis is the only major human parasitic infection that there appears to be immunity to reinfection
- CMI most important -little role for antibodies
- TH1 are most critical part of CMI
  - Produce cytokines for Mφ activation
  - Destroy intracellular parasites with Nitric Oxide (NO) and reactive oxygen intermediates (ROI)

**Trypanosomiasis: Trypanosoma brucei or T. cruzi**

- *T. cruzi* causes Chagas’ disease in S. and Central America
  - Intracellular parasite infects Mφ, muscle and nerve cells
  - Transmitted by bite and subsequent contact with reduviid bug feces or through mucus membrane contamination with feces
  - Activation of Mφ and generation of NO are critical for parasite killing
  - CTL, specific antibodies and complement are important to kill infected cells and parasites directly
  - *T. cruzi* antigens cross react with human cardiac muscle and mesenteric nerve antigens causing severe damage to these host tissues
  - Acute phase of Chagas’ disease may be asymptomatic, but chronic infection can lead to cardiac arrhythmias, cardiomyopathy, or megacolon/megaesophagus.

- *T. brucei* causes African sleeping sickness
  - Strictly extracellular parasite
  - Transmitted by the bite of the tsetse fly
  - Intermittent fevers associated with antigenic drift of the parasite
  - Invasion of central nervous system leads to coma and death if untreated

**Life Cycle of *Leishmania* species**

- Promastigotes from infected female sand fly bite enter wound
- Activate complement and are taken up by Mφ
- Inside the phagolysosome they transform to amastigotes and replicate -filling the cytoplasm
- They rupture the Mφ and are taken up by new Mφ
- Get sucked back up into another sand fly during another bite and develop into infectious promastigotes

**Trypanosomiasis: Life Cycle of *T. cruzi***

- Trypomastigotes from infected hematophagous reduviid bugs bite enter wound from bug feces (or infect eyes from contaminated hands)
- Infect a wide variety of cells and cause an inflammatory lesion or chagoma at site of parasite entry
- Inside the host cells they transform to amastigotes and replicate -filling the cytoplasm
- They rupture the host cells and are released to infect new host cells
- Get sucked back up into another reduviid bug during another bite and develop into infectious trypomastigotes
**Toxoplasmosis: Toxoplasma gondii**

- Intracellular pathogen that can infect virtually any warm-blooded animal
  - Where asexual reproduction occurs and pseudocyst formation occurs
- The definitive host is the cat (wild and domestic)
  - Where sexual reproduction and egg production of parasite occurs
- Other hosts become infected upon ingestion of eggs found in cat feces or eat undercooked meat of an infected animal
- Normally remains in encysted in CNS
- Infection is very common, 1/3 of the world has been exposed
- Generally asymptomatic, but 2 high risk groups
  - Immunocompromised and pregnant women
    - Toxoplastic encephalitis in HIV+
  - Primary infection during pregnancy can lead to infected fetus and spontaneous abortion or congenital disease including mental retardation and blindness

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**Table 20.1 Major parasitic infections of humans**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Major species</th>
<th>Areas of occurrence</th>
<th>Infections (millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Toxoplasma</em></td>
<td><em>Toxoplasma gondii</em></td>
<td>Worldwide in tropics and sub tropics</td>
<td>1,000</td>
</tr>
<tr>
<td><em>Hookworms</em></td>
<td><em>Ancylostoma duodenale</em></td>
<td>Worldwide in tropics and sub tropics</td>
<td>900</td>
</tr>
<tr>
<td><em>Trichuriasis</em></td>
<td><em>Trichuris trichiura</em></td>
<td>Worldwide in tropics and sub tropics</td>
<td>500</td>
</tr>
<tr>
<td><em>Enterobius</em></td>
<td><em>Enterobius vermicularis</em></td>
<td>Worldwide in tropics and sub tropics</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Strongyloides</em></td>
<td><em>Strongyloides stercoralis</em></td>
<td>Sub-Saharan Africa, Central and South America</td>
<td>20</td>
</tr>
<tr>
<td><em>Trichinella</em></td>
<td><em>Trichinella spiralis</em></td>
<td>Worldwide in tropics and sub tropics</td>
<td>&gt;200</td>
</tr>
<tr>
<td><em>Schistosomiasis</em></td>
<td><em>Schistosoma mansoni</em></td>
<td>Africa, Asia, South America, East and Southeast Asia</td>
<td>&gt;200</td>
</tr>
<tr>
<td><em>Filaria</em></td>
<td><em>Brugia malayi</em></td>
<td>Worldwide</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

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**Toxoplasmosis: Life Cycle of Toxoplasma gondii**

- Pseudocysts from meat or eggs from cat feces are ingested
- Become tachyzoites and infect and replicate in any nucleated cell
- Immune system clears tachyzoites but some transform into bradyzoites and form pseudocysts to evade T cell response
- Tachyzoites can infect the fetus, cause damage, and form pseudocysts here as well

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**Overview of parasitic diseases**

**Table 20.3 Major parasitic infections of humans**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mode of transmission</th>
<th>Site of parasitism</th>
<th>Duration of infection (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hookworms</em></td>
<td>Infective larvae in fecally contaminated soil</td>
<td>Small intestine</td>
<td>1–2</td>
</tr>
<tr>
<td><em>Trichuriasis</em></td>
<td>Infective eggs in fecally contaminated soil</td>
<td>Colon and rectum</td>
<td>3</td>
</tr>
<tr>
<td><em>Enterobius</em></td>
<td>Infective eggs in fecally contaminated soil</td>
<td>Subcutaneous</td>
<td>&gt;10</td>
</tr>
<tr>
<td><em>Toxocara</em></td>
<td>Larval bite</td>
<td>Subcutaneous</td>
<td>&gt;10</td>
</tr>
<tr>
<td><em>Filaria</em></td>
<td>Larval bite</td>
<td>Subcutaneous</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

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**Overview of parasitic diseases**
Helminths are multicellular eukaryotic organisms (worms)

- Trematodes or flukes
  - Schistosomiasis = Bowel, Bladder, and Liver Infection
    - Schistosoma mansoni or S. japonicum, S. haematobium, and ALL three res.

- Nematodes or roundworms
  - Lymphatic Filariasis = Intravascular Infection - “elephantitis”
    - Wuchereria bancrofti and Brugia species
  - Intestinal Nematodes
    - Trichuris trichiura

**Trematodes or flukes: Schistosomiasis**

- Caused by highly immunogenic eggs deposited in tissues
- Triggers chronic inflammation and immunopathic reactions
- T cell-mediated host reaction to eggs results in granuloma formation
- Cause hepatic fibrosis = liver scarring
- Portal hypertension = high blood pressure in liver vein
- Bladder and urinary tract fibrosis
- No strong immunity to schistosomes
- Decreased susceptibility to additional infection of adults
  - Selective immunity to the larvae
  - Does not decrease number of adult worms in residence
  - Adult worms induce immune response that are protective against the larvae but not the adult worms themselves

**Nematodes or roundworms**

- Lymphatic Filariasis = Intravascular Infection - “elephantitis”
  - Wuchereria bancrofti and Brugia species
  - Damage to lymphatic vessels
  - Episodic lymphatic inflammation, pain, and fever
  - Adult worms can cause lymph stasis and gross enlargement of the limbs, scrotum, or breast
  - Lymphatic lesions likely caused by mechanical damage by worms and immune mediated inflammatory responses

- Onchocerciasis = Tissue Infection - “river blindness”
  - Onchocerca volvulus
  - Severe eye disease and dermatisis in Africa and Central America
  - Transmitted by black flies near fast flowing waters
  - Adult worms reside in cutaneous tissues
  - Microfilariae migrate to ocular tissues
  - Disease is caused by immune responses to the circulating Microfilariae
Lymphatic Filariasis: Life Cycle of *Wuchereria bancrofti*

- Infective larvae from Culex, Anopheles, and Aedes mosquito bite
- Migrate to lymphatic vessels and lymph nodes where they mature into adult worms
- Adults produce offspring or microfilariae which enter the bloodstream
- Get sucked back up into another mosquito during another bite
- Chronic inflammation and vessel damage cause pathology

Nematodes or roundworms

**Intestinal Nematodes**
- *Ascaris lumbricoides*: hookworm, and *Trichuris trichiura*
  - Infect 1/4 of World’s people
  - Do not cause overt clinical problems in most cases
  - Problems with massive infections or inappropriate immune response

**Tissue Nematodes**
- *Trichinella spiralis*
  - Larvae become encysted in striated muscle
  - Eat undercooked meat, larve hatch, migrate to gut, mature into adult worms, lay eggs and larvae migrate to muscle

Both provoke TH2 immune response
- with cytokines IL-4, IL-9, and IL-13
- important for expelling worms from the gut

T cells and Cytokines Regulate Immune Responses in Parasitic Infections

- T cells are critical to control all parasitic infections
- Do not provide protective immunity against reinfection
- Are required to control parasitic infection
- Genetically deficient mice lacking B and T cells and athymic mice with few T cells are
  - unable to control infections and
  - develop overwhelming parasite burdens that are fatal
T cells and Cytokines Regulate Immune Responses in Parasitic Infections

- Development of tissue injury is often due to
  - Inappropriate immune response
  - rather than insufficient immune response

- T cells are usually responsible for immune mediated injury and disease progression

- TH cell subsets (TH1 and TH2) were discovered through
  - dissecting disease resolution versus disease progression
  - and immune-mediated injury in parasitic infections

Table 20.2 The potential role of T-cell subsets in parasitic diseases

<table>
<thead>
<tr>
<th>Parasite</th>
<th>T-cell subset(s)</th>
<th>Potential function in disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leishmania major</em></td>
<td>TH1</td>
<td>Protection</td>
</tr>
<tr>
<td><em>(murine)</em></td>
<td>TH2</td>
<td>Exacerbation</td>
</tr>
<tr>
<td><em>Trypanosoma cruzi</em></td>
<td>CD8⁺</td>
<td>Protection</td>
</tr>
<tr>
<td><em>Plasmodium</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver stage</td>
<td>CD8⁺</td>
<td>Protection</td>
</tr>
<tr>
<td>Cerebral</td>
<td>TH1, TH2</td>
<td>Immuneopathology</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>CD8⁺</td>
<td>Protection</td>
</tr>
<tr>
<td><em>Helminths</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>TH1, TH2</td>
<td>Protection</td>
</tr>
<tr>
<td><em>(murine)</em></td>
<td>Immuneopathology</td>
<td></td>
</tr>
<tr>
<td><em>Trichuris muris</em></td>
<td>TH1, TH2</td>
<td>Protection</td>
</tr>
<tr>
<td><em>(murine)</em></td>
<td>Protection</td>
<td></td>
</tr>
</tbody>
</table>

*The association of specific T-cell subsets with disease outcome derives from animal models and is not always mutually exclusive and generally is not as clear cut in human infections.*

TH cell subsets and their cytokines regulate immune responses to parasites

- Expansion of TH1 or TH2 cells in response to infection is dependent on
  - Nature of the invading organism
  - Genetics of the host

- Host genetic importance demonstrated in inbred mouse strains where difference in genetic background can determine whether an infection is
  - Harmless or Lethal

- First shown with *Leishmania*
  - Induction of TH1 leads to disease resolution
  - Induction of TH2 leads to disease progression

TH cell subsets and their cytokines regulate immune responses to parasites

- Many Studies have shown:
- Activation of TH1 lymphocytes is usually necessary for the destruction of protozoa
- Activation of TH2 lymphocytes occurs in Helminth infections
  - Characterized by increase in eosinophils and mast cells
  - and upregulated IgE levels
- TH2 bias has been studied in murine models and humans infected with schistosomes or filarial nematodes
- For tissue helminths, it is unclear whether the TH2 response benefits the host or the parasite
- For Gut nematodes, it is clear that TH2 cytokines are necessary for clearance of the parasites
**IL-12 drives TH1 responses**

- IL-12 initiates TH1-dependent cell-mediated immune responses
- IL-12 is produced by phagocytic cells and B cells
  - in response to infection with protozoan parasites
- IL-12 is a critical component in the early response to infection that drives TH1 cell expansion
- IL-12 directly stimulates the production of IFN-γ
  - by T cells and NK cells

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**IL-12 and parasite infection**

- Helminthic antigens induce the production of IL-4
- IL-4 drives TH2 development
- Helminthic parasites do not induce production of IL-12
- and may directly block production of IFN-γ
- TH2 cells produce IL-10 which directly blocks IL-12 production
- Intracellular parasites in Mϕ activate the production of IL-12
- Induces production of IFN-γ by T cells and NK cells
- Immediately activates more Mϕ
- IL-12 and IFN-γ favor development of more TH1 cells
- IL-12 and IFN-γ favor the additional production of IFN-γ by established TH1 cells

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**IL-12 and parasite infection**

- When exogenous IL-12 is added to helminthic infections:
  - Normal production of TH2 cells is prevented
  - TH1 cell development follows pathway of protozoan parasitic infection
IFN-γ

- For intracellular protozoan parasites:
  - IFN-γ is the major cytokine responsible for disease resolution
  - Because it activates macrophages

- Inbred mouse strains BALB/c and C57BL/6
  - BALB/c: produces IL-4 and TH2 response to L. major
  - C57BL/6: produces IL-12 and TH1 response to L. major

- TH2 is not protective and mice die (no IFN-γ)

- TH1 is protective and mice clear infection and remain resistant to reinfection (yes IFN-γ)

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IFN-γ

- BALB/c: IL-4/TH2 response/no IFN-γ/death
- C57BL/6: IL-12/TH1 response/yes IFN-γ/lives

- Give BALB/c mice anti-IL-4 antibody
  - Binds up all IL-4 so it cannot work
  - No TH2 response that usually downreg TH1 response
  - Get a TH1 response and yes IFN-γ
  - Mice live and are resistant to reinfection

- Give C57BL/6 mice anti-IFN-γ antibody
  - Binds up all IFN-γ so it cannot activate Mφ
  - No TH1 response to downreg TH2 response
  - Mice die

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IL-4 initiates TH2 response to Helminths

- Hallmarks of helminthic infection:
  - Elevated IgE
  - Elevated eosinophils in blood and tissue
  - Mast cell hyperplasia

- Above are induced by TH2 responses

- IL-4 is critical for inducing TH2 response

- Cells that can produce IL-4 include specialized T cells like NK1.1 and γδ, mast cells, basophils, and eosinophils

- After initial induction, TH2 cells take over IL-4 production
  - And IL-5, IL-6, IL-9, and IL-13

- It is not yet known what helminthic signal caused initial production of IL-4

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TH2 responses to Helminths clears parasites

- BALB.K: IL-4 / TH2 response / no IFN-γ / clears parasite
- B10.BR: IL-12 / TH1 response / yes IFN-γ / chronic infection

- Give BALB.K mice anti-IL-4 antibody
  - Binds up all IL-4 so it cannot work
  - No TH2 response that usually downreg TH1 response
  - Get a TH1 response and yes IFN-γ
  - Mice have chronic infection

- Give B10.BR mice anti-IFN-γ antibody
  - Binds up all IFN-γ so it cannot activate Mφ
  - No TH1 response to downreg TH2 response
  - Mice clear the parasites

TH2 responses to Helminths

- Universal feature of worm expulsion (nematode species) is the TH2 response

- The specific TH2 cytokines that are needed for expulsion differ for different parasite species

- Represents different effector mechanisms that mediate the expulsion of the different parasite species