به نام آنکه جان را فکرت آموخت

چراغ دل به نور جان بر افروخت

Vaccine development: from bench to phase 4

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2–3 million deaths each year

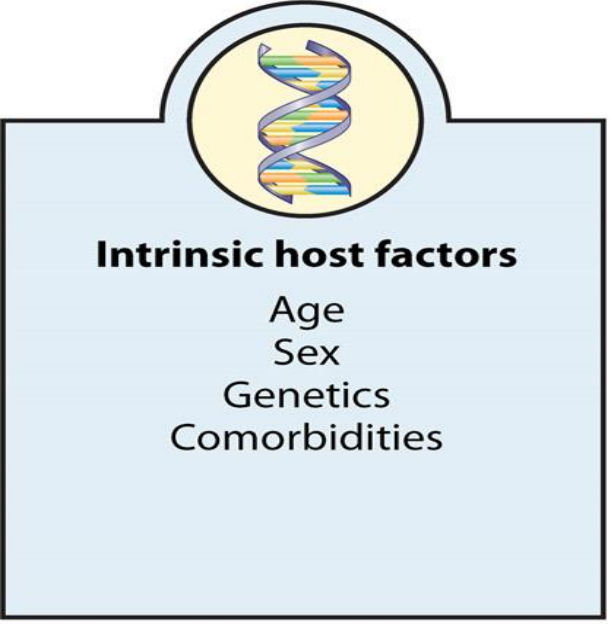
Eradicated smallpox, Reduced the prevalence of diseases like polio

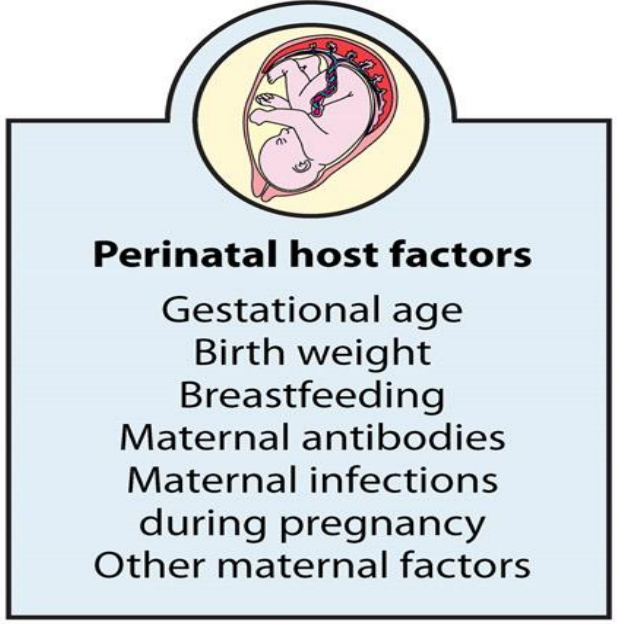
Brucellosis, Listeriosis, Salmonellosis, Q fever, Hepatitis A, Hepatitis E, Vibrio spp. Paragonimiasis, Clonorchiasis, Campylobacter spp., Pathogenic E. coli and other bacterial infections, Trichinosis, Toxoplasmosis, Cysticercosis, Fascioliasis, Malaria, Dengue fever, Chikungunya, Zika virus, Viral haemorrhagic fevers, Lymphatic filariasis, Japanese encephalitis, Yellow fever, Rickettsial spotted fevers, Lyme disease, Ehrlichiosis,

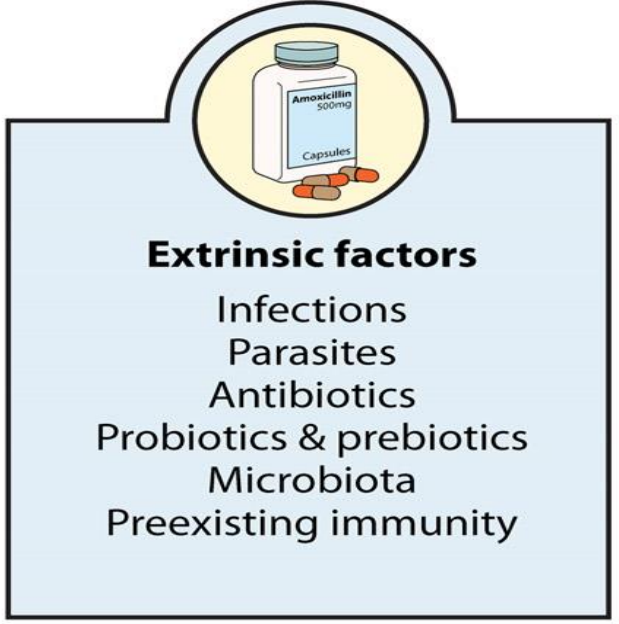
Babesiosis, Tularaemia, Congo-Crimean fever, Tick-borne encephalitis, Endemic relapsing fever, Tsetse fly African trypanosomiasis, Triatomine (‘kissing’) bugs, American trypanosomiasis, Sand flies Bartonella bacilliformis, Leishmaniasis (cutaneous and visceral), Sand fly fever, Murine typhus (Rickettsia typhi), Cat-scratch (Bartonella henselae), Mites Scrub typhus (Orientia tsutsugamushi), Black flies Onchocerciasis, Lice Epidemic relapsing fever, Epidemic typhus (Rickettsia prowazekii), Trench fever (Bartonella quintana), Horse and deer flies Loiasis

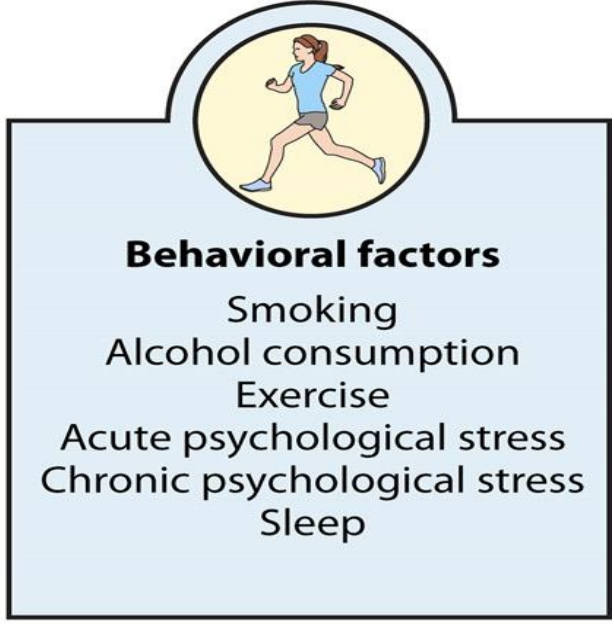
Schistosomiasis, Leptospirosis, Free living amoebae, Coccidioido mycosis, Paracoccidioidomycosis, Blastomycosis, Talaromyces (formerly Penicilliosis), Melioidosis, Histoplasmosis, Strongyloidiasis, Cutaneous larva migrans, Anthrax, Tularaemia, Rabies,

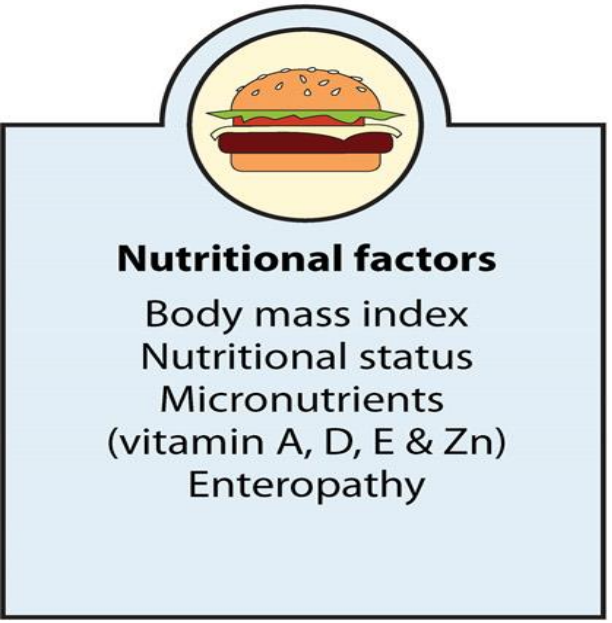
HIV, hepatitis B and C, syphilis, gonorrhoea, chlamydia, etc.

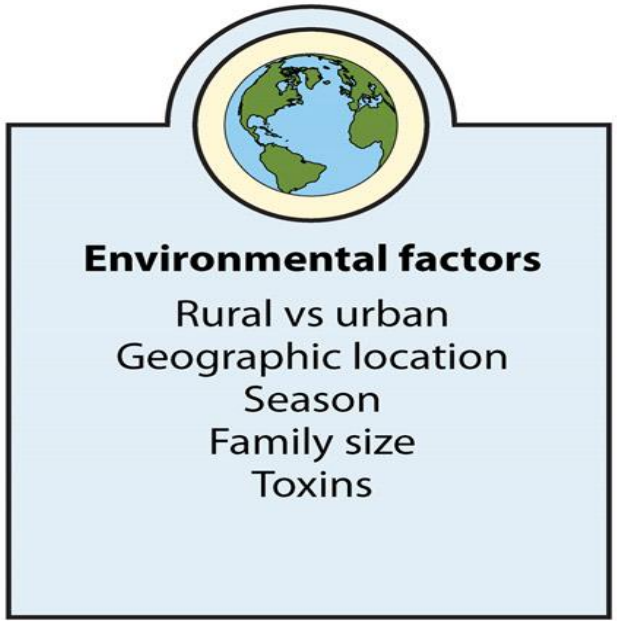


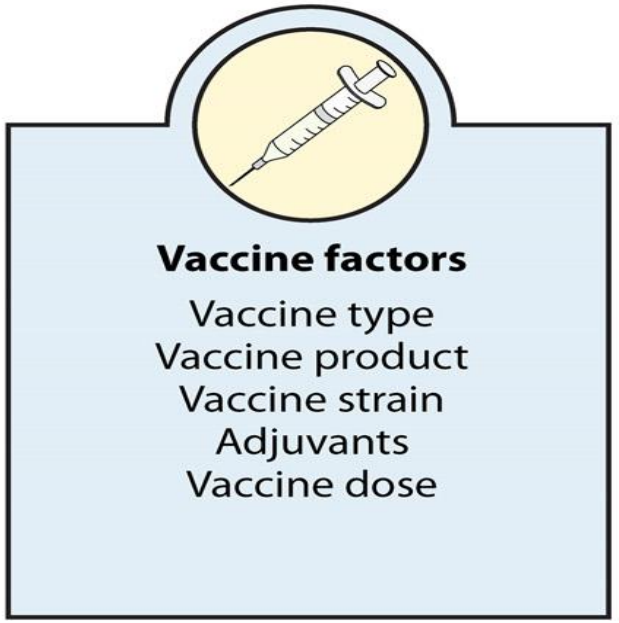


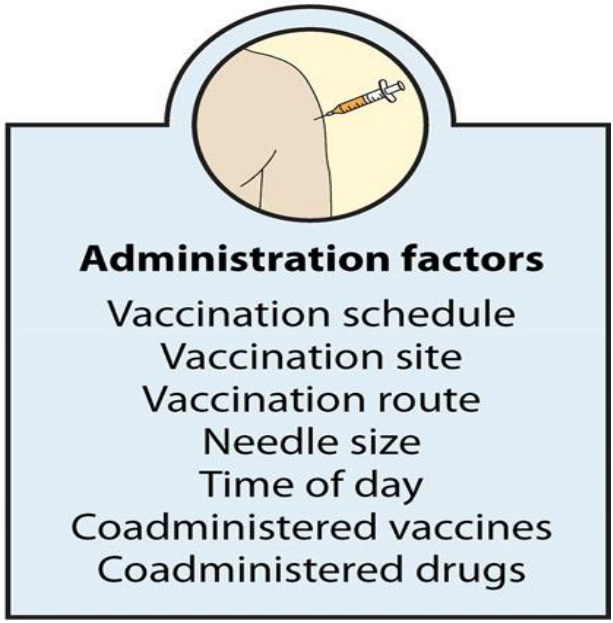












Zimmermann P, Curtis N. Factors That Influence the Immune Response to Vaccination. Clin Microbiol Rev. 2019 Mar 13;32(2):e00084-18.
doi: 10.1128/CMR.00084-18. PMID: 30867162; PMCID: PMC6431125

Factors affect vaccine efficacy?

- Intrinsic Host Factors
- Age, Sex, Genetics, Comorbidities, Perinatal Host Factors, Gestational age, Birth weight, Breastfeeding, Maternal antibodies , Maternal infections during pregnancy, Other maternal factors
- Extrinsic Factors
- Infections, Parasites, Antibiotics, probiotics and prebiotics,
- Microbiota
- Preexisting immunity, Behavioral Factors, Smoking, Alcohol consumption, Exercise, Acute psychological stress,
- Chronic psychological stress, Sleep, Nutritional Factors, Body mass index, Nutritional status, Micronutrients (vitamins A, D, and E and zinc), Enteropathy, Environmental Factors, Rural versus urban environment, Geographic location, Season, Family size, Toxins
- Vaccine Factors
- Vaccine type, product, and strain, Adjuvants, Vaccine dose,
- Administration Factors
- Vaccination schedule, Vaccination site, Vaccination route, Needle size, Time of day, Co-administered vaccines, Co-administered drugs

Reasons to develop a vaccine (1)

- Drugs
  - Unsatisfactory
  - Too expensive
  - Side effects
  - Etc….
- Control measures
  - Impractical
  - Unsafe for environment
- Reservoir control: impossible or impractical
- Etc

Reasons to develop a vaccine

Benefits: **- Protection against disease**

- Improved protection

- Fewer adverse events

- Lower cost / higher coverage

- Change the route

Risks: **- More (severe) adverse events**

- Lower protection

- Change epidemiology

OVER VIEW

- Drugs unsatisfactory or too expensive
- Vector control impractical, unsafe for environment
- Reservoir control: impossible or impractical

Short term success (China, S. Arabia, India, but…)

- VACCINE IS THE BEST SOLUTION

Why there is a need for Leishmania Vaccine?

- Efficacy of 10 and 65% at 4 and 8 weeks after initiation of treatment with Glucantime.
- Efficacy based on complete re-epithelialization and elimination of induration of all lesions was about 20% at 12 weeks after the initiation of treatment (*L. tropica*).
- Only 20% complete healing was seen at 6 weeks of initiation of weekly Intralesional injection of Glucantime (*L. major*)

Why vaccine is feasible?

- After recovery of CL, 90-95% are protected against re-infection
- Strong immune response developed after recovery

  in vivo (LST) **90-95%**

  in vitro (LTT, IFN-γ **80-85%**, etc.)
- Leishmanization is an effective prophylactic tool against CL
- Protection in animal models: mice, hamsters & non-human primates
- *Leishmania: e*asy to grow and genetically manipulation is

Types of Vaccines

A. 1st generation vaccines

- Attenuated live
- Killed parasites
- Parasites' fractions
- Toxoid, purified proteins
- Not well defined antigens

B. 2nd generation vaccines

- Recombinant protein antigens
- Well defined antigens

C. 3rd generation vaccines

- DNA, mRNA

Challenges of vaccine

Estimates:

For prophylactic vaccine development …….. $150 – 500 Million, 10-15 years, the fastest 5 years

Discovery	Preclinical	Clinical	Registration	Post-registration
5-10% 10 M	10-30% 20 M	60-80% 165 M	0.5% 1 M	1.5-3% 4 M

Pre-clinic of Drugs & Vaccines

Pre-discovery

- Goal: Understand the disease and choose a target molecule.
- How: Scientists in pharmaceutical research companies, government, academic and for-profit research institutions contribute to basic research.

Discovery

- Goal: Find a drug candidate.
- How: Create a new molecule or select an existing molecule as the starting point.
- Perform tests on that molecule and then optimize (change its structure) it to make it work better.

Preclinical

- Goal: Test extensively to determine if the drug is safe enough for human testing.
- How: Researchers test the safety and effectiveness in the lab and in animal models (mice, rat, rabbit and guinea pigs).

Every drug has benefits and risks

Requirements for clinical evaluation

Pre-clinical evaluation:

- Immunological principle,
- Immuno/safety data from animal model,
- Develop and validation of lab methodology for clinical immunology (ELISA, CMI,…..)

Safety procedures:

- Consultation with safety board

Regulatory requirements:

- Country specific (MoH, CTX, IND in US etc)

Study specific procedures:

- ERC approval, ICH guidelines, GCP

Preclinical (*Drugs* or *Vaccines*)

Chemical and or Biological Synthesis under GLP in certified premises

- **Procedures (SOP)**
- **Source of materials from the origin**
- **Animal products – certificates**
- **Maintenance of material (recorded)**
- **Reproducibility (yield, physical/chemical characteristics >3 lots)**
- **Purity**
- **Stability**
- **QA, QC (recorded)**

Drugs or *Vaccines*

Use GLP lots for

- **Potency**
- **Efficacy in Exp. Animals**
- **Toxicology**
- **Accelerated Stability**
- **Product Master file for clinical study**
- **Clinical Development Plan**

Preclinical

- **Exploratory Phase-1**
- **Phase-1**
- **GMP production is needed for Phase 2 and beyond**

Clinical

Exploratory IND studies to select the best lead

- **Very limited human exposure**
- **No therapeutic or diagnostic intent**
- **Compare animal studies with humans (e.g., a binding property or inhibition of an enzyme)**
- **Information on pharmacokinetics (PK)**
- **Explore a product's bio distribution with imaging technologies**
- **A limited number of subjects with a limited range of doses for a limited period of time.**
- **More flexibility with regard to the preclinical testing requirements**

Phase 1

- Safety trial of a new drug/antigen or a new adjuvant
- Small number healthy volunteers (~20-100)
- Usually adults, even for pediatric vaccines
- Healthy adults volunteers (hospital)
- Outside endemic areas (and in endemic areas)
- Dose needed for adequate immune response
- Clinical tolerability (reactogenicity profile)
- Safety assessments (biochemistry, hematology, urine analysis)

Phase 2 (phase2a, 2b)

- **Immunogenicity/Pharmacokinetics**
- **Trials in different target populations**
  - **infants, children, adolescents, adults, elderly**
  - **at risk groups, patients …**
- **Small number of subjects (~100-300)**
- **Evaluation of doses of drug/vaccine, schedule, vaccine formulation, route of administration, co-administration with other vaccines**
- **Immunogenicity/Pharmacokinetics (antibody persistence, reactogenicity and tolerability)**
- **Randomized double-blind placebo-controlled**

Phase 3

- **Efficacy trial**
  - **Population-based trials**
  - **Required a large number of participants (hundreds/thousand)**
  - **Evaluation of efficacy in the target population (Population at risk)**
  - **Randomized double-blind placebo controlled**
  - **Comparison with the best treatment available**
  - **Primary and secondary endpoints**
  - **Monitoring of rare side-effects**

Clinical evaluation of a vaccine

Objectives of the clinical evaluation:

- **To document the efficacy**
- **To evaluate possible risks**

Phases of vaccine/drug trials*
Phase IV

- **After registration, used in routine conditions**
- **Rare side-effects**
- **Epidemiological impact of vaccination/mass treatment**
- **Study design (case-control, stepped wedge, trend analysis)**
- **Cost-effectiveness analysis in different conditions**
- **Evaluation under different transmission pressure***
- **Long-term immune response vs. protection***
- **Predictions / modelling transmission vs. protection***
- **Social acceptability if repeated vaccination/treatment is required**
- **Duration of protection***
- **Efficacy in different subgroups of the population***
- **Effect on less common endpoints:**
  - **severity of disease**
  - **mortality**
- **Efficacy in different epidemiological conditions**

Types of vaccines

Historical and still the best (Leishmanization)

- **Live virulent *L. major***
- **Produce a self-healing lesion and immunity in endemic foci (Uzbekistan)**
- **Historical: Iran, Occupied Palestinian territories**

First Generation Vaccines
Ready to be tested in humans (decades ago)

Killed whole Parasite with /without adjuvants used in Humans (BCG, BCG+Alum)

Second/third Generation Vaccines
Mostly in discovery (one in development)

- **Recombinant proteins plus new adjuvants**
- **DNA vaccines**
- **DNA prime protein boost**

First Generation Vaccines

Killed whole Parasite with/without adjuvant in humans

- **Ready to be tested in humans.**
- Possible to produce locally in the developing countries
- Affordable
- Safety information
  - Early pioneering trials in Brazil 1930's (No GCP, GMP!)
  - Therapeutic trials in Venezuela (No GCP, GMP!)
  - Leishmanization experiences
  - Killed *Leishmania* had been used for decades for skin test (leishmanin)

Started development of GMP products in Brazil and Iran

Killed vaccines (1)

1. **Efficacy of killed whole-parasite vaccines in the prevention of leishmaniasis—A meta-analysis**

Sassan Noazina, Ali Khamesipour, Lawrence H. Moulton, Marcel Tannerd, Kiumarss Nasseri, Farrokh Modabber, Iraj Sharifig, E.A.G. Khalil, Ivan Dario Velez Bernal, Carlos M.F. Antunes, Peter G. Smith, Vaccine 27 (2009) 4747–4753

2. Vaccines for preventing cutaneous leishmaniasis
Narges Khanjani, Urbà González, Jo Leonardi-Bee, Mehri Saffari, Ali Khamesipour
Review number: #99 28,297 participants

Killed vaccines (2)

11 clinical trials are included,

Number of participants: 22,683 and 28,297

New World

Vaccine prepared from;3-strains (*L. guyanensis*, *L. brasiliensis*, *L. amazonensis*), 5-strain vaccine (species of *brasiliensis* and *mexicana* complexes, including *L. guyanensis and L. amazonensis*)

Single strain *L. amazonensis*

Against L. panamensis, L. guyanensis, *L. brasiliensis*, and *L. amazonensis*

Old World

Vaccine prepared from *L. major* Against *L. major, L. tropica, L. donovani*

Outcome: LST conversion 0-68% Efficacy -14 to 72%

Canine Experiment in Iran

ALM

- **1. Experimental**

- G1: *L.major* + BCG
- G2: *L. infantum* + BCG
- G3: BCG alone
- G4: PBS (4 dogs per group)

Challenge 2.5 X 10^6 L. infantum (IP)

G1 75% protection,G2 100% protection

- **2. Field trial**

- 119 dogs were vaccinated double blind randomly (four groups as above)
- ~ %20 protection

Mohebali, et al Arch. Razi, Inst.50, 87 – 92, 1999.

Leishmaniasis vaccine clinical trials

- Previous exposure of the volunteers
- Endemicity is not uniform in the whole site
- Time of exposure
- Site of inoculation
- Number of Parasite inoculated

Leishmanization

Historical and still the most effective (Leishmanization)

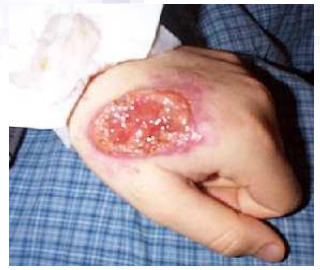
- **Live virulent *L. major***
- **Produce a self-healing lesion and immunity in endemic foci Historical:**
  - **Iran**
  - **Occupied Palestine**
  - **Uzbekistan**

What is leishmanization?

- Inoculation of virulent *L. major*
- At a predetermined site of the body
- Produce a self-healing lesion
- Subsequently LZ individuals are protected against multiple lesions at exposed parts of the body especially on the face

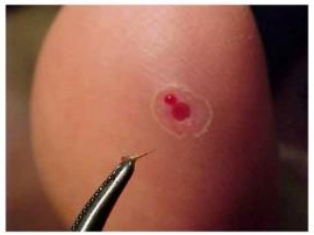
Sand fly

I



Scratch of the Lesion

II

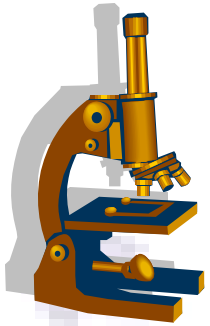


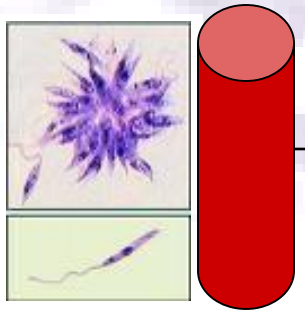
Scratch

New Host(s)

Expose rear end

III





Leishmanization in Iran

More than 2 million during 1982-1986

- **Soldiers and civilians adults and children**

  160,000 in the Isfahan area, 1,800,000 military personnel, 6,000 refugees, etc.

  - **Inoculated with** $2\text{-}3 \times 10^5$

  - Incubation period varies from 1-14 months

  - **Take rate varies 45% - 60% and even 15%**

  - Healing period was between 3-14 months

    - 1-3% active lesion at 2 year

    Late Nadim A., *et al.*

Efficacy of Leishmanization in Iran

Prevalence rate

0.8% for takes

10.5% for "non-takes"

40% for controls not leishmanized

Leishmanization during the Iran – Iraq war, Late Nadim, et al.

Challenges in Leishmaniasis Vaccine Development

Host

Epidemiological diversity nature of the leishmaniasis

Lack of information of immune response (ZCL, ACL, VL)???

Surrogate marker(s) of protection???

- o After recovery of CL, 90-95% are protected against re-infection
- o Strong immune response developed after recovery
  - *in vivo* (LST) 90-95%
  - *in vitro* (LTT, IFN-γ 80-85%, etc.)

No Suitable Adjuvant

No appropriate animal model

Challenges in Leishmaniasis Vaccine Development

Parasite

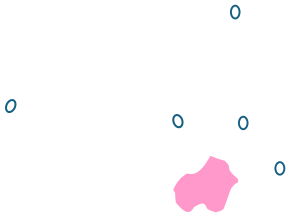
- Standardization
- Any correlate of virulence/pathogenicity
- Culture media with no serum
- Lack of an appropriate adjuvant
- Preservatives

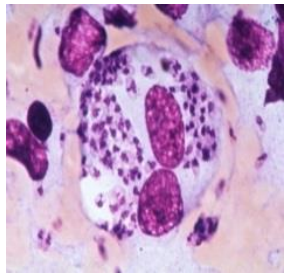
Evaluation

- Phase 1-2 in non endemic area
- Phase 3 in endemic area
- Exposure to sand fly bite

Leishmaniasis vaccine trials

- Endemicity in different location was not similar
- Time of exposure
- Inoculum site
- Inoculum No of parasites
- Time of exposure
- etc



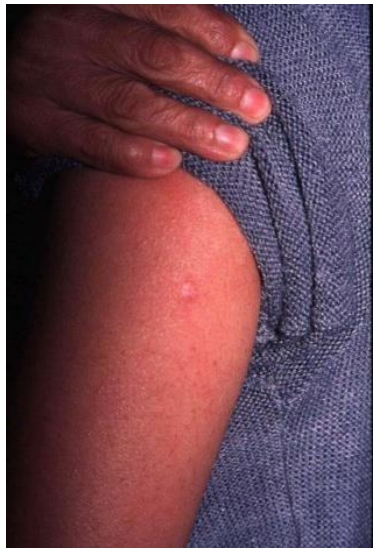


IF-γ
IL-12
No Ab
DTH^{+}

Prophylactic

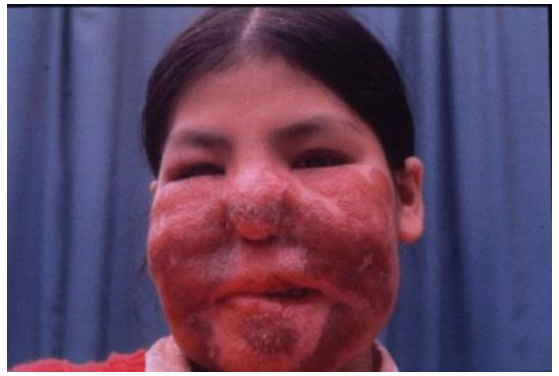
Th1

Th2



IL-4
IL-5
IL-10
High titre Ab
DTH$^{+/-}$

DTH $^{++}$

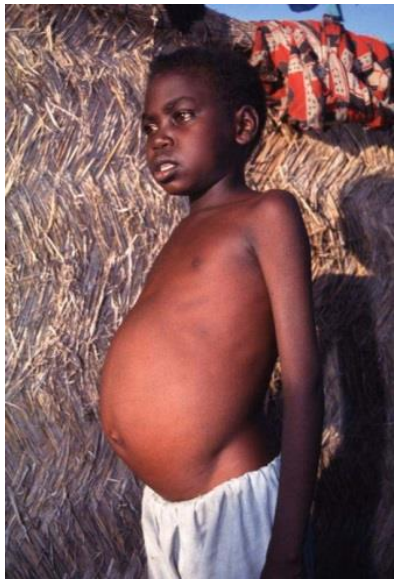


+

Th1

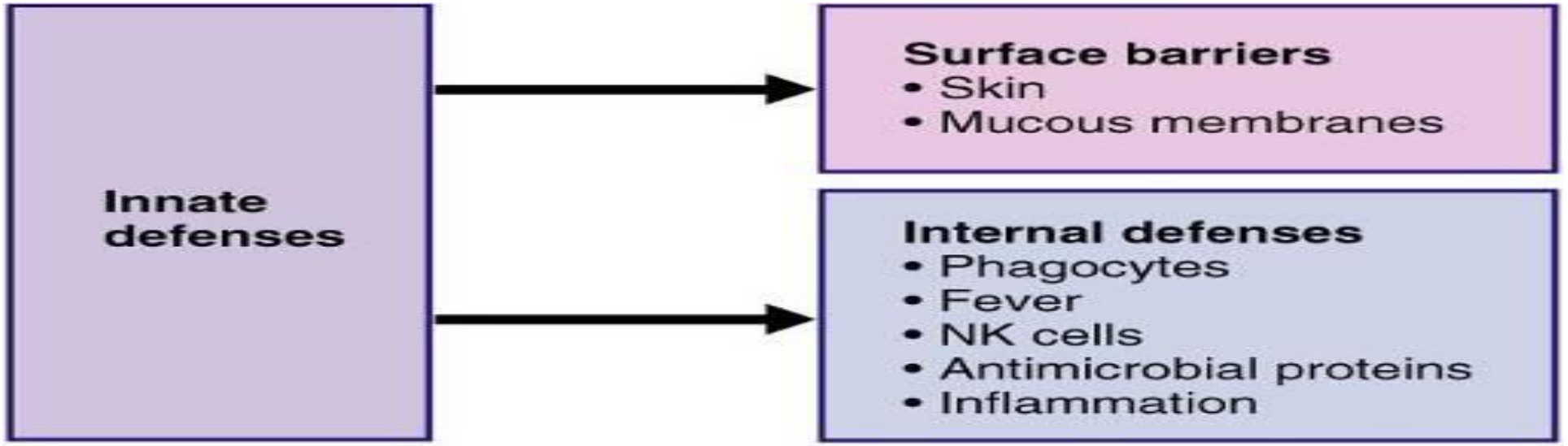
Therapeutic

Th2

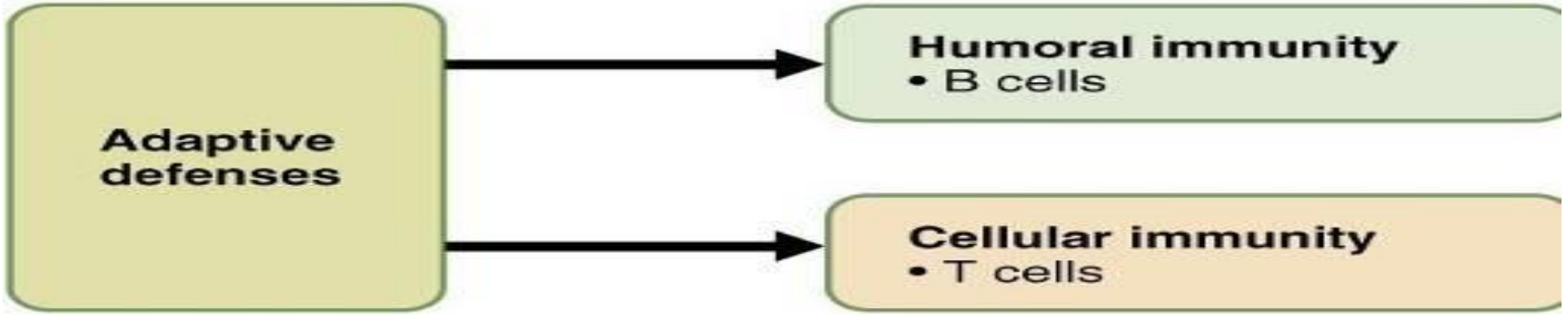


DTH $^{-}$

Immune System

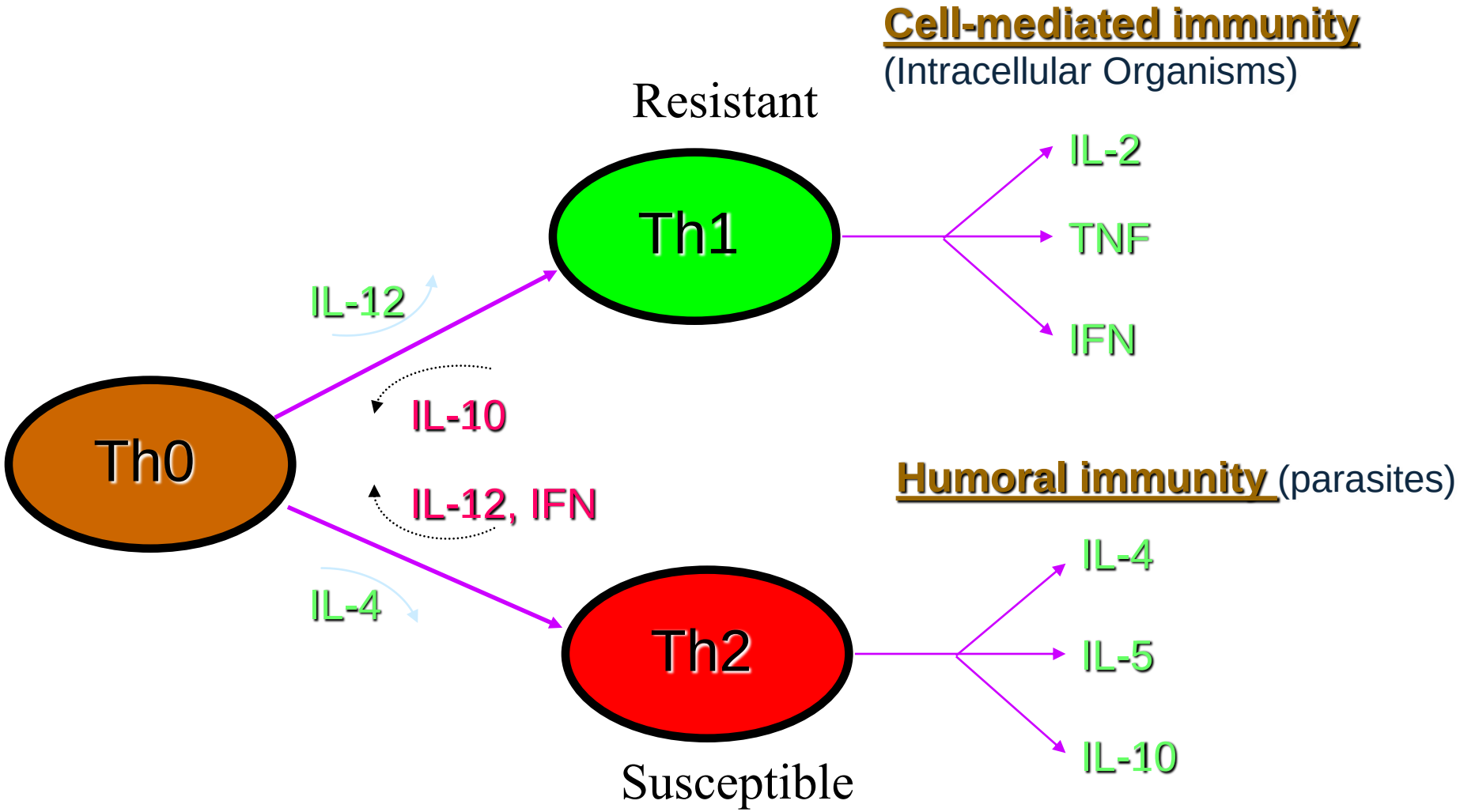


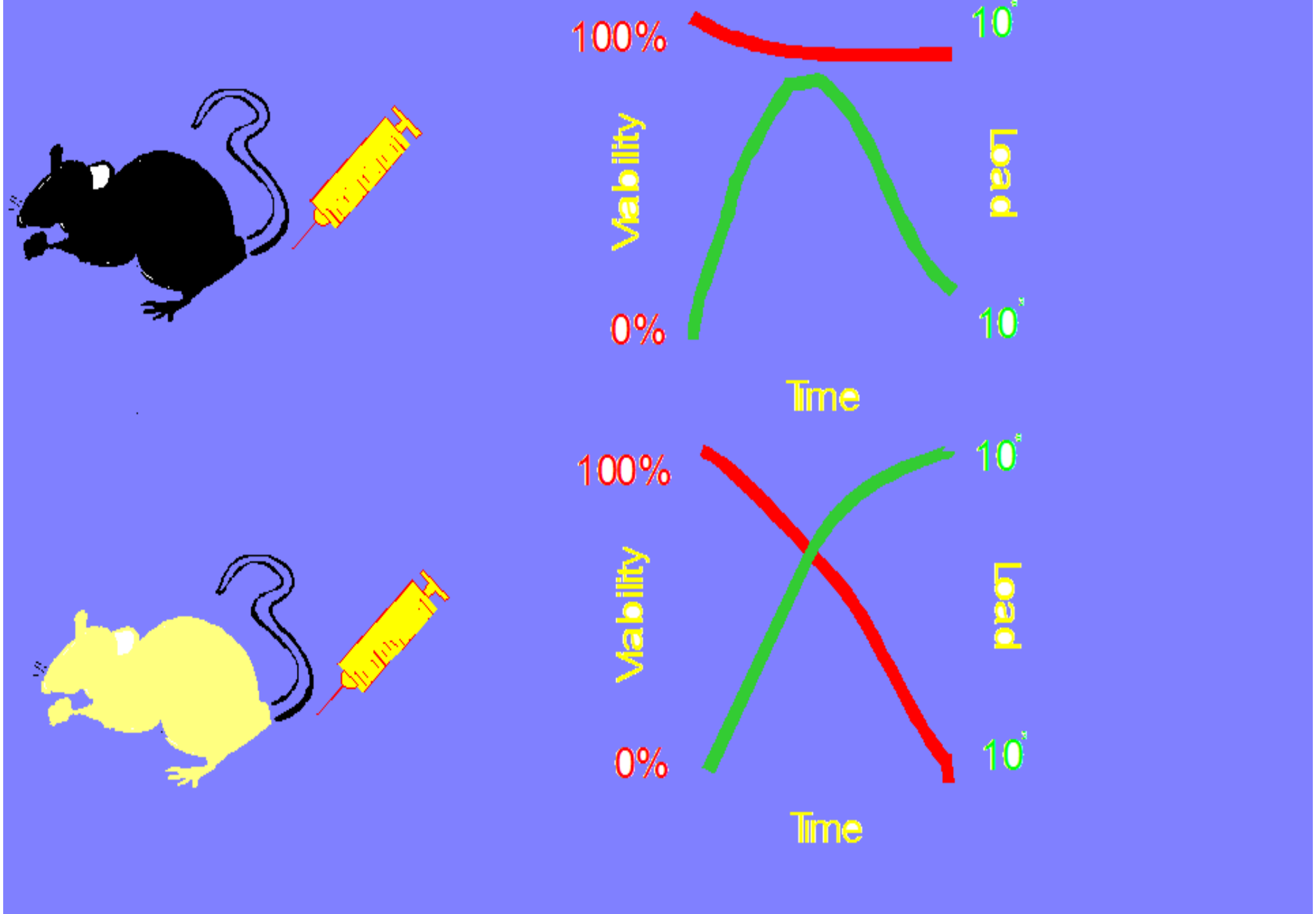
(a)



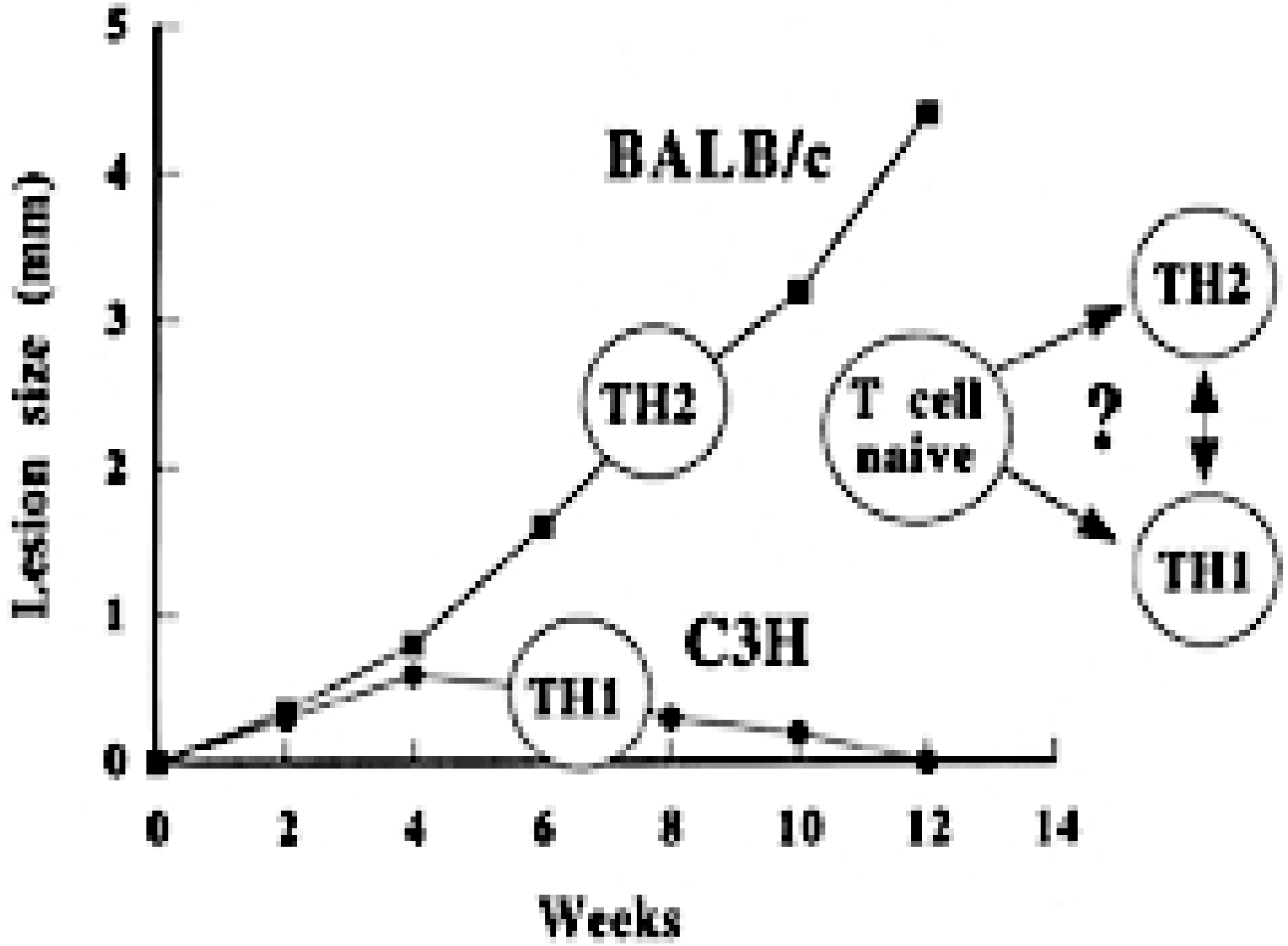
(b)

Th1/Th2 Paradigm

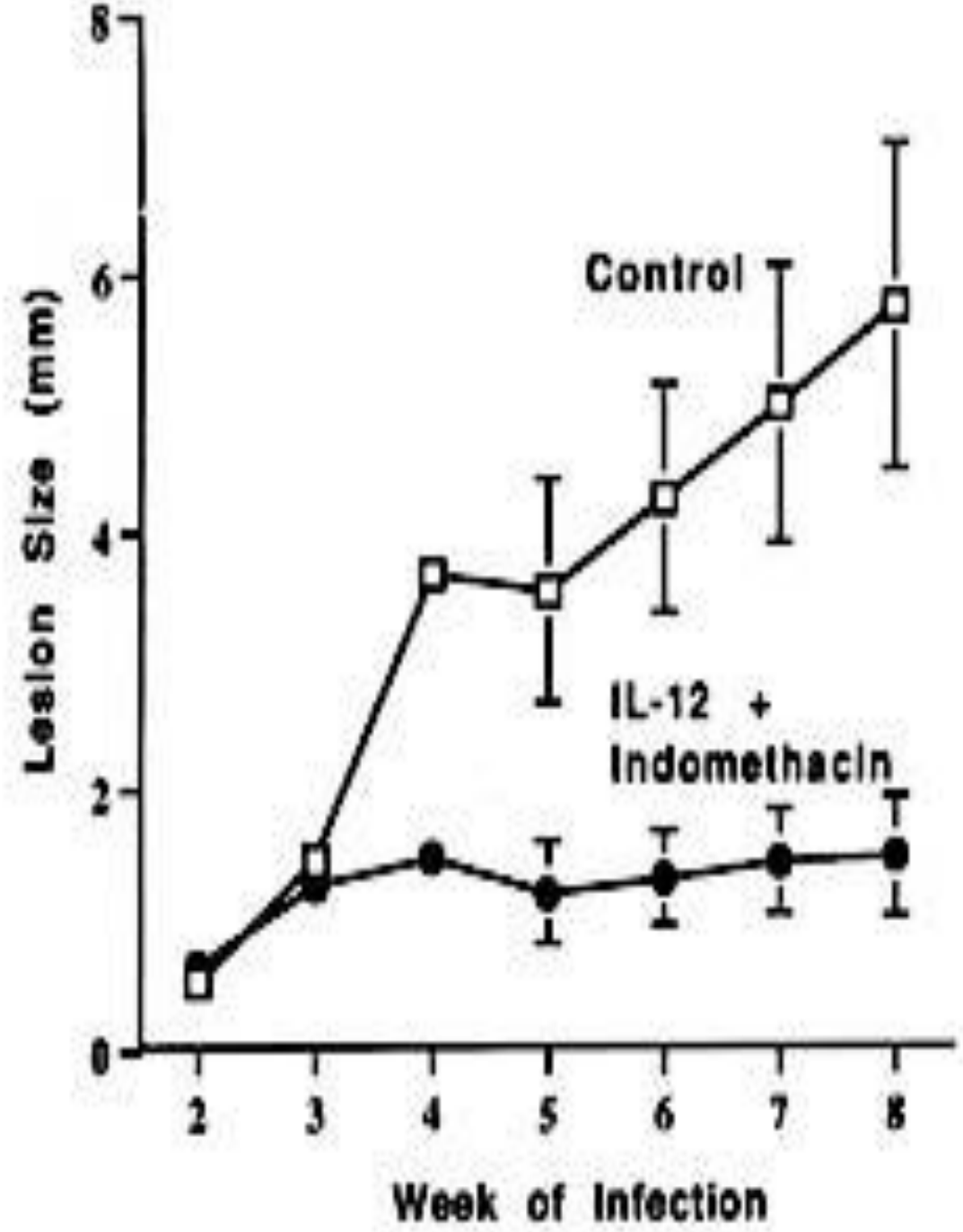


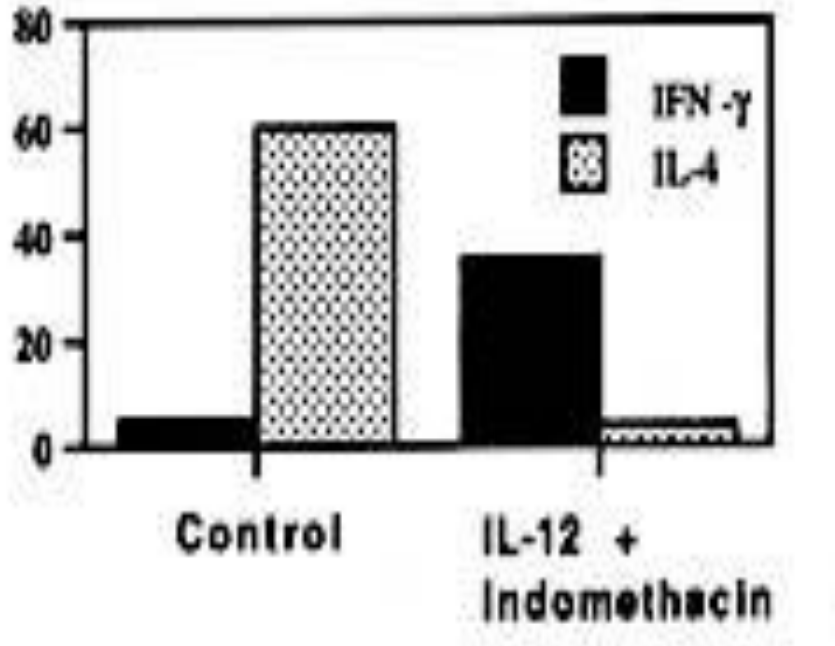

100%
0%
Viability
Load
Time
100%
0%
Viability
Load
Time

***Leishmania major* infection in mice: An *in vivo* model to study Th cell differentiation and regulation**



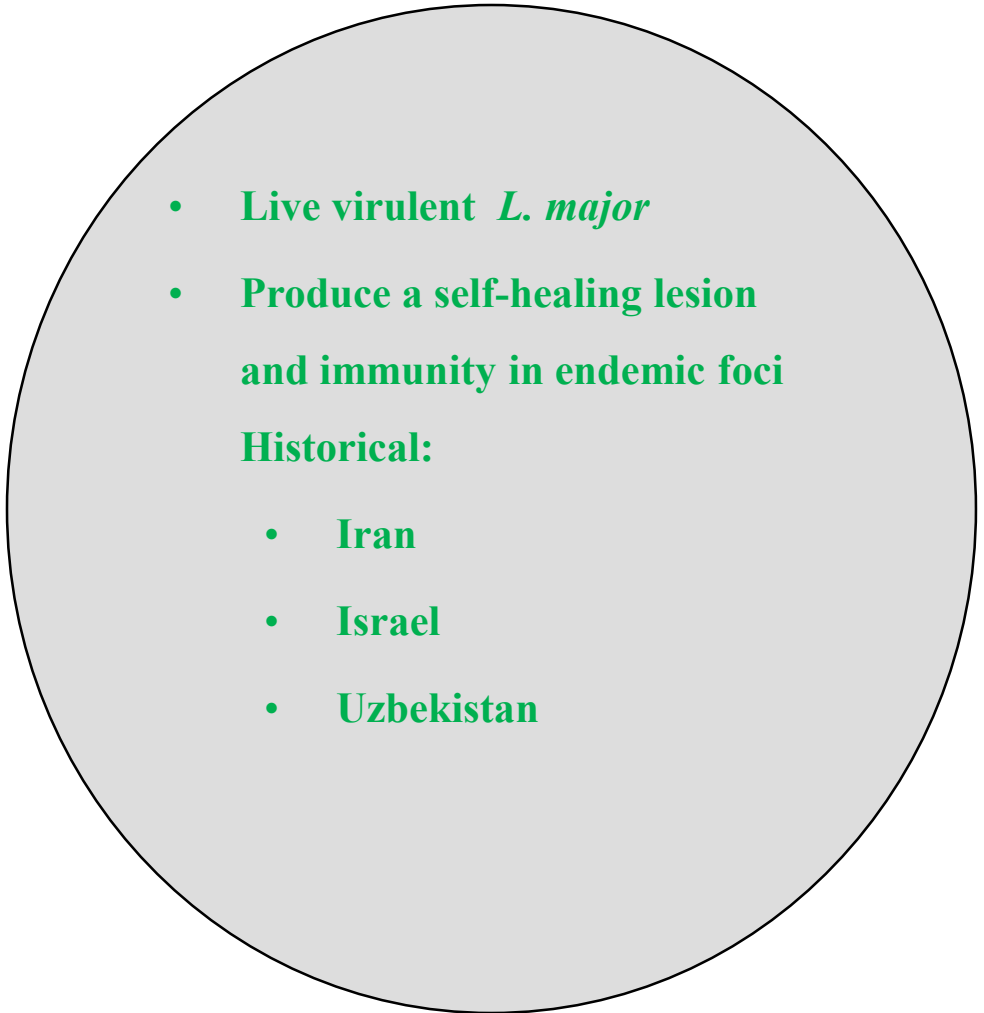
D


Lesion Size (mm)
0
2
4
6
8
Control
IL-12 +
Indomethacin
2
3
4
5
6
7
8
Week of Infection


0
20
40
60
80
IFN -γ
IL-4
Control
IL-12 +
Indomethacin

Leishmanization

Historical and still the most effective (Leishmanization)



First Generation Vaccines

First Generation Vaccines

- Ready to be tested in humans
- Safety profile

Killed whole Parasite
with /without adjuvants

KLM
ALM
Alum-ALM

BCG
Alum

Reasons in favour of leishmanization

- No effective drug is available against CL
- Vaccine is the most cost effective tool to control CL
- No vaccine is available
- Leishmanization is relatively safe except the lesion
- Historically Leishmanization showed to be the most effective tool to control CL

Problems (Host)

- Immune responses and correlate of protection
- Duration of protection
- Are some people more susceptible to if so why
- Cross protection?

Problems (inoculum)

- Reproducibility?
- Standardization?
- Stability?
- Delivery?

What needs to be done

Standardization of *Leishmania* parasite

Identify virulence factor(s) in *Leishmania*

Search for culture media without animal serum

Define preservatives allowed to be used in human

Studies on possibilities of lyophilisation of *Leishmania*

Attenuated *Leishmania*

Gene manipulated strains of *Leishmania*

Non Pathogen *Leishmania*

Identify surrogate marker(s) of protection

Cross protection

Current studies

Centrin KO *Leishmania* using CRISPR-Cas9
LmCen$^{-/-}$ are safe and induce protective Th1 response in hamsters and mice
The parasites do not cause disease in immunocompromised host
Immunization with *LmCen*$^{-/-}$ parasites confers protection against CL and VL
LmCen$^{-/-}$ can be grown under GMP conditions

L. major H strain gentamycin induced mutation ***Daneshvar*** *H,*

Non-pathogenic *L. tarentolea* **Rafati S,**

First generation vaccine
Liposomal SLA, induced protection treated *L. major* lesions in Balb/c mice Badiee A, NIMAD


Thanks for
LISTENING & Attention