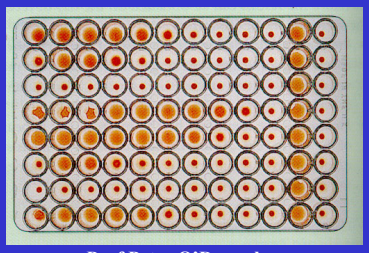
Biomedical Parasitology

Immuno-parasitology



Prof Peter O'Donoghue

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How are hosts protected?

- natural resistance
  - genetically determined
  - inherited (basis of breeding programs)
- acquired immunity
  - humoral responses (extracellular parasites)
  - cell-mediated responses (intracellular parasites)

review three lines of defense

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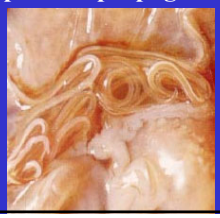
Interactions

PARASITE	HOST
- needs food supply	- resist infection
- place to develop	- moderate disease
- place to propagate	- develop protection



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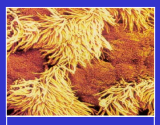
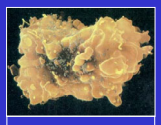
THREE LINES OF DEFENSE





first line BARRIER	second line INNATE	third line ADAPTIVE (ACQUIRED)
nonspecific	nonspecific	specific
parasites encounter external coverings and their secretions	parasites penetrating barriers encounter phagocytes & inflammation	surviving parasites encounter cell-mediated and humoral responses

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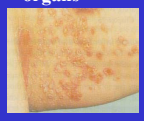
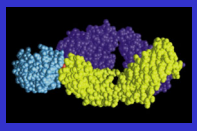
FIRST LINE

NONSPECIFIC EXTERNAL BARRIERS

Physical coverings
skin
mucous membranes
cilia in respiratory tract
flow through tubular organs



Chemical secretions
mucus, lysozyme
gastric juices, saliva
milk, sweat
urine



objective is to prevent entry of pathogens

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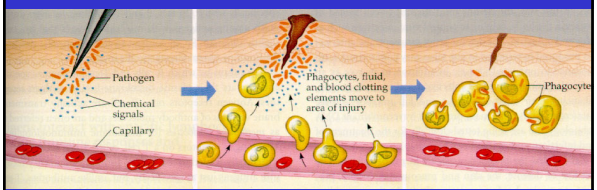
SECOND LINE

NONSPECIFIC INNATE IMMUNITY

phagocytes	- ingest pathogens
antimicrobial proteins	
complement (C')	- lyse organisms
interferon (IFN)	- inhibit spread
inflammation	- "setting on fire"



objective is to mop up pathogens that have entered

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THIRD LINE

ADAPTIVE (ACQUIRED) IMMUNITY

reliant on lymphocytes which provide:

- specificity
- diversity
- memory
- self tolerance

T cells	B cells
mature in thymus	mature in marrow
cell-mediated immunity	humoral immunity

objective is to target and destroy "nonself"

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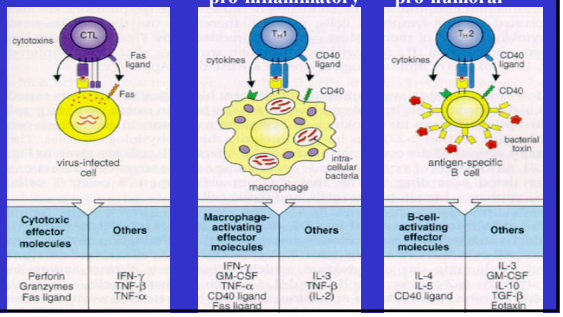
T-CELLS

Cytotoxic T cells	Helper T cells	
CD8 + MHC I	CD4 + MHC II	
(Killer cells)	(T_H1) pro-inflammatory	(T_H2) pro-humoral



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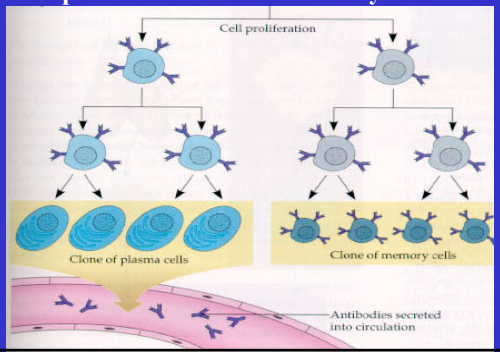
B-CELLS

antibody production

plasma cells memory cells



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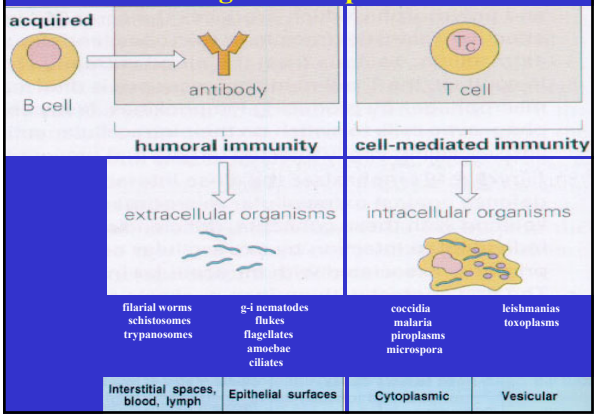
Integrated responses



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What happens if the immune responses are inappropriate or exaggerated?

HYPERSENSITIVITY REACTIONS

Type I (immediate)	IgE, mast cells	roundworm urticaria
Type II (cytotoxic)	IgG, IgM, C'	malaria anaemia Chaga's megacolon
Type III (immune complex)	IgG, Ag, C'	malaria nephritis trypanosomiasis schistosomiasis
Type IV (delayed)	T cells, mØ	filarial elephantitis swimmer's itch leishmaniasis

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How do parasites survive it all?

- Become less aggressive
  (parasite - commensal - symbiote)
- Learn to avoid host immune system
  (evasion mechanisms)
- Host-parasite evolutionary arms race
  (middle ground = enzootic stability)

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Evolutionary arms race

Host immune system works to:
- repel/destroy invaders
- undertake damage control
- protect against re-infection

Parasites develop survival strategies to avoid:
- innate immune responses
- acquired immune responses

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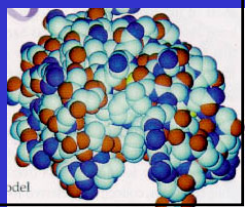
Functional level

Organismal - infectivity, survival
Cellular - reproductive, nutritional
Organellar - motility, energy transduction
Molecular - recognition, signalling

Operational via:
- parasite surface coat
- parasite secretions
- parasite excretions



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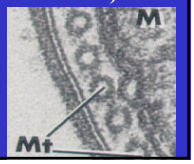
Parasite surface coat

- plasma membrane asymmetrical (polypeptides on inner and outer layers clearly different)
- external surface contains glycolipids, glycoproteins (rich in carbohydrate)
- other molecules may be adsorbed, esp. proteoglycans (acid mucopolysaccharides)
- surface coat may be delicate or evident as thick mat (up to 10% cell protein)



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Surface coat (glycocalyx)

- molecules anchored in plasma membrane by GPI (glycophosphatidylinositol) - precursor of all cell walls developed by animals/plants

Functions
- preservation of body shape
- mechanical/chemical barrier
- cell receptors (recognition/adhesion)
- biochemistry (enzymes, energy, transport)
- immune recognition (antigenic epitopes)

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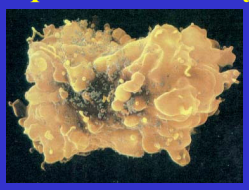
Innate immunity / Acquired immunity



nonspecific mechanisms: phagocytosis, inflammation

specific mechanisms: cell-mediated & humoral responses

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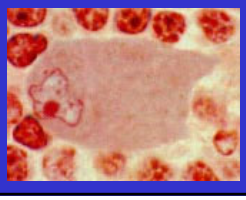
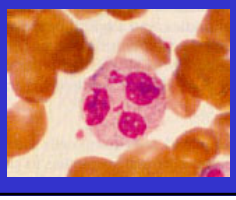
Innate immune mechanisms

phagocytes - ingest pathogens
natural killer cells - lyse infected cells
inflammation - deliver phagocytes to injured tissues





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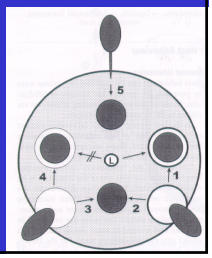
Strategies to avoid INNATE responses

- kill phagocytes using toxins (common for bacteria, suspected for some parasites)
- best to avoid phagocytic lysis altogether (common for protozoa)
  - develop in cytoplasm
  - parasitophorous vacuole
  - produce inhibitory proteins
  - produce anti-oxidants



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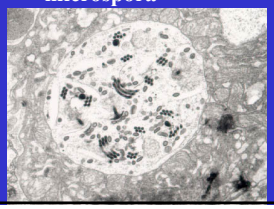
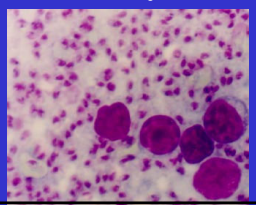
Intracytoplasmic development

- penetrate membrane directly into cytoplasm
  - microspora

escape from phagolysosome
  - amastigotes of *Leishmania*, *T. cruzi*





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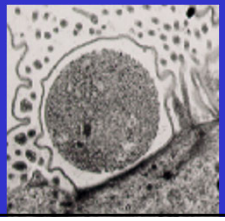
Parasitophorous vacuole

intracellular yet extracytoplasmic location

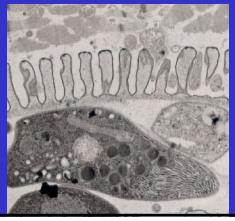
- live in vacuole lined by host membranes

Cryptosporidium



- or host + parasite materials

Sarcocystis



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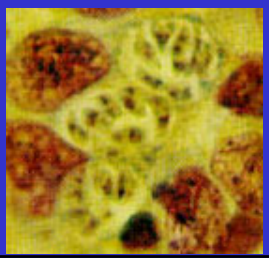
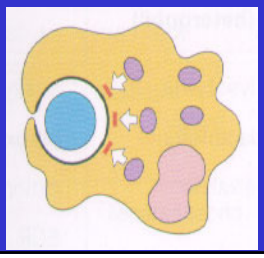
Inhibitory proteins

- fusion of phagosome and lysosome inhibited e.g. *Toxoplasma gondii* tachyzoites





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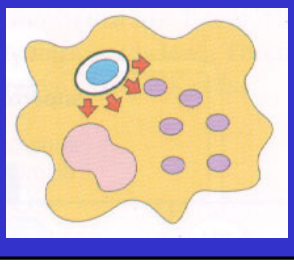
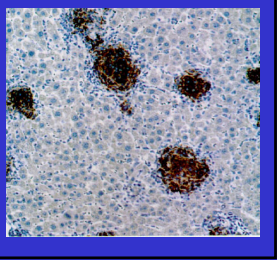
Anti-oxidants

- parasites resist killing by ROI and/or RNI (reactive oxygen/nitrogen intermediates) using anti-oxidants e.g. *Leishmania, Plasmodium*





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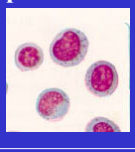
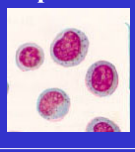
Acquired immune mechanisms

dependent on lymphocytes which provide:



- specificity
- diversity
- memory
- self tolerance



T cells	B cells
mature in thymus	mature in marrow
cell-mediated immunity	humoral immunity

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Strategies to avoid ACQUIRED responses

HIDE	- conceal antigens
CHANGE	- antigen variation
SUBVERT	- immunosuppress

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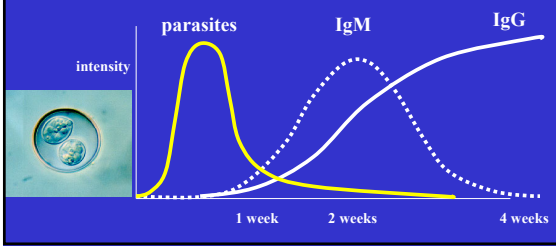
HIDE 1: hit-and-run

Rapid transient infections, e.g. coccidia

- short prepatency and patency
- outrace immune responses



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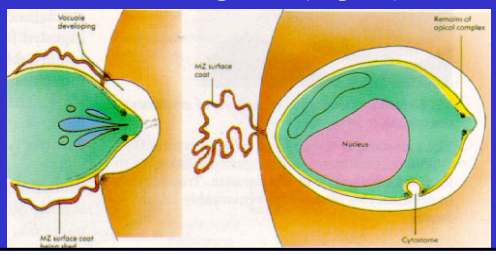
HIDE 2: antigen presentation

- do not express antigens (hide in cytoplasm)
- shed antigenic coat e.g. *Plasmodium*
- inhibit MHC recognition (implied)



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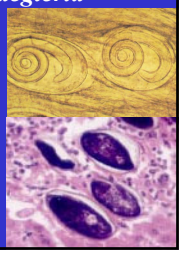
HIDE 3: inert sites

Infect host tissues where lymphocyte populations are absent or reduced

- gastro-intestinal lumen e.g. *Giardia*
- central nervous system e.g. *Naegleria*
- joints e.g. *Onchocerca*
- embryo e.g. *Toxoplasma*
- intragenomic e.g. *Karyolysus*
- intracystic e.g. hydatid cysts
- encapsulation e.g. *Trichinella*
- granuloma e.g. *Schistosoma*



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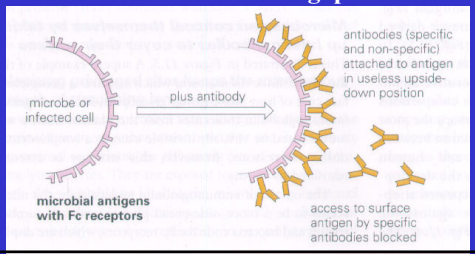
HIDE 4: antigenic mimicry

- mimic host antigens e.g. *Plasmodium*
- coat with host antigens e.g. *Schistosoma*
- coat with host antibodies e.g. protein A



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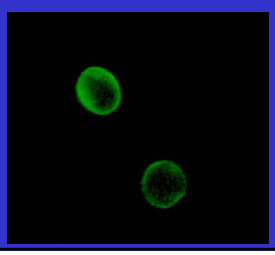
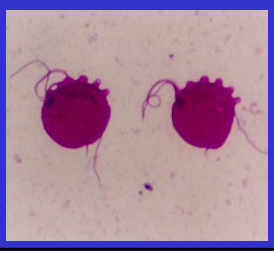
CHANGE 1: mutation

antigenic drift

Giardia axenic culture	*Trichomonas* culture
drift over >10 generations	drift over 20 generations





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CHANGE 2: recombination

genetic shift
(well known for human/avian influenza virus)

implicated for exceptionally virulent strains

- *Toxoplasma* RH (lacking cysts)
- *Giardia* 'Polly' (zoonotic, petechia)

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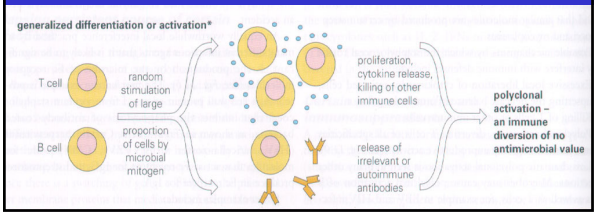
SUBVERT 2: produce exotoxins

- induce polyclonal activation (T cell mitogens)
- immunodiversion (saturate host with irrelevant antigens) (e.g. many worms)
- produce proteolytic enzymes (cleave Ig)



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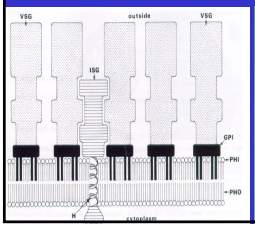
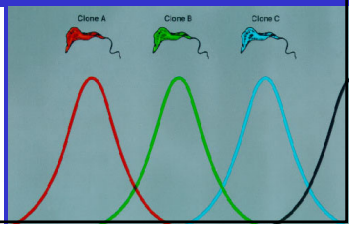
CHANGE 3: gene switching

- best known example: *Trypanosoma* VSG's (variant-specific glycoproteins) (up to 1,000 genes but only one governing expression)
- occurs for many other parasites (esp. protozoa)





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SUBVERT 3: immunosuppress host

- induction of suppressor cells
- proteinase destruction of host effector molecules
- inhibit host proteinases/cytokines

- malaria immunosuppression linked to down-regulation of cytotoxic T cells

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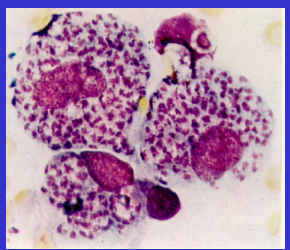
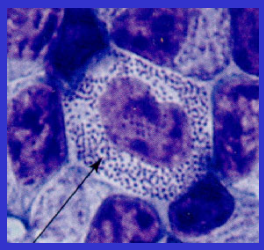
SUBVERT 1: target immune cells

- macrophages
  Toxoplasma, Leishmania
- lymphocytes
  Theileria





impaired function, impaired division, cell death

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Immuno-evasion

INNATE IMMUNITY

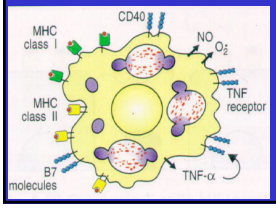
- develop in cytoplasm
- parasitophorous vacuole
- inhibitory proteins
- anti-oxidants

ACQUIRED IMMUNITY

HIDE (conceal antigens)
- antigen presentation
- inert sites
- antigenic mimicry

CHANGE (antigen variation)
- mutation
- recombination
- gene switching

SUBVERT (modulate)
- target immune cells
- produce exotoxins
- immunosuppress host



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Immuno-evasion

HIDE - CHANGE - SUBVERT

These strategies improve parasite survival (colonization, development, reproduction)

but still allows host immune system to limit disease and provide some protection

Remember: overt virulence resulting in host mortality is not in best interest of most parasites - better to be sneaky!

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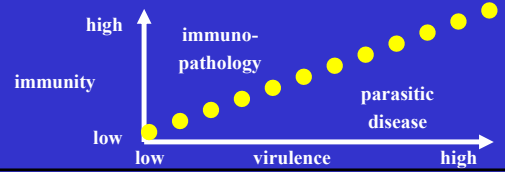
Evolutionary relationships

- evolutionary pressure to find balance between parasite aggressiveness (virulence) and host defenses (immunity)
- for parasites, this balance usually leads to clinical immunity rather than sterile immunity (enzootic stability)



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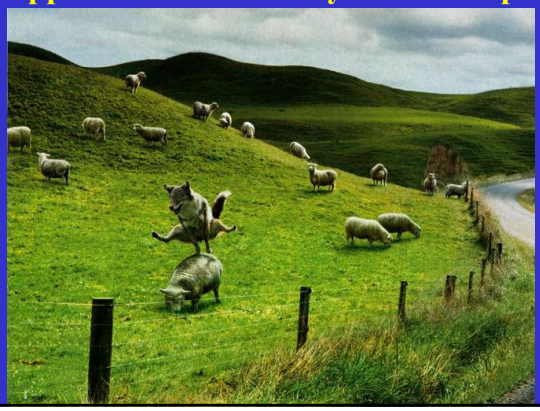
Appreciate evolutionary relationships



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How do hosts survive it all?

- Become resistant or tolerant
  (survival of fittest - Red Queen hypothesis)
- Ameliorate disease
  (minimize acute-chronic damage)
- Develop protective immunity
  (memory, premunition)

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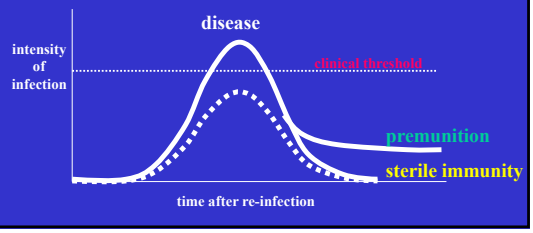
Three types of immunity

Previous exposure confers:

- clinical immunity (disease prevented)
- sterile immunity (parasites eradicated)
- concomitant immunity (some parasites persist)



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Protection against disease (if not infection)

ACTIVE IMMUNITY

- natural infection (subclinical)
- vaccination (stimulate immunity)

PASSIVE IMMUNITY (antibody transfer)

- natural (transplacental, colostral)
- artificial (immuno-prophylaxis/therapy - HIBC)

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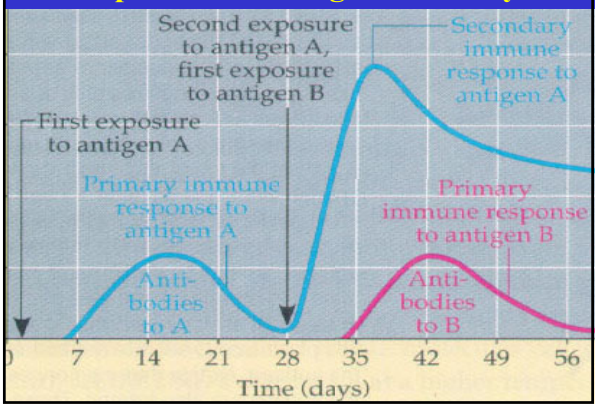
Exploit immunological memory



43

Objectives

Resultant immunity acts to:

- **prevent infection (block transmission)**
- **prevent disease (limit pathogenicity)**
- **eradicate infection (affect cure)**

Various success stories

- **most against bacterial or viral diseases**
- **few against parasites (yet!)**

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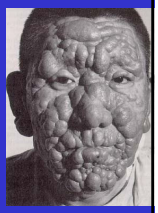
History

- **ancient Middle Eastern practice of "leishmanization"**

  deliberately infect children at inconspicuous site (buttocks)

  with *L. tropica* from mild cases resulting in self-healing lesion (Oriental sore)



- **10th century China - "variolation" infect children with mild cases of smallpox**

45

Vaccination

Jenner 1796-98 used cowpox (vaccinia) to protect against smallpox (variola)

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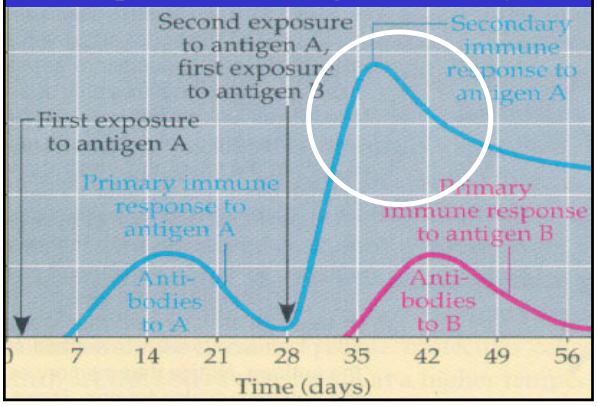
Exploit immunological memory



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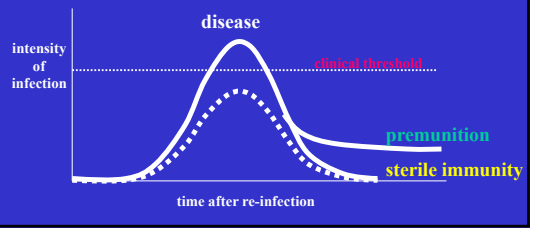
Three types of immunity

Previous exposure confers:

- **clinical immunity (disease prevented)**
- **sterile immunity (parasites eradicated)**
- **concomitant immunity (some parasites persist)**



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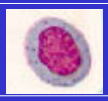
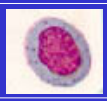
Dependent on adaptive (acquired) specific immunity

vaccination primes adaptive immune system to obtain rapid heightened response to re-infection/super-infection

prerequisite is development of memory cells



T lymphocytes
cell-mediated immunity



B lymphocytes
humoral immunity

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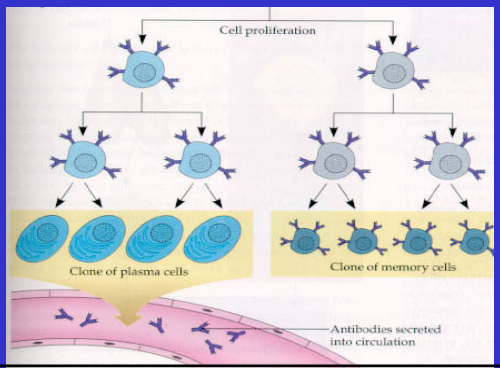
Clonal expansion following activation

- effector cells
- memory cells



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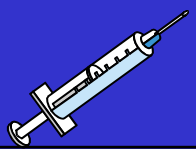
Best vaccines

- native/natural antigens
- contain multiple epitopes
- contain both T and B cell epitopes
- contribute to cooperative cell-mediated and humoral immunity (MHC class II needed for T cell responses)



51

Objectives

Resultant immunity acts to:

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- prevent disease (limit pathogenicity)
- eradicate infection (affect cure)

Various success stories

- most against bacterial or viral diseases
- few against parasites (yet!)

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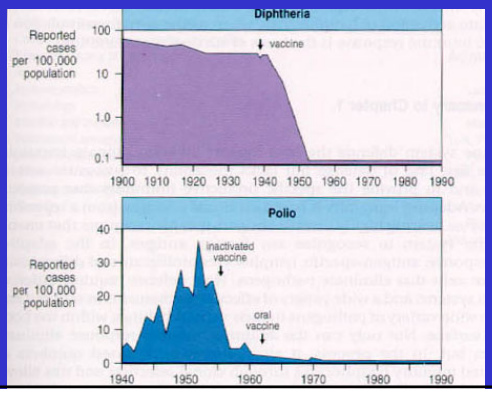
Vaccination programs



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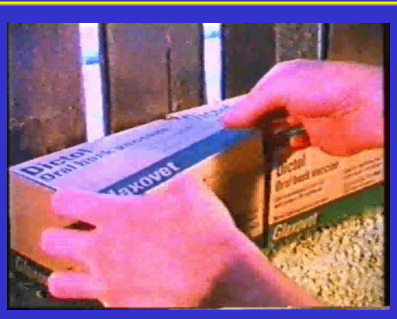
Success Story



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Vaccination

Vaccine type:	Problems:
• live attenuated organisms	• reversion
• killed organisms	• allergy
• subcellular vaccines	• contamination

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Live attenuated vaccines

Selection of induced mutants (genetic roulette)

- avirulent species/strains/clones
- precocious strains
- serial passage in animal models
- serial passage in tissue culture
- adaptation to low temperature
- chemical mutagenesis
- irradiation

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Killed vaccines

Inactivated through:

- chemical treatment
  - formaldehyde
  - phenol/acetone
  - β-propiolactone/ethylenimines/psoralens
- heat/cold
- irradiation
  - microwave
  - ultra-violet

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Subcellular vaccines

- surface coats
- membrane determinants
- cytosol fractions
- organelle extracts
- cytoskeletal elements
- secretory/excretory metabolic products
- inactivated toxins (toxoids)
- anti-idiotype vaccines (surrogate antigens)

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Vaccine production

Direct from parasites
(poor quantity, good quality)

- culture parasites
- harvest antigens
- purify immunogens

Via molecular biology
(good quantity, poor quality)

- recombinant vaccines
- DNA vaccines

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Recombinant vaccines

Expression vector used for bulk production but recombinant antigen often less immunogenic

- lacking glycosylation sites
- inappropriate presentation
- loss of epitopes during expression
- often stimulate B cell responses (not T cell)

- best presented as MAP (multiple antigenic peptides) - structure with branching lysine core large enough to eliminate need for carrier

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DNA vaccines

- cloned genes via microbial vectors (virus/bacteria)
- immunize with plasmid DNA encoding antigens
- use plasmids with promoters for high expression
- expression library immunization (single antigens often ineffective)
- application
  - injected (i/m, s/c)
  - needle-free (Biojector using CO_2)
  - particle bombardment (gene gun)

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Vaccine optimization

Small antigens cleared rapidly from host
Need to prolong exposure to achieve response

- use carrier molecules
- use adjuvants
- use delivery systems
- use slow release depots

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Adjuvants

Immunostimulants (additive/synergistic)

- inorganic salts (floccs)
  - aluminium hydroxide, beryllium hydroxide
  - aluminium phosphate, calcium phosphate
- saponins
- bacterial products
  - BCG (bacille Calmette-Guerin) tubercle bacillus
  - Freunds complete (bovine tuberculosis)
  - MDP (muramyl dipeptide)
- natural mediators
  - IL-1, IL-2, IFN-γ

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Delivery systems

- liposomes (phospholipid vesicles)
- proteosomes
- iscoms (immune stimulating complexes)
  - cage-like micelles of saponin derivative QuilA, cholesterol, phospholipids and antigen
- block polymers
  - polyoxyethylene
  - polyoxypropylene
- slow release formulations (bolus)

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Vaccine failure

- incomplete immunity (partial protection, disease in immunocompromised)
- short-term immunity (loss of protection)
- inappropriate responses (polyclonal activation)
- exaggerated responses (immunopathology)
- no clinical immunity (host disease)
- complete failure (host death)

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Vaccine pathology

- contamination
  (esp. with viruses)
- allergy/hypersensitivity
  (to egg proteins, horse serum)
- autoimmunity
  (arthritis)
- neurological side-effects
  (convulsions)
  (meningitis/encephalitis)

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The great debate

Chemotherapy	versus	Vaccination
• broad spectrum (targets whole groups)		• narrow activity (species specific)
• short-acting		• long-lasting
• re-infection possible		• re-infection prevented
• drug resistance		• reversion of virulence
• drug residues		• hypersensitivity
• environmental toxins		• contamination

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Parasite Vaccines

- roundworms (nematodes)
- flatworms (cestodes/trematodes)
- protozoa (flagellates/sporozoa)

- complex immune interactions cf. viruses and bacteria
- poorly understood

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PROTOZOAL VACCINES

- urgent need due to failure of many drugs
- more infections due to factory farming, inadequate effluent disposal, urbanization
- cause sudden onset, acute diseases
- masters at immuno-evasion
  - pernicious effect (over-stimulation/exhaustion)
  - evasion (antigen variation/intracellular dev.)
  - immuno-modulation (mitogens/superantigens)

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PROTOZOAL VACCINES

- need T and B cell activation and interaction
- antibodies implicated in:
  - inhibition/neutralization
  - interaction with NK, mØ, granulocytes to induce:
    - ADCC (antibody-dependent cell cytotoxicity)
    - ADCI (antibody-dependent cell inhibition)
    - opsonization
    - phagocytosis
- responses isotype specific and cytokine-dep.

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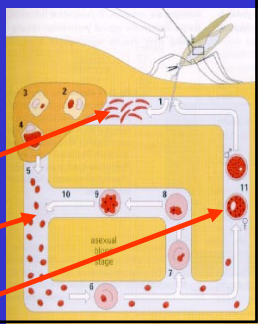
Malaria vaccines

- protective immunity acquired naturally, but not life-long
- three vaccination strategies adopted
  - prevent infection (sporozoite vaccines)
  - cure infections (merozoite vaccines)
  - block transmission
  - (gametocyte vaccines)



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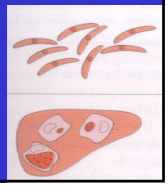
Sporozoite vaccines

- live attenuated (irradiated) sporozoites protective (but not heat-killed, formalin-inactivated or lysates)
- immunity involves antibodies, CD8 cells, IFN-γ + NO

CD8 → secretes → IFN-γ → activates → iNOS

- Partial success with:
  - Pf-CSP circumsporozoite protein
  - plasmid DNA encoding Pf-CSP
  - NYVAC-Pf7 vaccinia virus incorporating 7 Pf genes



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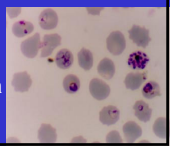
Merozoite vaccines

- immunity induced by drug cure
- protection afforded by adoptive transfer of antibodies or CD4 cells (esp. αβ cells)
- involves cytokines, γδ cells (IEL), NO
- candidate vaccines
  - ABRA: acidic/basic residues antigen
  - Pf155/RESA: 155kDA Pf antigen
  - GLURP: glutamate-rich protein
  - AMA-1: apical membrane antigen
  - MSP-1: merozoite surface protein



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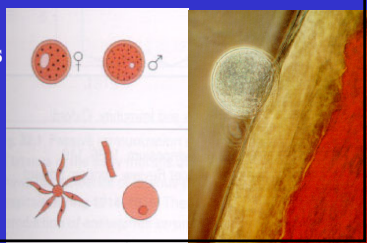
Gametocyte vaccines

- immunity limits sexual development
- but too late to prevent/cure damage
- best part of cocktail vaccine strategy
- little known
- few candidates



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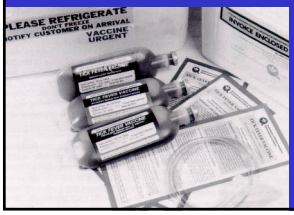
Tick fever (*Babesia*) vaccines



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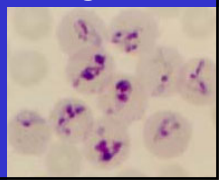
Tick fever (*Babesia*) vaccines

Molecular vaccines

- SBP1: spherical body protein
- RAP-1: rhoptry-associated protein
- MSA-1: 42kDa major surface antigen
- MSA-2: 44kDa major surface antigen
- all induce CD4 response, IFN-γ production and give partial protection



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Other protozoal vaccines

Trypanosoma	sleeping sickness, ngana
	Chagas disease
Theileria	East Coast fever
Eimeria	coccidiosis (esp. poultry)
Toxoplasma	toxoplasmosis
Neospora	bovine abortion
Ichthyophthirius	freshwater white spot
etc.	

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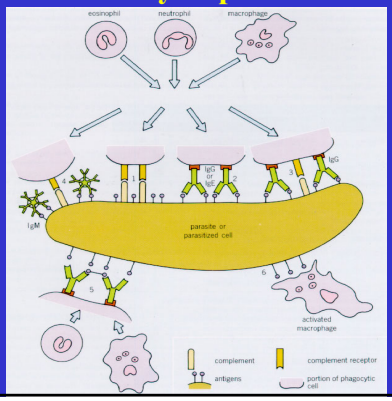
Immunity to parasites



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NEMATODE VACCINES

1958 - bovine lungworm *Dictyocaulus viviparus*

- live larvae protective, crude extracts not
- attenuation by irradiation (400 Gy)
- not sterile immunity (95-98% effective)
- not lifelong (lasts one year)
- good weaner vaccine

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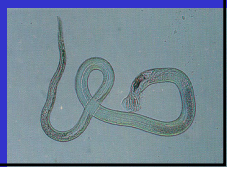
Nematode vaccines

Experimental attenuation of infective larvae successful for various nematodes, but few commercial successes due to:

- poor efficacy
- high costs
- variable results
- weaner susceptibility



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Targets

Natural antigens (seen by host immune system)

- ES (excretory/secretory) antigens
  Haemonchus, Ostertagia, Trichostrongylus
- tropomyosin (worm muscle cells)
  Trichostrongylus, Onchocerca
- acetyl choline esterases (worm metabolites)
  Dictyocaulus, Trichostrongylus, Haemonchus
- MSP (major sperm protein)
  Ascaris

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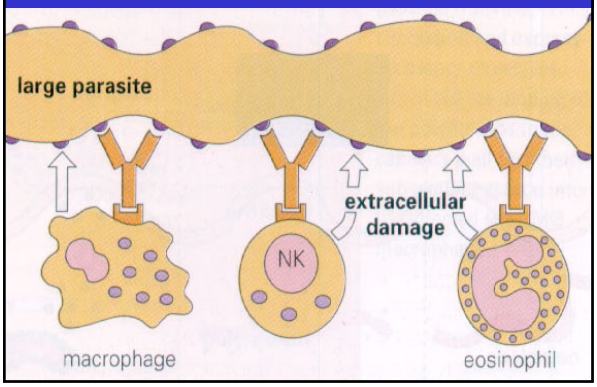
Protection involves cell immunity



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Targets

Hidden antigens (novel/concealed/covert)
(not normally seen by host immune system)

- gut antigens
  worms ingest antibodies against their own intestinal cells resulting in blockage/lysis
  Haemonchus contortin

- tick gut antigens
  Boophilus microplus

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FLATWORM VACCINES

- trematode/cestode infections accumulate causing chronic diseases
- multi-stage life-cycle involves multi-targets so little protective immunity develops
- hosts frequently re-infected/super-infected
- drug action curative, transient
- vaccine prophylactic, long-lasting
- need not be sterile immunity to reduce morbidity and transmission

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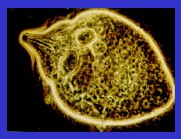
Targets

Parasite antigens (cytoskeletal proteins)

- paramyosin: major component of thick filament in invertebrate muscles, 97kDa antigen from *Schistosoma* tegument was 30-80% protective



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Targets

Parasite antigens (enzymes)

- GST: glutathione-S-transferase involved in detoxification of xenobiotics, extracts from *Schistosoma/Fasciola* tegument were 70% protective
- TPI: triose phosphate isomerase involved in glycolysis, 28kDa from *Schistosoma* was 40% protective
- cathepsin: two proteolytic enzymes produced by *Fasciola* able to cleave Ig were 50-70% protective

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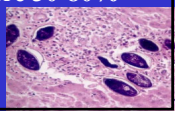
Targets

Parasite antigens (similar to host proteins)

- S23: integral membrane protein 23kDa from *Schistosoma* was 40-60% protective
- FABP: fatty acid binding protein involved in intracellular transport in lipid/vitelline droplets in *Schistosoma*, *Fasciola* and *Echinococcus* were 30-80% protective



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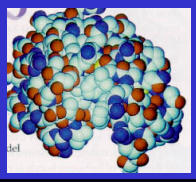
Targets

Elements considered as candidate vaccines

- internal proteins
- cytoskeletal proteins
- parasite enzymes
- molecules similar to host proteins



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Targets

Parasite antigens (internal proteins)

- 45W: oncosphere 45kDa antigen of *Taenia* - 94% protective

- EG95: oncosphere 95kDa antigen of *Echinococcus* - promising

- hp200: 200 kDa haemoglobin-like protein of *Fasciola* - 40% protective

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Vaccination - summary

- live organisms
  - pathogenic (+ chemotherapy)
  - nonpathogenic
  - attenuated
- killed organisms
- subcellular vaccines
  - crude fractions
  - organelle/membrane determinants
  - secretory/excretory metabolic products
  - recombinant antigens
  - DNA vaccines (immunogen expression)

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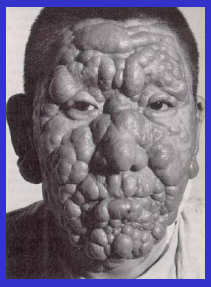
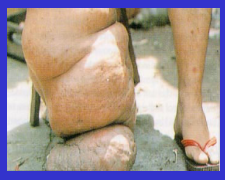
Take home message

Prevention is better than cure!





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Control of parasitic disease

Treatment (intervention)
[demands knowledge of biochemistry/physiology]
- chemotherapy (treat parasites)
- supportive therapy (treat symptoms)

Prophylaxis (prevention)
[demands knowledge of biology/immunology]
- management (disrupt transmission)
- vaccination (induce protection)

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Current problems

- Rapid emergence of drug resistance
  (variations on a theme)
- Complexity of immune interactions
  (humoral + cell-mediated responses)
- Few candidate vaccines
  (many antigens - few immunogens)
- Diminished immunocompetency
  - congenital immunodeficiencies
  - acquired immunodeficiencies
  - immunosuppressive chemotherapy

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Three phases of immunity

- host resistance/susceptibility
  - risk assessment
  - breeding for resistance
- modulation/eradication of active infection
  - affect cure (or symptomatic resolution)
  - moderation of pathogenicity
- acquisition of protection
  - identify effector mechanisms
  - generate life-long immunity

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Protective immunity

Active (self-generated)
- natural infection (subclinical)
- vaccination (stimulate immunity)

Passive (inter-host transfer)
- natural (transplacental, colostral)
- artificial (cells/antibodies) - immunotherapy

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Immunotherapy

Endogenous
- constitutive (modulate existing function)
- restitutive (restore absent function)
- delimiting (splenectomy)

Exogenous
- immune cells (lymphophoresis)
- immunoglobulins (antibody transfer)
- soluble factors (cytokine therapy)
- immunostimulants

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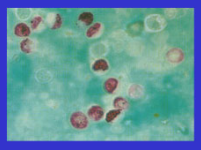
Immunobiology in action

Exemplar

Cryptosporidium parvum



- newly recognized enteropathogen
- protozoan parasite similar to coccidia
- causes significant morbidity, some mortality
- anthroponotic, zoonotic, water-borne
- no effective chemotherapy

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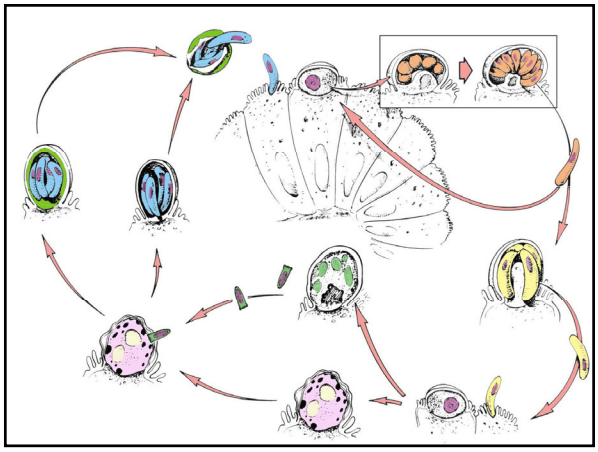

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Pathogenesis

- **villus atrophy**
- **microvillus destruction**
- **impaired glucose and electrolyte transport**
- **impaired carbohydrate and protein digestion**
- **malabsorptive and maldigestive disease**

- **pernicious cycle (cyclic merogony)**
- **auto-infection (chronic infections)**

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Host susceptibility/resistance

Age-related
- clinical infections most common in neonates
- rapid development of resistance in animals

Acquisition of mature intestinal flora
- severe infections in germ-free/gnotobiotic animals

Malnutrition
- depleted iron status, low protein diet

Immunological maturity
- immature - senescent

100

Humoral immunity

Serum antibodies (acute-convalescent)
- serological tests (IFAT, ELISA)
- transient IgM, IgA, IgE (weeks)
- prolonged IgG (months)

Copro-antibodies (patent infections)
- local/secretory IgA, IgM, IgG 5-16 dpi

B cell deficiencies
- hypo-, a-gammaglobulinaemia
- selective immunodeficiencies

Antibodies alone not protective (strong responses in AIDS patients with chronic infections)

101

Cell-mediated immunity

inflammation/infiltration
- neutrophils, macrophages, lymphocytes, plasma cells

T-cell deficiencies
- low CD4 (helper)/AIDS patients - chronic infections
- CD4 depletion in animals - chronic infections
- CD4 restitution - limits duration of infection

- CD8 (cytotoxic) modulation - no effect
- NK (natural killer) cell modulation - no effect
- SCID mice, nude mice/rats - chronic infections

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Cytokines

IFNγ (interferon-gamma)

- selective depletion by neutralizing mAb's leads to severe infections
- restoration moderates infection severity
- deficient C57/BL6 mice develop non-resolving fatal infections compared to asymptomatic self-limiting infections in normal wild type
- PBMC (peripheral blood mononuclear cells) produce IFNγ in immunocompetent patients but not in AIDS patients

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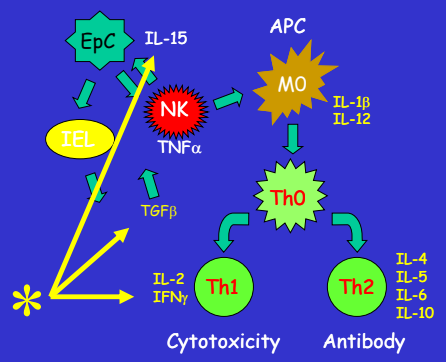
Cytokines



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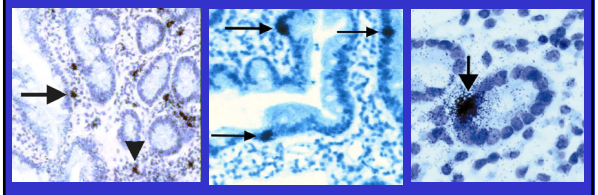
Interferon IFNγ | Interleukin IL-15 | Transforming growth factor TGFβ



- vital for resolution
- reduced oocyst output
- shortened infections

- required for protection
- activates CD8, NK cells
- enhances CD8 γδ T-cell (IEL) activity

- stimulates IgA
- involved in repair

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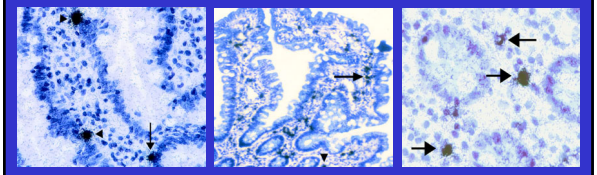
Tumour necrosis factor TNFα | Interleukin IL-4 | Interleukin IL-1β



little effect on infection/disease kinetics/dynamics

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Cytokine immunotherapy

Improve Th1 cytokine levels

- IFNγ: therapeutic application truncated infection (but oocyst shedding recommenced after treatment)
- IL-2: little effect

Improve macrophage activation

- IL-12: better antigen presentation

BUT, adverse effects unknown

Therapy cost-prohibitive

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Lactogenic immunity

Observations from surveys of neonates

- fewer infections in breast-fed children than in bottle-fed children
- more severe infections in colostrum-deprived calves, lambs

Passive transfer studies

- colostrum neutralizes sporozoites
- colostrum protects against severe disease
- colostrum helps resolve symptoms

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Colostrum

- maternal milk produced post-partum
- nutritionally-rich (protein/fat)
- immunologically-rich (antibodies)
- plentiful source (dairy industry)

Source

- uninfected cows - low titre (1:100)
- infected cows - medium titre (1:1000)
- devise immunization schedule to improve titre

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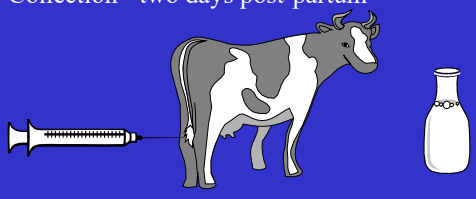
Immunization

Route - intramammary infusion

Dose - soluble oocyst-sporozoite extracts

Schedule - weekly pre-partum

Collection - two days post-partum



HIBC = hyper immune bovine colostrum

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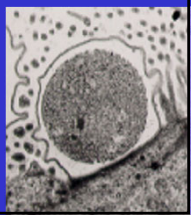
Characterization

Antibodies

- titres up to 1:400,000
- isotypes IgG_1, IgG_2, IgM, IgA
- reactivity against antigens from:
  - sporozoites
  - merozoites
  - gametocytes
- intracellular activity?



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Activity

Prophylaxis (administered before infection)

- partial protection in animals
- reduce severity of infection

Therapy (administered after infection)

- patent period reduced
- oocyst production reduced
- clinical resolution/ symptomatic improvement

Undergoing clinical trials (FDA, TGA)

Problem with lactose intolerance

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HIBC immunotherapy

- similar strategy used for other enteropathogens e.g. rotavirus
- protective activity of colostrum well known in animal industries/veterinary science as prophylaxis against neonatal diarrhoea esp. in piggeries

Alternative strategies

- mouse monoclonal antibodies
- hyperimmune egg yolks

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Have a cow!

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