PROSPECTS FOR DEVELOPMENT OF VACCINES AGAINST FUNGAL DISEASE

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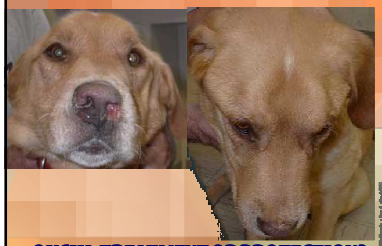
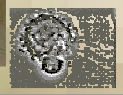
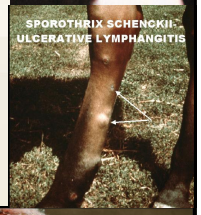




OUCH! TREATMENT OR PROTECTION?

Fungal diseases

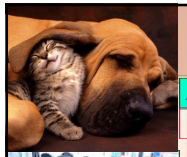
- Dermatophytosis
- Subcutaneous mycoses
  - Chromomycosis
  - Sporothrichosis
- Systemic mycoses
  - Aspergillosis
  - Pathogenic yeasts
  - Dimorphic fungi
  - Phycomycoses
    - Mucomycoses
    - Entomophthomycoses
- Mycotoxicosis

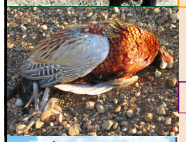


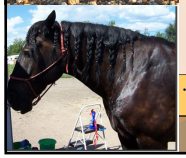
SPOROTHRIX SCHENCKII-ULCERATIVE LYMPHANGITIS

HISTOPLASMOSIS-SKIN

Mycoses in domestic animals







Aspergillosis, Dermatophytoses, Otitis

Dermatophytoses, Cryptococcosis

Fungal abortion caused by *Aspergillus, Candida, Zygomycetes*

Dermatophytoses

Cryptococcosis

•Pnuemonic cryptococcosis

•Aspergillosis

- *Pnuemocystic carinii* and arthritis

Blanco, 2006

ANTIFUNGAL DRUGS--by mode of action

•Membrane disrupting agents

Amphotericin B, nystatin

•Ergosterol synthesis inhibitors

Azoles, allylamines, morpholine

•Nucleic acid inhibitor

Flucytosine

•Anti-mitotic (spindle disruption)

Griseofulvin

•Glucan synthesis inhibitor

Echinocandins

•Chitin synthesis inhibitor

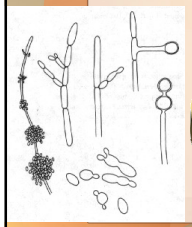
Nikkomycin

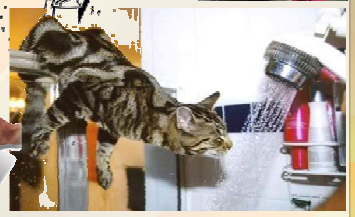
•Protein synthesis inhibitor

Sordarins, azasordarins

Antifungal therapies.

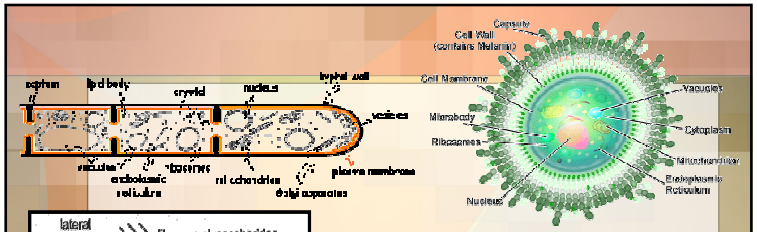
- Undetectable causes
- A Long term
- High adverse
- High cost
- polymorphism

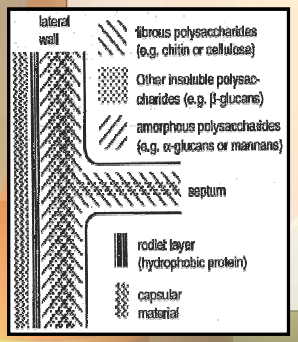




Have a guess?

- ***Aspergillus fumigatus***
  Nystatin, Amphotericin B, Itraconazole
- ***Candida albicans***
  Nystatin, Clotrimazole Amphotericin B, Ketoconazole
- ***Cryptococcus neoformans***
  Amphotericin B+Flucytosine, Fluconazole
- ***Histoplasma capsulatum***
  Amphotericin B, Ketoconazole, Itraconazole





HSP90

Glucuronoxylomannan (GXN)

Histone 2B

Phosphomannoprotein

Laminarin (1,3 β glucan)

Adhesin

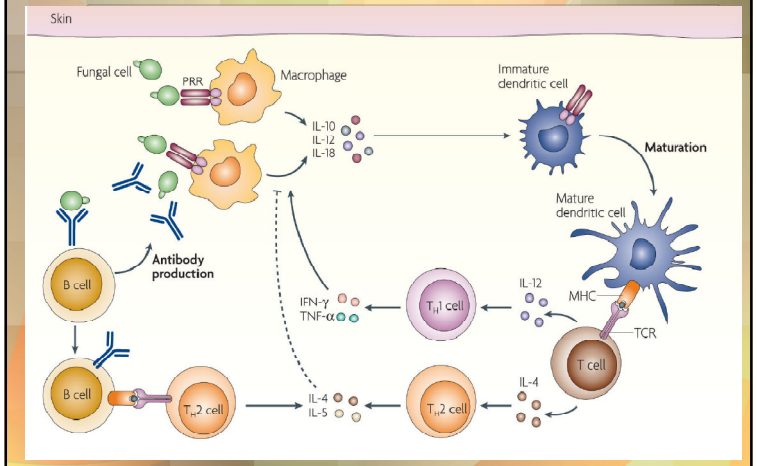
Special structures: conidia, hyphae, spherules.

Yeast and Mold Antigens

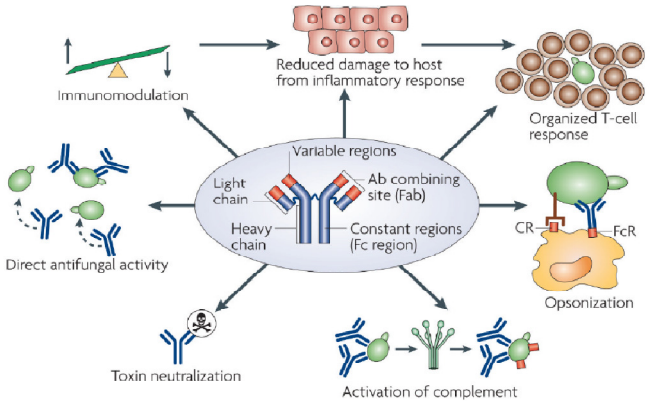
Capsular polysaccharide

- **glucuronoxylomannan**
- **Ab unresponsive to Ag**
- **Inhibit leukocyte migration**
- **Enhance HIV**
- **Induce shedding L-selectin**
- **Induce T-cell secrete immunosuppressive molecule**
- **Dysregulation of cytoine**
- **Inhibit phagocytosis**

Host response to fungi



Host response to fungi



Immune response to specific fungal antigens

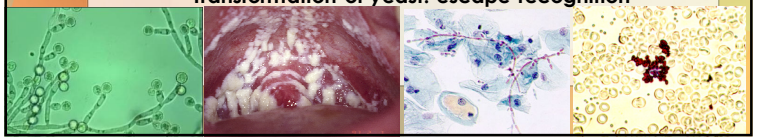
Cutaneous Candidiasis

Difference of CMI and HMI response to pathogen

Yeast	Germinating
↓	↓
Ab in normal	Ab in patient

Cause of CMI deficiency has still not elucidated in chronic form.

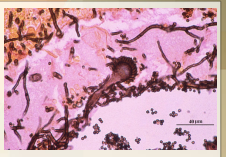
Transformation of yeast: escape recognition



Dermatophytosis

- **Major antigenic substance: chitin, glucan, glycopeptide, CHO and keratinase → DTH**
  - **IgG**
- **Broadly cross-reactive to other fungi**
  - **Immediate hypersensitivity to air-born fungi and penicillin.**

Invasive Aspergillosis



Fungus triggers immune phenomena

Allergic rhinitis, asthma, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis, aspergilloma.

Normal Host	Susceptible host
Maintain only conidia form	Switch to hyphae form

Specific immune response to fungi

Major defense mechanism esp. for intracellular fungi.

CD4+ and CD8+ T cell limit dissimilation of fungi to other organs.

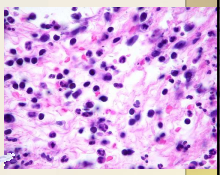
Th_1	Th_2
Phagocytic dependence dependenc	Non-phagocytic
Phagocytosis, inflammation IgG production	Tissue injury, granulomatous, IgE
Host resistance	Host susceptible

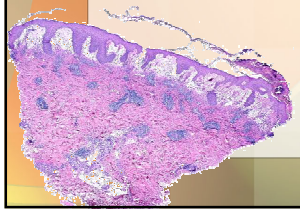
GM-CSF, IFN, TNF, IL1, IL6, IL12, IL18, MIP1, MCP

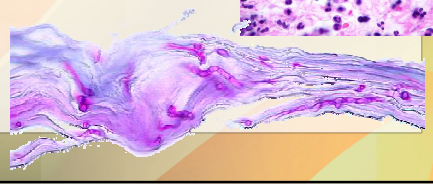
IL4, IL10

Vaccination

- Passive immunotherapy
- Vaccination to induce humeral immunity
- Vaccination to induce cell-mediated immunity.
- Dendritic cell vaccination







Passive immunotherapy

- Neutralize an infection by (monoclonal) antibody.
  - Mycograb® bind to hsp90 of *Candida* sp.
    Cryptococcus sp.
    Aspergillus sp.

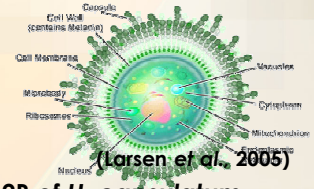
(Matthews and Burnie, 2004)

TABLE 1. Homologies to the *C. albicans HSP90* gene sequence[a]

Gene	Identity (%)	Length of overlap (bp)
Saccharomyces cerevisiae hsc82	79.0	2,262
Saccharomyces cerevisiae hsp82	78.1	1,568
Histoplasma capsulatum hsp82	67.0	1,153
Lycopersicon esculentum hsc80	64.8	1,894
Arabidopsis thalia hsp83	64.5	2,204
Pharbitis nil hsp83	64.1	1,617
Arabidopsis thalia hsp81	63.8	1,662
Chicken *hsp90*	63.7	1,608
Human *hsp90*	63.3	1,545
Rat *hsp90*	61.5	1,510
Mouse *hsp84*	61.0	1,507

Passive Immunotherapy

- Murine Mab 18B7 to GXM *Cryptococcus neoformans*
  - Optimum dose
  - HAMAs
  - Adverse effects
  - Recovery rate



(Larsen et al., 2005)

- Mab against Histone2B of *H. capsulatum*.
  - Opsonsophagocytosis and killing of yeast (via CR3)

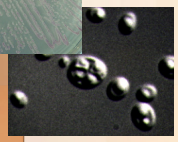
(Nosanchuk et al., 2003)

- Mab against β-1,2 mannotriose of *Candida*.

(Han et al., 2000)

Novel Passive Immunotherapy

Use of *Pichia anomala* killer toxin = KT Ag



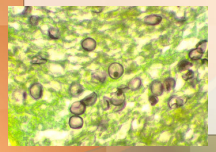
↓

Anti-idiotypic KT Ab

↓

Protection of vaginal Candidiasis,
P. carinii pneumonia.

(Magliani et al., 2005)



Vaccination to induce humeral immunity

Phosphomannoprotein from cell wall

(Han et al., 2001)

B-(1,3)- glucans from *Laminaria digitata* conjugated to diphtheria toxoid

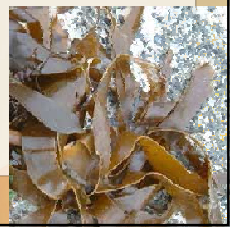
(Torosantucci et al., 2005)

A prophylactic vaccine in human for *C. albicans*?

GXM linked to protein carrier

(Devi et al., 1993, May et al., 2003)

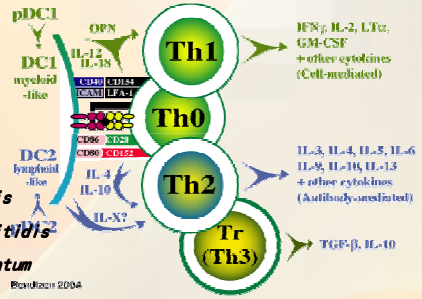
(Oscarson et al., 2005)



Vaccination to induce cell-mediated immunity.

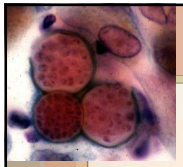
Responsive GOALS

Antigen specific T-cell response



Coccidioides immitis
Blastomyces dermatitidis
Histoplasma capsulatum
Pythium insidiosum
Cryptococcus neoformans

Vaccine candidates



Formadehyde-killed C. *immitis* spherule

(Pappagianis, 1993)

A live attenuated *B. dermatitidis*,

bad1 gene: adhesion receptor, inhibit TNF-α

bad1 Mutant

CD8+Tcell memory response Proinflammatory cytokine

(Finkel-Jimenez et al., 2001 and Wüthrich et al. 2003)

Vaccine development in Immune-deficient hosts

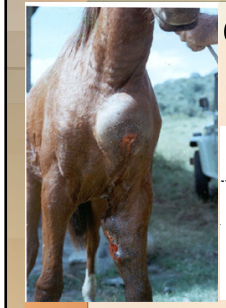
CD4+Tcells are dispensable for vaccine-induced resistance against fungal pnuemonia

B. dermatitidis
H. capsulatum

↑ TNF α ↑ IFN-γ ↑ GM-CSF

Wüthrich et al., 2003

Vaccine candidates



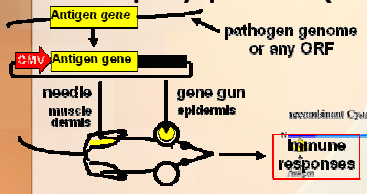
Crude soluble antigen vaccine for human and equine pythiosis.

Successful Tx associated with a shift from Th2 response to Th1 response

(Mendoza and Newton, 2005)

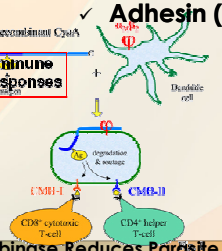
Bacterium-expressed recombinant vaccine

- *Coccidioides posadasii*
  - ✓β-1,3-glucanosyltransferase (*GEL1*) : rGel1p
  - ✓Aspartyl protease (*PEP1*) :



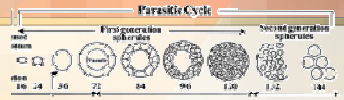
Candida albicans

✓ Adhesin (ALS1)



Recombinant Aspartic Hemoglobinase Reduces Parasite Load and Blood Loss after Hookworm Infection in Dogs

Bacterium-expressed recombinant vaccine



E. coli derived GEL1 (Delgado et al., 2003)
E. coli derived PEP1 (Tarcha et al., 2006)
E. coli derived *H. capsulatum* hsp60 (Gomez et al., 1995)
S. cerevisiae derived ALS1 (Ibrahim et al., 2005)

Protective T helper immune response promotes IFN-γ and IL12

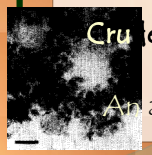
Vaccination to induce HMI and CMI.

The protocol failed to prevent fungal invasion or development of dermatophytic lesions.

Microsporum canis recombinant keratinolytic metalloprotease (r-MEP3) (Vermout et al., 2004)

Killed *Microsporum canis* cell-wall vaccine (DeBoer and Moreillo 1995)

Decreased 50% of the dermatophytosis incidence

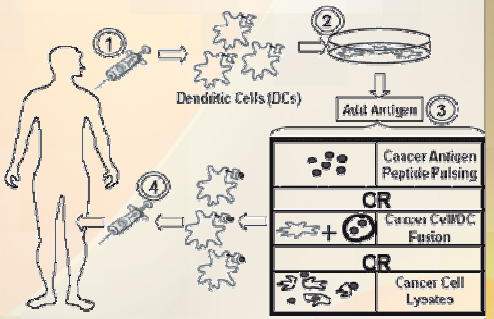


Crude ribosomal fraction *(CRF)* of *T. verrucosum* (Elad and Segal, 1995)

An attenuated strain of *Trichophyton verrucosum* (LTF 130) (Gudding and Lund, 1995)

Dendritic cell vaccination

· A unique vehicle for vaccination



(Banchereau and Palucka, 2005)

Dendritic cell vaccination

CMI response

Candida **RNA transfect DC generated Th1 response with IFN γ** (Bacci et al., 2000)

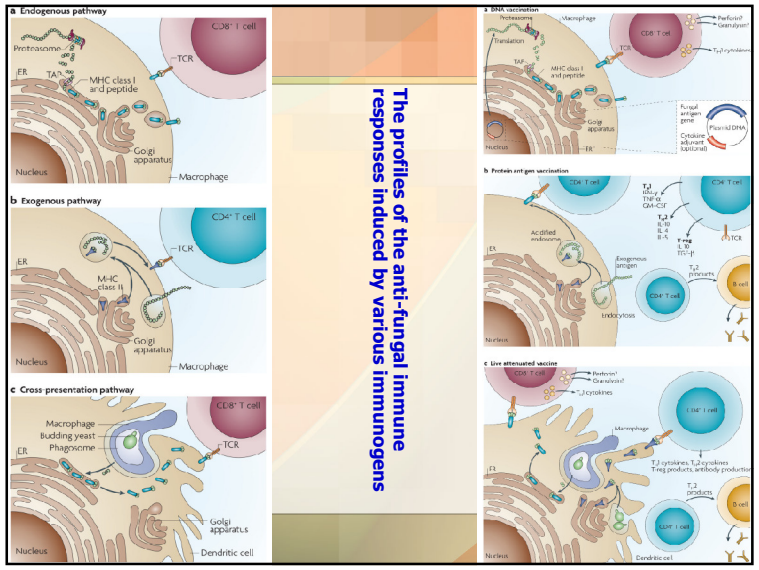
Route IV or SC ? (Eggert et al., 1999)

Conidia-derived RNA generated a Th1 response to *Aspergillus fumigatus.* (Bozza, 2003)

HMI response

(adenovirus-mediated transduction of CD40L+DCs)

CD40L+ DC pulsed with *P. carinii* cysts (Zheng et al., 2001)



Conclusion

Vaccinating should be associated—

- **Be worthwhile?**
- **Geographic distribution**
- **Nature of microbes**
  - Normal flora, opportunistic, pathogen
- **Host strengths**
  - Healthy, tissue transplantation, immune deficiency
- **Fragmentation of Pathogens**
  - Whole cells, surface associated, recombinant proteins,
- **Technology**
  - Detection, DC vaccine, DNA vaccine
- **Cost!!!**