Biomedicine & Pharmacotherapy 109 (2019) 333–344

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Review

Fungal vaccines, mechanism of actions and immunology: A comprehensive review

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ARTICLE INFO

Keywords:
Fungal infections
Vaccine
Immune response

ABSTRACT

Fungal infections include a wide range of opportunistic and invasive diseases. Two of four major fatal diseases in patients with human immunodeficiency virus (HIV) infection are related to the fungal infections, cryptococcosis, and pneumocystosis. Disseminated candidiasis and different clinical forms of aspergillosis annually impose expensive medical costs to governments and hospitalized patients and ultimately lead to high mortality rates. Therefore, urgent implementations are necessary to prevent the expansion of these diseases. Designing an effective vaccine is one of the most important approaches in this field. So far, numerous efforts have been carried out in developing an effective vaccine against fungal infections. Some of these challenges engaged in different stages of clinical trials but none of them could be approved by the United States Food and Drug Administration (FDA). Here, in addition to have a comprehensive overview on the data from studied vaccine programs, we will discuss the immunology response against fungal infections. Moreover, it will be attempted to clarify the underlying immune mechanisms of vaccines targeting different fungal infections that are crucial for designing an effective vaccination strategy.

1. Introduction

Nowadays, the importance of preventive and treatment methods for fungal infections is highlighted by increasing number of the high-risk groups exposed to invasive fungal infections (IFIs), including cancer patients under chemotherapy, bone marrow transplantation, acquired immune deficiency syndrome patients (AIDS), and all other diseases with immune deficiency following long-term hospitalizations [1,2]. IFIs could also be found in patients treated with a wide range of antibiotics and intravenously or intra-arterially catheter treatment methods [3], in premature infants, and the hospitalized patients in intensive care units [4]. Furthermore, 40% of patients with hematologic malignancies are exposed to IFIs [5]. Very low birth weight (VLBW) infants are at high risk to IFIs [6]. To prevent the expansion of IFIs, these infants require extensive therapies, such as intravenous catheters, long-term antibiotic regimes, and more importantly, postnatal steroid therapies. Most prevalent IFIs in VLBW infants are, *Candida* species (spp), *Malassezia* spp, *Aspergillus* spp, and *Zygomycetes* [6]. It has been shown that *Candida* spp are the fourth and first causative agents of nosocomial bloodstream infections in the US and the European countries, respectively [7]. Furthermore, despite an experimental therapy, the mortality rate of invasive candidiasis is about 30–40% [7]. HIV/AIDS patients show a high mortality rate following opportunistic fungal infections. *Cryptococcus neoformans* (*C. neoformans*) is also a most common yeast that infects these patients [8]. The outbreak of cryptococcosis in HIV/AIDS patients was increased at the beginning of the 1990s in the US, before the utilization of highly active antiretroviral therapy. Using this therapy between 1993–2000, a 92% decrease in the outbreak rate of this infection in HIV/AIDS patients was reported [9]. Generally, IFIs are responsible for 50% of the mortality cases which encompasses 1.5 million

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https://doi.org/10.1016/j.biopha.2018.10.075
Received 3 August 2018; Received in revised form 2 October 2018; Accepted 14 October 2018

subjects per year. Such a high mortality rate is followed by nonspecific clinical signs and symptoms, scarcity of the preventive methods, appropriate diagnosis, and sufficient antifungal medicines [10]. Considering an increasing population of immunocompromised patients and application of immunosuppressive treatments, we have been facing with extremely dramatic increase in the life-threatening infections even by the coexistent species, such as *Candida albicans* (*C. albicans*) [11]. Therefore, it is essential to review how the immune system controls the fungal infection.

2. Immunology of fungal infections

2.1. Innate immunity

The frontline battlefield of the immune system with fungi pathogen is the physical barriers, chiefly the skin and the mucosal epithelial surfaces, existing in mouth, upper airways, and the gastrointestinal and genitourinary tracts, which are constantly exposed to environmental organisms [12]. Moreover, epithelial cells play pivotal roles in launching the effective antifungal responses through discriminating pathogenic and non-pathogenic fungal morphotypes [13,14].

The critical step in the initiation of an immune response is the recognition of the specific components of fungi, called pathogen-associated molecular patterns (PAMPs), by pattern recognition receptors (PRRs) (Fig. 1). Different types of innate immune cells, including macrophages (MQs) and dendritic cells (DCs) express a vast repertoire of PRRs, such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs), and RIG-like receptors (RLRs) [15]. Among them, TLR2, TLR4, and Dectin-1 have prominent roles in detecting fungal cell wall PAMPs. Previously we showed that TLR2 gene expression increased in mice group with systemic candidiasis (SC) and also in cyclophosphamide-dependent immunosuppressed mice with SC [16]. However, our recently published data showed that TLR2 had no significant role in launching the immune responses in immunosuppressed mice [17]. Phagocytic cells, MQs, DCs, and neutrophils, are able to recognize the fungi at the first stages of infection through a variety of receptors (including PRRs) and combat with fungi through phagocyting and releasing antimicrobial components, such as oxygen radicals. Additionally, phagocytic cells are able to produce cytokines, which induce the maturation of $CD4^+$ T cells toward specific subtypes (Fig. 2) [18–20].

Complement system and other humoral factors, such as antifungal peptides, mannose-binding lectins (MBLs), defensins, and collectins also provide fundamental defense mechanisms through opsonization of fungi [12,19,21]. For example, recognition of deposited complement particles on β-(1,6)- glucans of the fungus surface by complement receptor 3 (CR3; a heterodimer of CD11b and CD18 which is expressed on different types of immune cells, such as neutrophils, monocyte/macrophages, and natural killer (NK) cells) leads to elimination of pathogens by phagocytic cells [19], a process called opsonophagocytosis. Defensins (which are secreted by the epithelium and paneth cells in gut) and collectins are involved in opsonizing and also induction of inflammatory responses in collaborating with helper T (Th)-17 profile cytokines (Fig. 2) [3].

2.2. Acquired immunity

In addition to recognizing different regions of fungal cells through different types of PRRs, antigen-presenting cells (APCs), including DCs, MQs, and B cells present the antigenic epitopes on major histocompatibility complex (MHC) class II or class I molecules (which are expressed on APC surfaces) to $CD4^+$ or $CD8^+$ T cells, respectively [17,22,23]. This way, these cells stimulate acquired immune response (Fig. 2).

During this process, environmental cytokines produced at the site of APC-T cell binding trigger the differentiation of $CD4^+$ T cells into a specific Th cell subtypes through activating different signaling pathways. STAT1/STAT4 transcription factors are needed for Th1 differentiation, while STAT3/ROR-γt are required for Th17 development, GATA3/STAT6 are involved in Th2 development (Fig. 2) [12,18,19,24]. If DCs (known as the major APC) release interleukin (IL)-12, the $CD4^+$ T cells will be differentiated to Th1 cells. Different sets of immune responses will emanate from Th-triggered cytokines [19]. Th1 and Th17 cytokines, chiefly, gamma-interferon (IFN-γ) and IL-17, produce protective and protective-inflammatory responses, respectively [25,26]. More precisely, IFN-γ induces cell-mediated immunity through stimulating phagocytes and Th17 cells release IL-17 and IL-22 cytokines that initiate the neutrophilic response and release antimicrobial peptides peptides like defensins to the site of infection [27]. Finally, at the end stages of immune responses, $Foxp3^+/CD4^+$ T cells, which are called regulatory T (Treg) cells, release the transforming growth factor (TGF)-β and IL-10 in order to repress the elevated levels of inflammatory responses (Fig. 2) [28].

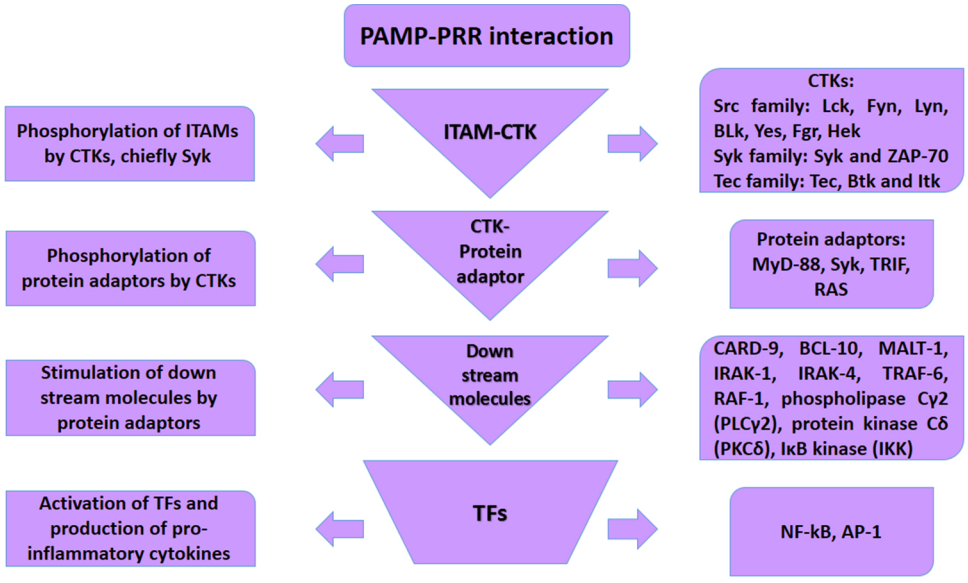

Fig. 1. Signaling pathway illustration during fungal sensing and processing. Following PAMP-PRR interaction, CTKs phosphorylate both central tyrosine of ITAM and also protein adaptors which triggers the further stimulation of downstream signaling mediators and eventually leads to the production of pro-inflammatory cytokines and other soluble mediators through activation of TFs. CTK; cytoplasmic tyrosine kinase. ITAM; immunoreceptor tyrosine-based activation motif. TFs; transcription factors.

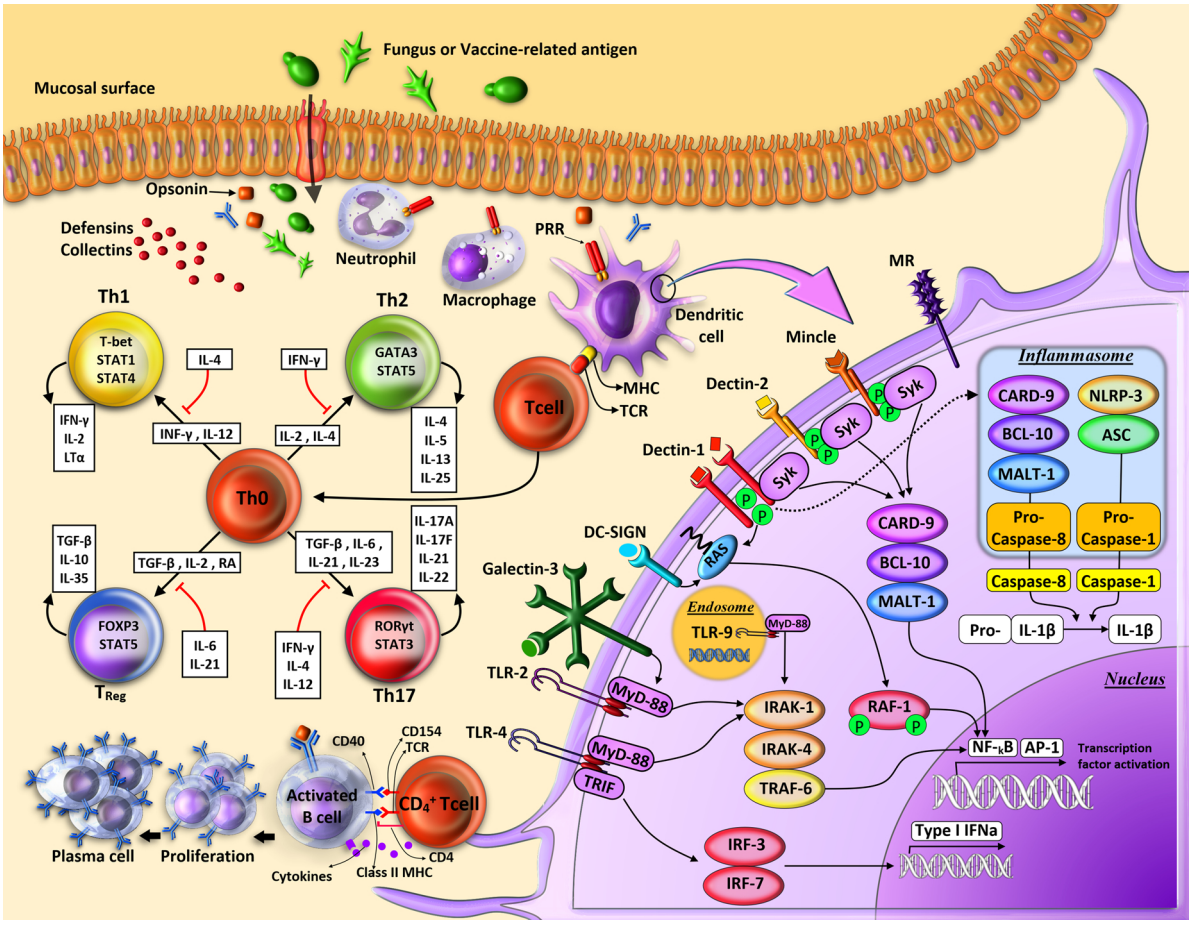

Fig. 2. Immune responses induced by fungus or vaccine-related antigens. After the failure of epithelial surfaces, as the first defense line against fungal infections, the immune response starts a new phase. Following the PRRs (TLRs, Dectins, Galectin-3, Mannos receptors, DC-SIGN, and Mincle) and PAMPs interaction, some specific signaling pathways are stimulated in the APC, eventually leading to the production of different cytokines. In this regard, the protein adaptors, such as MyD88, Syk, RAS, and TRIF are activated through connecting to the cytoplasmic stimulatory domain of PRRs (such as TIR for TLRs), which then trigger the downstream adaptor proteins (CARD-9, BCL-10, MALT-1, IRAK-1, IRAK-4, TRAF-6, and RAF-1). This, in turn, ultimately activate the transcription factors (NF-kB, AP-1, IRF-3, and IRF-7), resulting in the production of cytokines. Moreover, Dectin-1 is able to stimulate the inflammasomes (consisted of different adapter proteins) and finally triggers Caspase-8 and Caspase-1, which catalyze the production of IL-1β from pro-IL-1β. At the next step, the processed antigen in the APC (chiefly DCs) are presented to naive T cells. According to the cytokines resulted from PAMP-PRR interaction, the class of T cell is formed. For example, IL-23, IL-6, and TGF-β trigger the stimulation of the Th1 profile (through T-bet, STAT1, and STAT4 transcription factors) that induce the Th17 cytokines, such as IL-17 and IL-21, which are the main players of inflammatory response. B cell responds to fungal antigens through two different ways. One is through T-independent (TI) response against non-protein antigens (polysaccharides, lipids, glycolipids, acid nucleic). Due to the absence of T cell responses, no immunological memory, secondary response, affinity maturation, and isotype switching (usually conducted by cytokines of T cell) are occurred, eventually leading to the production of IgM isotype antibodies with low-affinity and low half-life. B cell also responds to protein antigens through the T-dependent (TD) immunity. The processed antigen is presented to the $CD4^+$ T cell by MHC class II molecules and CD40-CD40 L (CD154) interaction. Following the activation of $CD4^+$ T cells, the desired help package is released (consisting different cytokines and molecules) to the B cells. Afterwards, B cell responds to the help through triggering the specific signal transduction, which eventually leads to the production of various antibody isotypes (isotype switching) with high affinity (affinity maturation) and high half-life. This also includes the immunological memory and secondary responses. Nowadays, TD immunization is followed by many research projects (subunit and conjugate vaccines) through binding fungal polysaccharides to the engineered/synthesized proteins.

3. Fungal vaccines/main categories

Based on the analysis of different kinds of vaccines against infectious agents, it has been reported that vaccines annually prevent 6 million deaths all around the world [29]. The aim of this review is providing the comprehensive review of antifungal vaccines and their immune mechanisms. Here, different kinds of vaccines which are used for prevention of fungal infections are classified into three main groups. We discuss live-attenuated, recombinant, and conjugate vaccines (Fig. 3). Finally, almost all of the studied anti-fungal vaccine programs are gathered and presented in Table 1 to form an overall view.

3.1. Live-attenuated vaccines

According to the similarity of live-attenuated vaccines with infectious agents, they launch long-term and strong immune responses, which can be efficient in the immunocompetent patients. However, consideration of the precautionary aspects seems to be necessary. Vaccinologists have designed several products of live-attenuated vaccine strategies, which are very efficacious to combat with highly-infectious disease, mainly infectious viruses containing influenza, polio, mumps, rubella, measles, varicella, and rotavirus [30]. During infection with different pathogenic fungi, such as *Histoplasma capsulatum* (*H. capsulatum*), Blastomyces *dermatitidis* (*B. dermatitidis*), *Paracoccidioides brasiliensis* (*P. brasiliensis*), *Pneumocystis carinii* (*P. carinii*), and *C. neoformans*, these strategies are highly effective through triggering

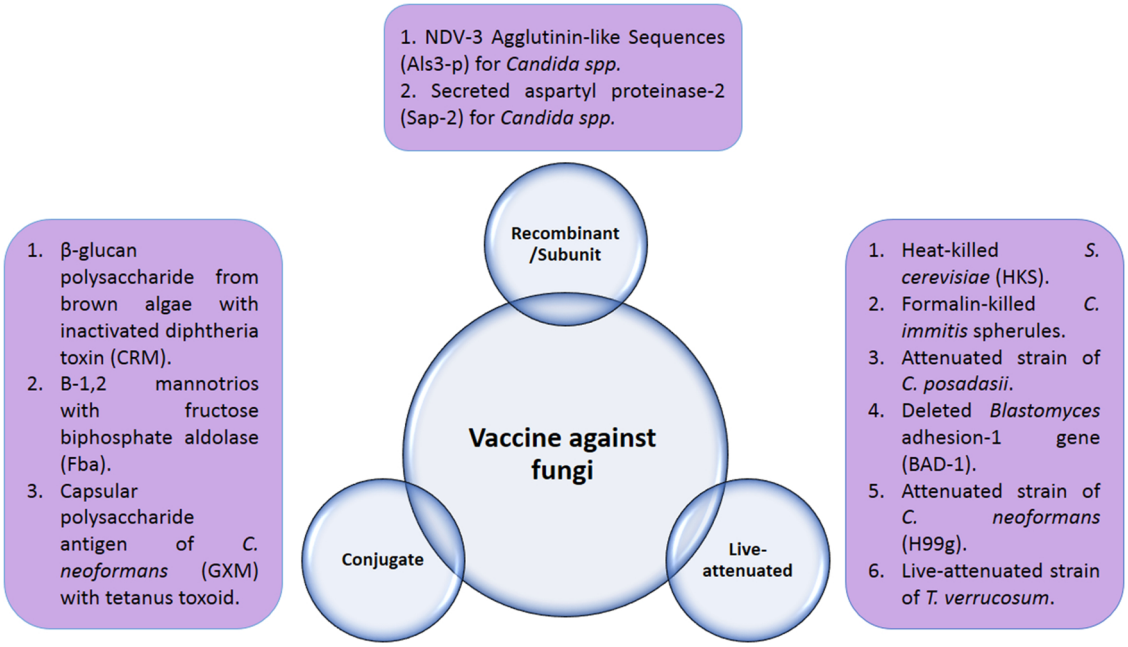

Fig. 3. Three main category of vaccine against fungi.

protective immune responses via common pathways (Table 1) [30,31]. This type is the first vaccine used in human subjects. There are several studies in this field evaluating the efficiency of killed and attenuated fungi (Table 1). These vaccines will be applicable for endemic fungal pathogen prevention in the future in subjects with healthy immune system who live in endemic areas [32,33]. One important finding is the heat-killed *Saccharomyces cerevisiae* (HKS) vaccine, which plays an important role in protection against different fungal infections as a pan-fungal vaccine plan (discussed below) [34,35]. Vaccination with HKS through a subcutaneous route has been shown to be effective in protection against virulent strains of the endemic fungus *Coccidioides posadasii* (C. *posadasii*) [32], *C. albicans* [35] and *Aspergillus fumigatus (A. fumigatus*) [35]. In addition, a study reviewed the clinical efforts about developing of whole recombinant *S. cerevisiae*-based therapeutic method for the treatment of cancer and viral diseases together with cytotoxic drugs to achieve more clinical responses [36]. One major issue is the specificity of the vaccine, which limits the spectrum of its effects [34].

Formalin-killed *Coccidioides immitis* (*C. immitis*) spherules (FKS) is another vaccine type in this category. Previously, a placebo-controlled phase III trial has been carried out to evaluate the efficacy of FKS that was unsuccessful to prevent the harshness of infection [36]. Later, a study showed that vaccination (subcutaneously or by oral gavage with or without adjuvants) with HKS protected 100% of CD1 mice from a lethal *C. immitis* challenge through prolonging survival and reducing fungal burden. Oral live *Saccharomyces*, but not HKS, prolonged survival without reduction in fungal burden. Survival of mice given HKS was equal with FKS. This study indicates that HKS was superior to a successful recombinant vaccine with adjuvant [32]. Moreover, a study showed promising results of subcutaneous immunization of mice model with an attenuated strain of *C. posadasii* [33]. This strain was unable to transform to pathogenic spherule form and endosporulation process, following deletion of two chitinase genes.

Deletion of *Blastomyces* adhesion 1 (BAD-1) gene presents an attenuated vaccine which has been shown to recruit multiple arms of the host immune response (Fig. 2) [37–40]. A study tested an immunization plan for the BAD-1 vaccine in $CD4^+$ T cell deficient host like HIV/AIDS patients. In the absence of T helper cells, fungal PAMPs activate memory $CD8^+$ cells via interaction between MHC class I and $CD8^+$ T cell that leading to secretion of their cytokines, such as tumor necrosis factor (TNF)-α, IFN-γ, and granulocyte/macrophage colony-stimulating factor (GMCSF). This study indicates that $CD8^+$ T cells could also rely on alternate mechanisms for robust vaccine immunity against experimental fungal pulmonary infections with two agents, B. dermatitidis and H. capsulatum [41]. In the same framework, the genetically engineered BAD-1 attenuated strain has also been tested that eventually leads to the failure in binding or entry of yeasts into macrophages and adherence to lung tissue, and also reduction of virulence in mice. [42]. Another study showed that subcutaneous administration of the BAD-1 live yeast without any adjuvant elevated the survival rate of mice from lethal challenge of *B. dermatitidis* [43]. Mice immunized with recombinant BAD-1 yeasts, alone or in combination with IL-12 as an adjuvant, showed acceptable efficacy in launching immune responses (Fig. 2) [44,45].

Another attenuated vaccine strategy, which is named H99 g, has previously been shown to protect $CD4^+$ T cell-deficient mice from infection with a virulent strain of *C. neoformans* through inducing murine IFN-γ and Th1 responses [46]. The H99 g strain is regarded as a live vaccination plan which is a potent stimulator of host cytokine production and, therefore, could not be usable in human subjects. A similar work reported the critical role of both $CD4^+$ and $CD8^+$ T cells in the protection of mice against C. *neoformans* infection [47]. The safety of attenuated vaccines in the immunosuppressed hosts has not been guaranteed. But these two recent strategies (BAD-1 and H99 g) may immunize the $CD4^+$ T cell-deficient subjects, particularly HIV/AIDS patients [48].

As a first live attenuated plan, a vaccine was designed for the prevention of ringworm caused by *Trichophyton verrucosum* (T. *verrucosum*) in cattle [30,49]. This study was carried out in a 5-year period on over than 400,000 cattle and demonstrated the efficacy of this immunization-immunoprophylaxis strategy. The results of these studies clear the perspectives about the future utilization of antifungal vaccines for subjects with $CD4^+$ T cell deficiencies, such as patients with HIV infection.

The main challenge is that the application of attenuated vaccines should not lead to other kinds of diseases in immunosuppressed patients.

Table 1
Studies conducted in the design of antifungal vaccine strategy (Nd; non-determined).

Target Pathogen	Antigen/Strain	Adjuant/carrier/ Vehicle	Vaccine Type	Model	Route of injection	Underlying immune mechanism	Human clinical trial	Reference(s)
Candidisis	Als3p Als1p	Aluminium hydroxide (Alum)	Recombinant protein(NDV-3)	Mice/Human	Oropharyngeal, vaginal and intravenous	IgA1, IgG IL17 A, IFN-γ	Phase I	[57], [58], [60,82], [83] [84],
	SAP2	Cholera toxin (CT)/Virosomal carrier	Recombinant protein	Mice/ Human	Intravaginally	Protective antibodies	Yes (delivered by intramuscular)	[60,85]
	secreted aspartyl proteinase protein, Sap2p PEV-7	Cholera toxin (CT)	Recombinant	Rat	Intravaginally	Antibodies	–	[85]
	Tet-NRG1 *(C. albicans* strain)	Nd (Not defined)	Genetically engineered/Live attenuated	Nd	Nd	T-cell mediated immunity		[86,87]
	C. albicans PCA-2 strain	Nd	Live-attenuated	Mice	Intravenously	Increased PMNs and macrophage activity	–	[88]
	Cell wall surface proteins (CWSP)	Liposomal adjuvant	Subunit	Mice	Subcutaneously	Antibodies, Th17	–	[89]
	C.albicans Mannan extracts	–	Mannan-protein conjugate	Mice	Intravaginaly	Protective antibody responses	–	[90]
	Laminarin (Lam) β-glucan	Complete Freund's adjuvant (CFA)	Lam- diphtheria toxoid CRM197 conjugate	Mice	Priming dose: Subcutaneously Booster: Intranasally	Passive protection by anti β-glucan antibodies Cross protection against *A.fumigatus* infection	–	[67,90,91,92,93,94]
	C. dubliniensis mannan/ Human serum albumin (HSA)	Nd	Conjugate	Rabbit	Intravenously	Th1/Antibodies: IgG and IgA	–	[95]
	Fructose bisphosphate aldolase (Fba) (cytosolic and cell wall peptides)	CFA	Subunit	Mice	Intraperitoneally/ Subcutaneously	Antibodies	–	[96] [97],
	C. albicans serotypes a and b ribosomes	Nonencapsulated *Klebsiella pneumoniae* proteoglycan	Recombinant/ Conjugate capsule	Women with vulvovaginal candidiasis (VVC)	Oral	Nd	phase II	[98]
	Heat-killed *C. albicans* (HK-CA)	*Detoxified Escherichia coli: LT(R192 G)*	Recombinant/ Conjugate	Mice	Intranasally/ Intravenously	Antibody(IgG, IgA)-Th1	–	[99]
	Glycolytic enzyme enolase	complete Freund	Recombinant	Mice	Subcutaneously	Antibody- Th1/2	–	[100]
	65 kDa mannoprotein (Camp65p)	*Plasmid: pRLV130-139-140-161-162-169*	Subunit/ Glycoconjucates	Mice	Intravaginally/ Intravenously	Adhesin-neutralising antibodies	–	[101]
	C. albicans cell surface protein Hyr1	Alum	Recombinant N-terminus of Hyr1 protein (rHyr1p-N)	Mice	Subcutaneously	Antibodies	–	[102]
	Combining β-mannan and peptide epitopes	CFA	Subunit/Conjugate	Mice	Intravenously	Th1, antibodies	–	[103]

(*continued on next page*)

Table 1 (*continued*)

Target Pathogen	Antigen/Strain	Adjuant/carrier/ Vehicle	Vaccine Type	Model	Route of injection	Underlying immune mechanism	Human clinical trial	Reference(s)
Aspergillusis	*Aspergillus fumigatus* crude culture filtrate Ags	Nd	Sonicate and filtrate Ags	Mice	Intranasally	Th1 cells producing IFN-γ and IL-2	–	[104]
	Asp f3	Incomplete Freund's adjuvant	Subunit/ recombinant	Mice	Subcutaneously	Antibodies/$CD4^+$ T cells		[105]
	Aspergillus fumigatus viable conidia	Nd	Sonicate and filtrate Ags	Corticosteroid ImmunosuppressedMice	Intranasally/ subcutaneously	Unknown	–	[106]
	Aspergillus fumigatus hyphal sonicate (HS)	Aspergillus fumigatus allergen Asp f 3	Recombinant	Mice	Subcutaneously	Antibody & cellular immunity responses	–	[107]
	Heat killed yeast (HKY) of *S. cerevisiae*	Nd	Live-attenuated	Antibody knockout mice	Subcutaneously	Th1, Th2, Th17	–	[108,109]
	A. fumigatus epitope p41 from the cell wall glucanase (Crf1)	Murine cytosine guanine dinucleotide (CpG) oligodeoxynucleotide(ODN)	Subunit	Mice	Intranasally/ Intragastricly	1. MHC II alleles that induces memory CD4 + TH1 cells. 2. cross-protection against lethal infection with *C. albicans* that is mediated by the same epitope as in humans		[110]
	Asp 16 f	Unmethylated CpG oligodeoxynucleotides (ODNs)	Recombinant/ subunit (DCs) pulsed with *Aspergillus* antigens	Mice	Intranasally	Th1	–	[111]
	Asp 3 f	TiterMax (TM)	Recombinant/ subunit	Mice	Subcutaneously	Th1	–	[107]
	Proteins: Gel1p, Crf1p, Pep1p, Cat1p, Sod1p, Dpp5p, RNUp, Mep1p, Polysaccharides: _1–3 glucan, _1–3 glucan, GM, Glycolipids: GSL, LGM	CpG oligodeoxynucleotide	*Recombinant/ Subunit*	*Bone marrow transplanted mice*	Intranasally	Th1	–	[112]
Panfungal	β-glucans of *S. cerevisiae*	Nd	Heat Killed Yeast (HKY)	Mice	Subcutaneous	Th1, Th17, Antibodies to glucan and mannan	–	[34]
Blastomycosis	Adhesin BAD1 gene	Nd	Whole organism/ Live-attenuated	Mice (T $CD4^+$ depleted)	Subcutaneously	$CD8^+$ T cells, MHC I, Th1 immunity	–	[41]
Paracoccidioidomycosis (PCM)	gp 43 (P10)	Plasmid vector	DNA vaccine (pcDNA3-P10)	Mice	Nd	T-reg cells Immunological memory	–	[1,113]
	gp 43 (P10)	*S. cerevisiae* expressing gp43 (yMAgp43)	Recombinant protein	Mice	Intraperitoneally	Th1 immunity/ elevation of IL-12 and IFN-γ	–	[114]
	P10- FliC fusion protein	CFA/MAP	Recombinant	Mice	Intratracheally	Th1	–	[115]
	rPb27	Corynebacterium parvum/ aluminum hydroxide Al(OH)3	Recombinant	Mice	Subcutaneously	Antibodies	–	[116]
	Heat shock protein 60 (HSP60)	Adjuvant containing monophosphoryl lipid A, synthetic trehalose dicorynomycolate, and cell wall skeleton	Recombinant	Mice	Subcutaneously	Th1	–	[117]
	Mycobacterium leprae-derived HSP65	Vector pVAX1/	Recombinant DNA	Mice	Intramuscular	Th1	–	[118]

(*continued on next page*)

Table 1 (*continued*)

Target Pathogen	Antigen/Strain	Adjuant/carrier/ Vehicle	Vaccine Type	Model	Route of injection	Underlying immune mechanism	Human clinical trial	Reference(s)
Coccidiomycosis	Killed spheroles	Nd	Whole organism/ Inactivated	Human	Intramuscularly in the deltoideus	Nd	Phase 3	[81]
	C. immitis spherule-phase genes	pBK-CMV phagemid vector	DNA(expression library immunization: ELI)	Mice	Intramuscularly/ Intradermaly	$CD4^+$ and $CD8^+$ T cells/T-helper 1 cells	–	[119,120]
	T-cell epitopes Antigen 2/ proline rich Ag (Ag2/ PRA)/Chimeric polyprotein	Adjuvant: CpG Vector: YEp-FLAG-1	Recombinant protein	Mice	Subcutaneously	Th1, Th17, Th2	–	[121,122,123]
	Attenuated mutant (ΔT vaccine strain)	Nd	Live-attenuated	Mice	Subcutaneously	Th1, Th17, Th2	–	[124]
	Immunodominant T cell epitopes	Adjuvant: CpG	Recombinant	Mice	Subcutaneously	Th1, Th17, Th2	–	[125]
	C. posadasii Gel-1 (β 1,3 glucosyltransferase)	CpG ODN	Recombinant protein	Mice	Subcutaneously	Th1. Antibody	–	[126]
	Urease (rURE)	Plasmid vector :pSecTag2A/ Adjuvant: CpG ODN	Recombinant	Mice	Subcutaneously	(Th1)/Th2	–	[127]
	Spherule phase of *C. posadasii* Peroxisomal matrix protein (Pmp1)	Monophosphoryl lipid A-stable emulsion (MPL-SE) adjuvant vector: YEp-FLAG-1	Recombinant	Mice	Subcutaneously	Elevated IgG titer	–	[128]
	Chimeric protein-aspartyl proteinase, phospholipase B and α mannosidase	Adjuvant: CpG ODN Vector: pGEM-TE	recombinant protein	Mice	Subcutaneously	Nd	–	[129]
Histoplasmosis	Water-soluble ethylenediamine extract from cell wall	CFA	Inactivated-filtrated Ags/ Soluble antigenic fractions	Mice	Intraperitoneally	Nd	–	[130]
	Ribosomes or live yeast cells of H. capsulatum	Incomplete Freund	Live-attenuated	Mice	Intravenously/ Subcutaneously	Lymphoid cells	–	[131]
	Cell wall and cell membrane of yeast-phase H. capsulatum G217B	Incomplete Freund	Live-attenuated	Mice	Subcutaneously/ Intraperitoneal/ Intravenously	Nd	–	[132]
	Histone H2B–like protein	Incomplete Freund's	Live-attenuated/ Recombinant	Mice	Intraperitoneally	Th1/Antibody	–	[133]
	Heat Shock Protein 60 (HSP-60)	–	Recombinant	Mice	Subcutaneously	Th1	–	[134,135]
	HIS-62	Bovine serum albumin (BSA)	Recombinant protein rHIS-62	Mice	Subcutaneously	Cellular immune response	–	[136]
	80-kilodalton antigen	Incomplete /Complete Freund's	Recombinant	Mice	Subcutaneously	Antibody/Cellular responses	–	[137]
	Sec31 homologue	Monophosphoryl lipid A, trehalose dicorynomycolate, and cell wall skeleton	Recombinant	Mice	Subcutaneously	T cell mediated	–	[138]
	H antigen(H.capsulatom)	Adjuvant containing monophosphoryl lipid A, synthetic trehalose dicorynomycolate, and cell wall skeleton and bovine serum albumin (BSA)	Recombinant antigen	Mice	Subcutaneously	Th1.Th2/CD8+	–	[139]

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Table 1 (*continued*)

Target Pathogen	Antigen/Strain	Adjuant/carrier/ Vehicle	Vaccine Type	Model	Route of injection	Underlying immune mechanism	Human clinical trial	Reference(s)
Cryptococcusis	GXM	Tetanus toxoid (GXM-TT)	Conjugate/Soluble antigenic fractions	Mice	Subcutaneously	Anti-GMX antibodies (Active immunization)	–	[66]
	GalXM	Quil A/ Freund's complete/ BSA	Subunit/Conjugate	Mice	Subcutaneously/ Intraperitoneally	Antibody: IgG, IgM	–	[140]
	C. neoformans strain H99γ (serotype A, *Matα*)	Nd	Live-attenuated	T-cell depleted mice	Nasal inhalation	$CD4^+$ and $CD8^+$ T cells	–	[47]
	Mutant *C. neoformans* strain lacking the enzyme sterylglucosidase 1 named (Δsgl1)	Plasmid pCR II-TOPO 4.0 kb	Live- attenuated-recombinant	$CD4^+$ T cells depleted /immunocompetent mice	Intranasally	$CD4^+$ and $CD8^+$ T cells	–	[141]
	CneF (culture filtrate Ags), Mannoprotein	Nd	Subunit/ Recombinant	Mice	Nd	Th1, Antibodies	–	[62]
	GXM	CFA	GXM–protein conjugate	Mice	Intraperitoneally	High-titer IgG responses	–	[142]
	P13 (a peptide mimetic of GXM)	Tetanus toxoid (TT) or diphtheria toxoid (DT)	Conjugated	Mice	Subcutaneously	Anti GXM antibody response(IgG2 & IgG4)	–	[143,144,145,146,147,148]
	Laminaran	Nd	Subunit (algal β-glucan based)	Mice	Nd	Passive immunity		[149]
Pneumocystosis	Kexin genes	Adjuvant: CD40L Vector: CMV to express Antigene EF-1α to express CD40L	Kexin-CD40 L DNA vaccine	CD4-deficient mice	Intramuscularly	Elevated IgG titers	–	[120]
	P55 protein (major surface glycoprotein)	Titermax	Recombinant protein	Mice	Subcutaneously	Th1-Th2 responses	–	[150]
	Major surface glycoprotein (also known as gp120)	Titermax	Recombinant protein	Mice	Subcutaneously	Antibody and T-cell dependent	–	[151]

3.2. Recombinant (subunit) vaccines

Subunit vaccines are the most investigated sorts of fungal vaccines that consist of one or more purified recombinant proteins or polysaccharides of fungi. Genetic engineering and also increased knowledge in the microbial pathogenesis and fundamentals of immunology help scientists to develop efficient subunit vaccines. Scientific basis of this technology is transferring and expressing of a gene encoding an immunogenic antigen, in order to trigger the desired immune response. In fact, in this approach, a gene which is transmitted encodes a portion related to virulence and pathogenicity of organism. These protein antigens are often combined with an appropriate adjuvant or protein carrier, mostly bacterial toxoids, to establish an efficient immune response and prolonged immunization (Fig. 2) [31,48,50]. Alum (aluminum salts, such as aluminum hydroxide and aluminum phosphate) is one of the most common adjuvants in this field which induces strong antibody responses [48,51,52]. Recombinant subunit vaccines has several advantages, such as absence of the pathogenic agent and, therefore, application of these vaccines becomes safer particularly in immunocompromised patients [48,53]. By merging the DNA engineering and recombination technologies, vaccines have been carefully designed, purified, and produced, which leads to the engineering of highly specialized antigens [54,55].

A study showed that an invasion protein, agglutinin-like sequence 3 (Als3p) conjugated with alum, which is called NDV-3, conferred anti-Candida protection through preventing yeast-epithelial/endothelial attachment [56]. In addition, NDV-3 induced a cross-protection against highly infectious bacterial pathogens, such as *Staphylococcus aureus* (S. *aureus*) due to the structural homology between Als3p and clumping factor-A of S. *aureus*. Most importantly, NDV-3 successfully passed the phase I clinical trial and was found to be safe and protective in human subjects through triggering the antigen-specific T cells that released IFN-γ and IL-17 A cytokines [57]. This vaccine has also been approved to elicit a protection in animal models of oropharyngeal, vaginal, and invasive candidiasis [48,58,59].

Another study showed that secreted aspartyl proteinase-2 (Sap-2), a highly expressed virulence factor secreted by different *Candida spp*, displayed protective roles against recurrent vaginal candidiasis in a virosome-based format of the vaccine [48,60]. This vaccine was applied in the rat model of vaginal candidiasis and also a phase I clinical trial and showed effective results [48,60]. However, there are several problems in the commercialization of recombinant vaccines, such as healthy status of a subject (both immunocompromised and immunocompetent hosts), economic issues in targeting the human subject (high costs of application in clinical trials), and also the method of synthesis of the vaccine, such as glycosylation, which directly affects the immunization circumstances [48,61,62].

3.3. Conjugate vaccines

A conjugate vaccine is produced by covalent attaching of a poor antigen to a strong antigen, commonly polysaccharide to protein, respectively. This is carried out in order to generate a potent immune response [63]. B cells, in confronting with polysaccharide antigens, develop antibody responses without contribution of T cells, which is called T-independent immune response. In fact, polysaccharide epitopes are recognized by B cell receptors, but for the presentation of antigens to T cells, they should bind to peptides (hapten-carrier system) and the peptide is required to be presented by MHC complexes expressed on the APCs. Immunity stimulated by T cells is a strong and durable. Through conjugating a polysaccharide to a protein carrier, MHC molecules are able to bind proteins and eventually induce the T cell responses (Fig. 2) [48,64].

One major advantage of conjugate vaccine strategy is that these vaccines are based on targeting the polysaccharide epitopes, which are common in all fungi, especially β-glucans. Therefore, this technique could be applicable to produce and commercialize pan-fungal vaccines. This is very crucial for immunosuppressed patients which are at high risk for various form of IFIs [63,65].

The first fungal conjugate vaccine was designed against *C. neoformans* that contained glucuronoxylomannan (GXM), a capsular polysaccharide, and tetanus toxoid (TT) [66]. These two particles are linked by a covalent bond and a monophosphoryl lipid A (MPL) is used as an adjuvant in the vaccine complex. Immune mechanism of this vaccine is based on the antibody (especially IgA and IgG) responses. Additionally, a pan-fungal vaccine, designed by conjugating a β-glucan polysaccharide extracted from brown algae, to inactivated diphtheria toxin (CRM) and complete freund's adjuvant (CFA), showed effective roles in protection against invasive candidiasis and aspergillosis [67]. Another conjugate anti-Candida vaccine was constructed by conjugating β-1,2-mannotriose to a peptide segment from fructose-bisphosphate aldolase (Fba), which is the surface antigens of *Candida* spp. Various forms of this vaccine have been applied in different studies (with or without alum adjuvant) [68] (see Table 1).

4. Novel strategies

4.1. DNA vaccines

By entering the cDNA encoding the desired antigen into a plasmid and transferring the gene containing plasmid to the host's APCs (mainly DCs), the antigen is expressed and eventually generates a desired immune response. Bacterial plasmids contain non-methylated CpGs, which are recognized by TLR9 (expressed on DCs), and further stimulate the acquired immune responses. In addition to the antigen coding gene, the gene which codes the co-stimulatory molecules and also cytokines can join to the plasmids. This vaccine could also be applied without any adjuvant. However, despite the current theories on safety, immunogenicity, and efficacy, the application of this type of vaccine for human faced with some major challenges [30,69]. Previously, DNA vaccines have also been examined through transferring one or more antigen coding plasmids [70–72]. The first fungal DNA vaccine may be related to ringworm caused by *T. verrocosum*, which was discussed above [49].

4.2. Immunotherapeutic products

Two novel vaccine strategies share immunotherapeutic nature based on the application of fungus antigen-primed DCs and/or fungus-specific T cell clones [31,73,74]. In these programs, fungus-protective antigens were identified, regenerated, primed to the DCs ex vivo, and eventually infused to the host in order to selective priming-activation of DCs and formation of highly specific T cell clones. Immune responses produced by these strategies have clearly potent effects and precision. Romani and colleagues described the benefits of these approaches [31,73,74].

4.3. Pan-fungal vaccine strategy

Fungal cell wall contains common epitopes. By inactivating and conjugating the fungal common polysaccharides with different immunogenic peptides, we are able to form a type of vaccine which protects the host from different types of fungal infections. Nowadays, two pan-fungal vaccine plans have been developed. Killed S. *cerevisiae* triggers the protective immune response against glucans and mannans, which are common fungal polysaccharide epitopes. This vaccine also launches the cross-reactive immune responses against homologous proteins which exist on the fungal cell walls [34]. Another universal vaccine strategy is made up from conjugating β-glucans to an inactivated version of diphtheria toxin (CRM), which was mentioned above [67].

5. Vaccine based immunity against fungal infections

One of the most interesting and fascinating topics is to predict the immune responses before and after exposure to the vaccine agent in both healthy and infected hosts. In other words, each vaccine stimulates the immune responses through different and more-specified ways, which scrutinizing them is one major point in progressing the vaccine strategies against fungal infections. We previously discussed the immune responses against fungi. Totally, T cell-mediated responses are the main arms of the immune response in combating fungi. Furthermore, there are several cellular (macrophages, neutrophils) and soluble (antimicrobial peptides, cytokines, and chemokines) tools. Vaccines not only launch but also amplify the immune responses, particularly the vaccines used in conjugation with an adjuvant. Some of the antifungal vaccines enhance antibody responses and others mostly intensify Th responses. But most of them simultaneously boost both types of immune responses (Fig. 2). The immune responses used for each vaccine are extracted and listed in Table 1. Here, we review the mechanisms of some important vaccines.

5.1. Antibody mediated vaccine responses

Studies showed that specific antibodies cause protection against C. *neoformans* by triggering classical opsonophagocytosis, complement activation, and direct neutralization of adhesins or enzymes, which are totally humoral immunity [62,75]. There are several studies about antibody-mediated immunity to fungal infections induced by passive vaccination strategies (Table 1). The immune mechanism emerged from NDV3 and above discussed conjugate vaccine for cryptococcosis [66] are based on IgG and IgA antibodies. Alum adjuvant is also a potent stimulator of antibody responses (discussed above).

5.2. Th mediated vaccine responses

Th1/Th17 profiles play major roles in eliciting protective/inflammatory responses, which are triggered by different types of fungal vaccines (Fig. 2). Therefore, vaccinologists have focused on this field more precisely. A lot of studies in this field showed that the IFN-γ/IL-17 responses and also other receptors and cytokines, which are crucial for Th1 and Th17 responses, play pivotal roles in vaccine mechanisms. For example H99 g induces Th1 profile cytokines, chiefly IFN-γ [46], and another vaccines triggering both $CD4^+$ and $CD8^+$ T cells (discussed above) [47]. Predominanly, these studies have been conducted more specifically for candidiasis and aspergillosis [76], which indicated that Th1/Th17 mediated immunity by vaccines are far important than other factors, such as neutralizing antibodies. DNA vaccines are able to stimulate both $CD4^+$ and $CD8^+$ T cell responses through the MHC class I and MHC class II pathways (Fig. 2). They also activate the phagocytic/cytotoxic effectors and humoral responses. Another interesting topic in this area is the upgraded collaboration between Th17 and neutralizing antibodies by different types of the vaccines [76–78]. As discussed above, the subunit vaccines are typical examples of the vaccines that induce multiple immune responses, including T cells and antibodies. Almost all of the existing vaccines mediate the protection through both Th17 and neutralizing antibody-mediated mechanisms [79].

6. Conclusion

In recent decades, a wide range of studies tested vaccines for fungal infections, such as *Candida* spp, *Pneumocystis jiroveci/carinii*, A. *fumigatus*, and *C. neoformans*. But there are a lot of limitations in this field. The main limitation is the emerging of IFIs in patients with immune deficiency who are not able to produce effective response against vaccines. Another limitation is triggering the allergic responses by specific vaccines in sensitive people [65,80]. However, some studies carried out on the endemic fungal infections, histoplasmosis, blastomycosis, coccidioidomycosis, and paracoccidioidomycosis [2]. In the 1980s, clinical trials were conducted on the coccidioidomycosis vaccines and these trials have continued until now [81]. Apart from all these efforts for producing a suitable vaccine with active or passive immunization, none of the fungal vaccines have been confirmed by FDA.

The first step in designing an effective strategy for vaccination against fungal infections is to improve our knowledge of the immune system. Following the profound knowledge of the mechanisms of immune responses to fungal infections, it is possible to design and apply the immunological products which termed immunotherapy. Immune adjuvants, especially TLR-ligands, along with monoclonal antibodies are the most important products of immunotherapeutics. Monoclonal antibodies, in spite of high costs, shows acceptable results in conjunction with vaccines. Nowadays, this strategy is expanding by many research projects. Targeting the pan-fungal antigens also presented acceptable results in the production of universal fungal vaccines. However, application of new techniques, such as DNA vaccines and immunotherapeutic products, with significant advances in the fields of genomics, vaccinomics, and proteomics will be useful to open new avenues for the success of vaccine strategies in clinical trials.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors are grateful to Department of Medical Mycology and Parasitology- laboratory of Medical Mycology, School of Medicine, Tabriz University of Medical Sciences and also dear Dr. Behzad Baradaran, the director of Immunology Research Center, Tabriz University of Medical Sciences for critical reading of the paper.

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