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REVIEW

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β-glucan as a new tool in vaccine development

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Abstract

Vaccination constitutes one of the major breakthroughs in human medicine. At the same time, development of more immunogenic vaccine alternatives to using aluminium-based adjuvants is one of the most important phases of vaccination development. Among different sources of carbohydrate polymers, including plants, microbes and synthetic sources tested, glucans were found to be the most promising vaccine adjuvant, as they alone stimulate various immune reactions including antibody production without any negative side effects. The use of glucan particles as a delivery system is a viable option based on the documented efficient antigen loading and receptor-targeted uptake in antigen-presenting cells. In addition to particles, soluble glucans can be used as novel hydrogels or as direct immunocyte-targeting delivery systems employing novel complexes with oligodeoxynucleotides. This review focuses on recent advances in glucan-based vaccine development from glucan-based conjugates to a glucan-based delivery and adjuvant platform.

1 | INTRODUCTION

Natural products useful in disease prevention and treatment have been highly sought after throughout human history. A major problem in the characterization of many natural products is that they represent a complex mixture of ingredients, any one of which may contribute to their bioactivity. β-Glucans from fungi, yeast and seaweed are well-known biologic response modifiers that function as immunostimulants against infectious diseases and cancer.[1,2] Unlike most other natural products, purified β-glucans retain their bioactivity, which permits the characterization of how β-glucans work on cellular and molecular levels. Several decades of intensive research on the biological effects of β-glucan show they exert strong immunomodulatory properties and are among other substances acting through an organism's own biological response mechanisms as biological response modifiers.[3,4]

β-1,3-glucans are structurally complex homopolymers of glucose, usually isolated from yeast and fungal cell walls. Yeast are characterized by a high glucan content (more than 85% β-1,3-D-glucan polymers) with a small admixture of chitin (about 2%) and lipids (<1%).[5] The isolation from various types of mushrooms was a logical follow-up of the folk remedy use of mushrooms in numerous nations. The number of different glucan structures is almost as great as the number of sources used for their isolation. Different physicochemical parameters, such as solubility, primary structure, molecular weight, branching and polymer charge, influence the biological activities of β-glucans. It is therefore imperative to use only highly purified and sufficiently characterized glucans.

2 | GLUCAN AND ITS EFFECTS ON IMMUNITY

Research with β-glucans has shown that they function through stimulation of granulocytes, monocytes, macrophages and natural killer cells. Two membrane β-glucan receptors that trigger responses to β-glucans have been characterized on a molecular level. The first to be reported was the iC3b receptor known as complement receptor 3 (CR3), and the second was the dectin-1 receptor.[6-8] Despite years of research, it is not clear whether there are two separate receptors for glucan or a single receptor of both CR3 (CD11b/CD18) and dectin-1 proteins.[9] As biological effects of glucans appear to be multifactorial, it is not surprising that glucans also influence the production and secretion of cytokines.

Soluble β-1,3-D glucans have been shown to protect against infection with both bacteria and protozoa in several experimental models and to enhance antibiotic efficacy in infections with antibiotic-resistant bacteria. The protective effect of glucans has been seen in experimental infection with *Leishmania major*, *L donovani*, *Candida albicans*, *Toxoplasma gondii*, *Streptococcus suis*, *Plasmodium berghei*, *Staphylococcus aureus*, *Escherichia coli*, *Mesocestoides corti* and *Trypanosoma cruzi*.[10-19] It is particularly interesting that glucan has been found to protect against anthrax infection.[20] Moreover, glucan-mediated protection against lethal infections can be passively transferred.[21]

In addition to the protection against infection, glucan is a well-known biological response modifier that has been used as an immunoadjuvant therapy for cancer since 1980, mostly in Japan.[7,22-24] Another glucan activity, demonstrated during 1980s, was stimulation of haemopoiesis in an analogous manner as granulocyte-monocyte colony-stimulating factor.[25] Both particulate and soluble glucans, when administered intravenously, caused significantly enhanced recovery of blood cell counts after gamma irradiation.[26] Other researchers showed that glucan could reverse the myelosuppression caused by chemotherapeutic treatment.[27]

Glucan was originally administered solely by injection. Subsequently, the oral immune modulatory activities of glucans have been reported. However, more research has been devoted to demonstrate that orally given glucan is as active as injected glucan but only a limited number of publications have focused on its mechanism of action. The available data do suggest that glucan, when given orally, might have similar effects as glucan administered by either intraperitoneal or intravenous route.[20,28-31] There is no information about the influence of glucan administered in long-lasting preventive oral delivery on humoral and cell immunity parameters. Generally, preventive oral programmes with immunomodulators are intended for an optimization of anti-infectious immunity within endangered populations (allergic children, population affected by environmental stress, seniors and patients in post-operational recovery (well-being), workers in polluted environments, etc, or as a widely applied prevention before the onset of highly transmissible airway infectious disease incidents. Further research in preventive oral supplementation is done in agreement with the "WHO Declaration" and the new global health policy "Health for All in the 21st Century".

Orally administered β-glucans increased the numbers of intestinal intraepithelial lymphocytes and potentiated the production of cytokines, namely interferon-γ (IFN-γ).[32] It was found that soluble glucan upregulated leucocyte activity and cytokine secretion. These properties, together with prolongation of survival in some infections, have led us to question the efficacy of β-glucans in oral infections caused by intracellular bacterial pathogens, namely *Salmonella enterica* and *Francisella tularensis*. Oral route of infection is most common for these two bacterial species. The protection mediated by locally produced IFN-γ is the main mechanism of early natural immunity after infection with these microbes.

An additional advantage of using glucan is its marked absence of toxicity or negative side effects and the GRAS (generally recognized as safe) approval by the FDA.

3 | VACCINES

Vaccination is the most effective intervention in modern medicine and still plays a fundamental role in the prevention, and sometimes eradication, of infectious diseases. Vaccine development includes not only the development of new vaccines against diseases such as AIDS, tuberculosis and malaria, but also the development of one-time and needle-free vaccines. In addition, therapeutic cancer vaccines using the specificity of the immune system are novel, highly promising strategies for improving cancer therapy. It is not surprising that WHO encourages the speedy development of oral vaccine formulations to simplify their transport, storage and administration.

At present, there are more than 70 licensed vaccines for preventive or therapeutic disposition of almost 30 species of pathogenic viruses, bacteria and fungi. The first vaccines were based on the neutralization or attenuation of pathogenicity or toxicity of disease-causing agents. Expanding scientific knowledge, especially in infectious immunology, and new biotechnologies have enabled the development of newer and safer vaccine subunits composed of proteins, peptides or nucleic acids.[33] On the other hand, their reduced immunogenicity has demanded the use of potent substances that strengthen the immune response, principally working as adjuvants. Antigen encapsulation in polymer-based particles is a primordial tool for superior vaccine delivery to mucosal sites.

Mucosal epithelia represent the main gateway for penetration of pathogenic vectors inside the organism. From this point of view, oral vaccination may be the most important for protection against enteric pathogens and partially against respiratory pathogens. Orally administered vaccines containing whole attenuated pathogenic micro-organisms in some circumstances may be less effective (eg, in some immunocompromised patients) and may provoke outbreaks of infectious diseases similar to the 2000 outbreak of polio in several countries.[34] A new and more effective vaccination strategy consists of microparticulate antigen carriers which can be used for delivery and adjuvant integration, increasing the immune response.[35]

4 | GLUCAN-BASED ADJUVANT ENHANCES IMMUNE RESPONSES TO VACCINES

Adjuvants are simple or more complex compounds, which, when added to antigens used for immunization or vaccination, enhance their immunogenicity.[36] Numerous substances have been tested for their ability to act as potential adjuvants in animal models and human clinical studies, and have provided many potential adjuvants candidates.[37,38] Complete information about adjuvants—their composition and use in vaccination—is available in a web-based database (Vaxjo).[39,40] Few adjuvants have been approved for human use, although none for mucosal vaccination.[41] In this respect, glucans, due to their biological activities (specifically antioxidant, anti-inflammatory and immunomodulatory properties), appear to be the most desirable candidates for use as adjuvants.[42,43] As traditional adjuvants, particularly aluminium, can only induce Th2-type immune response, novel adjuvants are deeply needed. Carbohydrate-based polysaccharides might be an excellent alternative; for review, see Li and Wang.[44]

The immunogenic capacity of β-glucans lies in their molecular structure. Therefore, they are recognized as pathogen-associated molecular patterns by immune cell receptors on neutrophils, macrophages and dendritic cells such as toll-like receptors, dectin-1, CR3 and CD5. These interactions trigger intracellular signalling activation followed by expression of immune molecular factors regulating non-specific natural and specific adaptive immune responses.[45] Innate immunostimulatory activity of β-glucan microparticles of baker's yeasts origin was studied in mice experimental models.[46-48] Daily oral doses of 0.1 mg/kg of microparticulate β-glucan for 2 weeks significantly increased the phagocytic activity of peritoneal macrophages. β-Glucan microparticles applied in vitro enhanced the T cell activation and proliferation. In addition, other studies have demonstrated enhanced phagocytosis of β-glucan microparticles by peritoneal macrophages that was followed by secretion of the pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β).[49,50] There are similar results documenting stimulation of natural immunity factors in rat pulmonary macrophages and human mononuclear cells.[51,52] The adjuvant and immunomodulatory effect of β-glucans have also been described in fish, which possess the same type of immunity as all vertebrates.[53] β-Glucan with recombinant glyceraldehyde-3-phosphate dehydrogenase was administered intramuscularly, and consequently, the high-level antibody and upregulated transcription levels of immunomodulatory molecules involved in innate and adaptive immune responses increased significantly compared with controls immunized only by glyceraldehyde-3-phosphate dehydrogenase.[54] Sulphated β-glucan from *Saccharomyces cerevisiae* induced higher proliferation of chicken lymphoid splenic cells in vitro. After administered as the adjuvant with Newcastle disease vaccine to chickens, it enhanced serum antibodies and serum cytokine levels (IL-2 and IFN-γ) concentrations.[55] Also, the curdlan, the β-glucan from the bacteria of *Alcaligenes faecalis* var. *myxogenes* 103K, exerted good immunomodulating activity.[56] Curdlan sulphate stimulated proliferation of spleen lymphoid cells, murine macrophage cell line RAW 264.7, dendritic cells maturation and increased cytokine production (TNF-α, IL-6, and IL-1β).[57]

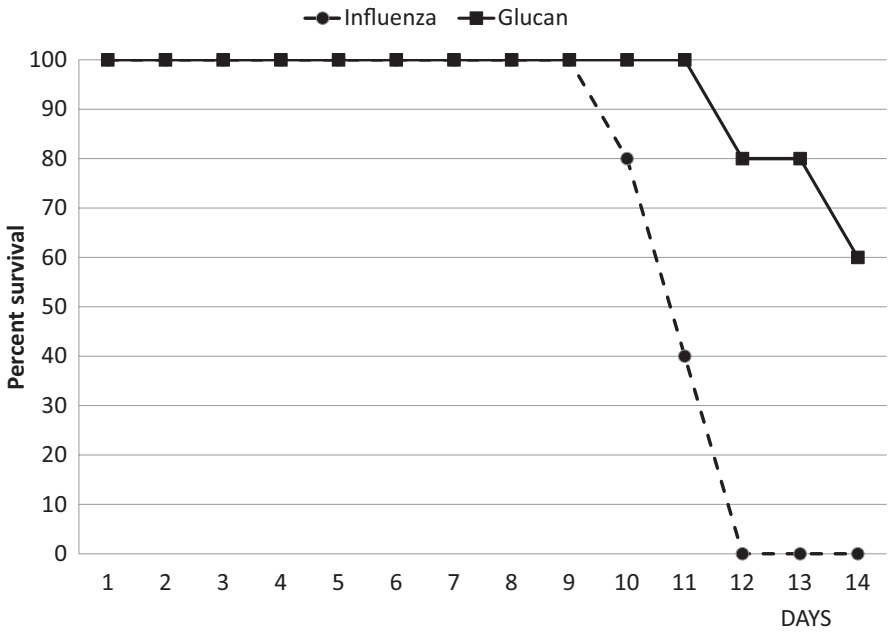

FIGURE 1 The oral administration of glucan mixture protects mice from lethal infection. All mice were infected with influenza. Glucan group was fed with glucan, influenza group was fed with PBS. Ten mice/group. From[94]

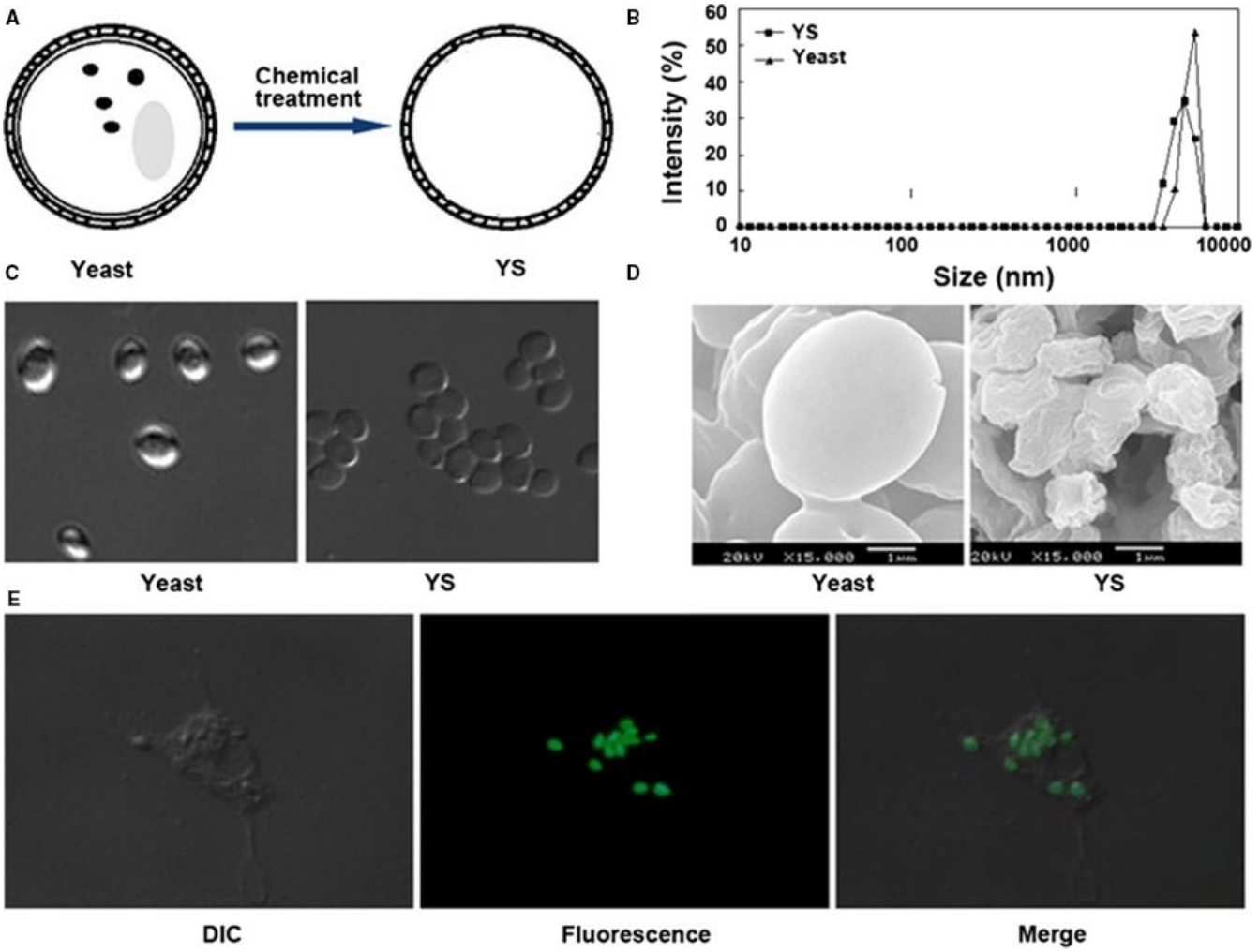

FIGURE 2 Preparation and characterization of the yeast shell. A, Yeast shell (YS) microparticles were prepared by a chemical treatment of yeast. B, The particle size distribution of yeast and YS. C, The light microscope images of yeast and YS. D, The SEM images of yeast and YS. E, The DC-uptake of FITC-YS. From[63]

Glucan supplementation used in addition to vaccination against *Yersinia ruckeri* significantly improved immune response of trout, suggesting that glucan does not need to be direct part of vaccine.[58] Similar findings were found in a model Birnagen Forte 3 vaccine, where glucan supplementation antagonized immune inhibitory effects of hypoxia and increased the levels of transcripts of key genes involved in both aspects of immunity.[59]

Curdlan, when compared to aluminium adjuvant, enhanced hepatitis B vaccine immunogenicity and supported immunity response in mice experimental model.[60] Co-administration of curdlan and anti-influenza vaccine enhanced influx of macrophages and dendritic cells, increased the levels of antigen-specific T cells and stimulated proliferation of splenocytes. Addition of β-glucan improved immune responses to influenza vaccine through stimulation of lymphocyte proliferation, enhanced antibody titres and promotion of cytokine production.[61] It is important to note that β-glucan alone can also significantly improve the immune response against an influenza challenge in mice (Figure 1).[62] Similar results were achieved with whole glucan particles as a part of a vaccine against systemic aspergillosis and coccidioidomycosis, suggesting a possibility to use whole glucan particles protection as a basis for the development of a pan-fungal vaccine.[63] Vaccination with recombinant *Cryptococcus* proteins in glucan particles protects mice against cryptococcosis in a manner dependent upon mouse strain and cryptococcal species.[64] For a detailed description of preparation of glucan particles for use as vaccine adjuvant carriers, see Mirza et al[65] Figure 2 shows preparation and characterization of these yeast shell microparticles. β-Glucans have repeatedly been proven as efficient for encapsulation and preserving antigen immunogenicity.[50,66] In a study by Levitz's group, they confirmed increased TNF-α production in human dendritic cells primed by IFN-γ, which may decrease the response threshold of competent cells after stimulation by β-glucan microparticles.[67]

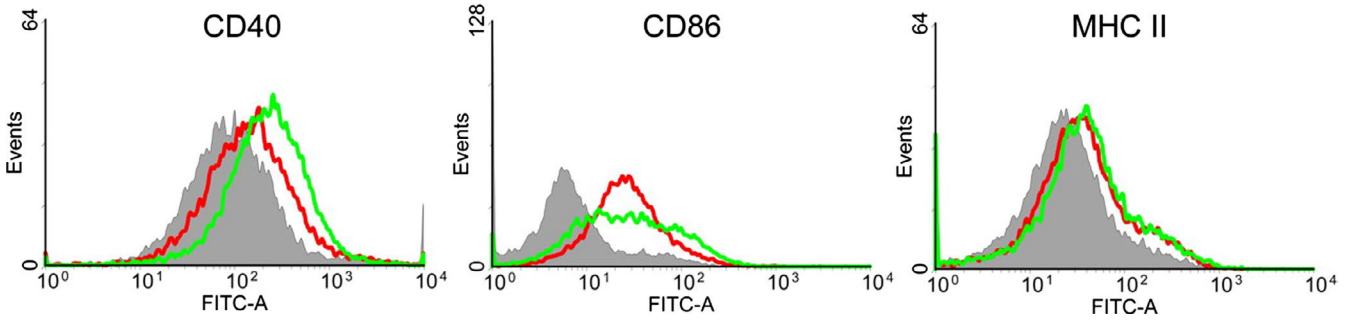

FIGURE 3 DC maturation stimulated by GP-OVA. DCs were left unstimulated (grey area) or stimulated with GP-OVA (10:1 particle-to-BMDC ratio; red line) or LPS (1 μg/mL; green line). Surface expression of CD40, CD86 and MHC-II was analysed by FACS. Data shown are representative histograms resulting from three independent experiments. From[64]

Adjuvant activity of glucan can be enhanced by cross-linking of an antigen, suggesting that the final configuration of the antigen-nanogel complex affects the strength of the immune response.[68] A recent approach was to combine β-glucan particles as shells for aluminium-based colloid vaccine.[69] The advantage is the improvement of immunogenicity and at least partial switch to Th1 immunity, but at the same time, denying one of the advantages of using glucan in vaccines (ie, substituting aluminium detested by various anti-vax movements with a natural molecule).

5 | GLUCAN-BASED DRUG AND VACCINE DELIVERY

Numerous bioactive molecules used in clinical practice suffer from limitations such as short half-life and lack of direct localizing. Conjugation with glucan molecules, which itself offers significant benefits, is one of the possibilities of how to improve biological and therapeutic activities of established drugs. For example, carboxymethyl glucan used in conjugation with the anti-cancer drug, gemcitabine showed that these conjugates offered significantly enhanced activities, particularly in lung cancer cases.[70]

In an experimental glioblastoma stem cell therapy study, β-glucan–anchored, paclitaxel-loaded, chitosan nanocarriers provided enhanced compatibility with good therapy and overcame serious limitation with systemic delivery of paclitaxel, which has usually undesirable haemolytic effects.[71] β-Glucan–based hydrogels have been successfully used in delivery of B_{12} to the intestinal tract, and the good bio-release rate makes this carrier a potential carrier for oral vaccination.[72] For a review summarizing the current knowledge of nanoengineering of polysaccharide-based vaccines, see Cordeiro et al[73]

A novel antigen delivery system using antigen-loaded glucan particles showed strong humoral and cytotoxic T cell immune responses and, in particular, activation of dendritic cells.[74] An experiment using glucan-based delivery systems for HBsAg immunization showed very high titres of specific antibodies, particularly when oral administration followed subcutaneous priming.[75] β-Glucan microparticles conjugated to bovine serum albumin (BSA) used for vaccination in experimental murine model, either by intradermal

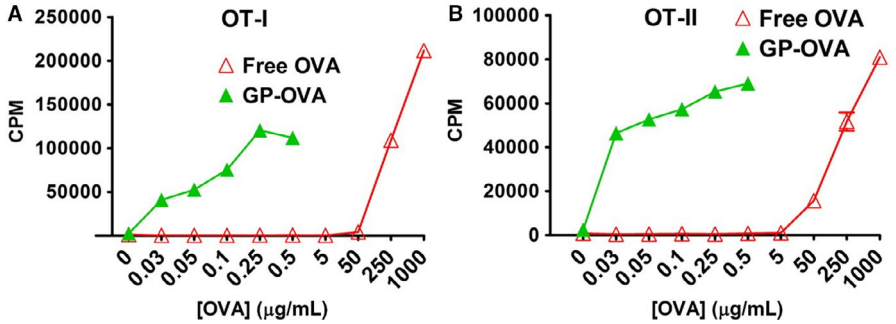

FIGURE 4 Lymphocyte proliferation stimulated by free OVA compared with that stimulated by OVA complexed in GPs (GP-OVA). T cells (105 cells/well) purified from the lymph nodes and spleens obtained from OT-I (CD8+) (A) and OT-II (CD4+) (B) mice were incubated for 4 d with mitomycin C-treated BMDCs (104 cells/well) and free OVA or GP-OVA over the indicated OVA concentration range. For wells containing GP-OVA, 105 GP-OVA (containing 6, 10, 20, 50 or 100 μg OVA per 108 GPs) were added to yield the indicated concentration of OVA. [3H] Thymidine was added 24 h before harvesting. Data are the means ± SE from a representative experiment (out of three); for each experiment, each sample was tested in triplicate. From[64]

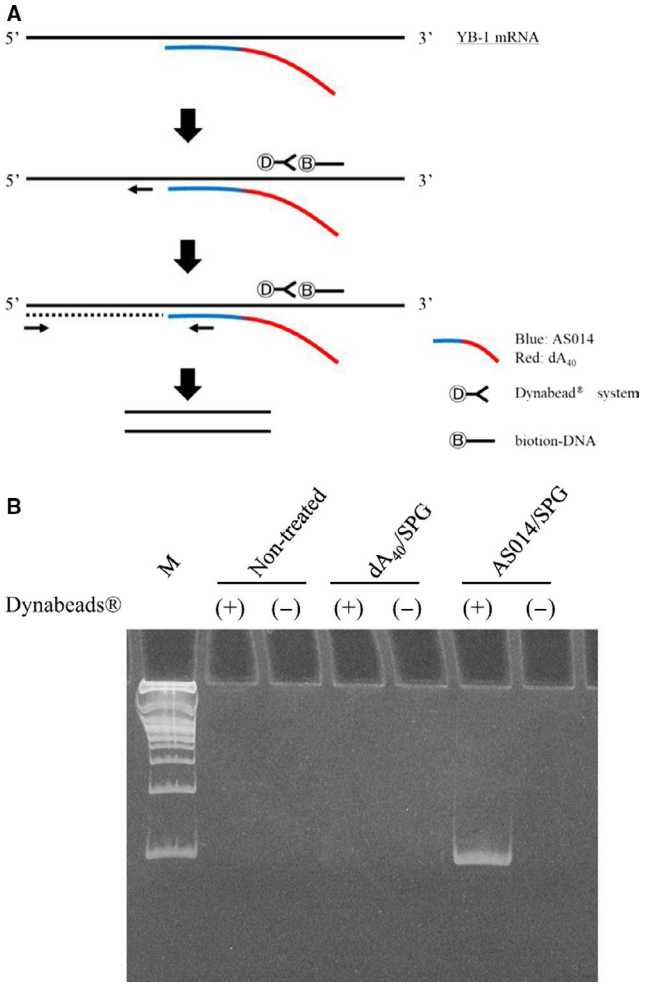

FIGURE 5 Binding of AS014 to YB-1 mRNA in the cytoplasm. A, Schematic illustration of the RT-PCR protocol using AS014 as a primer for reverse transcription. B, The amplification level of UB-1 using the dA40/SPG or AS014/SPG complex after treatment with or without Dyna-beads. From[73] With permission

or oral administration in the form of food pellets coated with BSA-β-glucan microparticles conjugate, enhanced IgG titres against BSA up to a 100-fold in comparison to controls after booster immunization.[50] When ovalbumin (OVA)-β-glucan microparticles were used for oral immunization of mice, the OVA-specific intestinal IgA production was increased in animals restimulated after 2 and 4 weeks. Adoptive transfer experiments showed increased proliferation of splenic ovalbumin-specific $CD4^+$ T cells; additionally, a significantly increased IL-17 and trend towards increased IFN-γ production in the spleens were both detected after antigen restimulation of mice.[76] These results concur with findings of another study, in which, after subcutaneous administrations of β-glucan microparticular formulations, strong antigen-specific antibody production and T cell responses were elicited, including the cytokine production (IFN-γ and IL17a) by $CD4^+$ Th1 and Th17 cells.[77] Capsular yeast shell microparticles were used to deliver OVA to dendritic cells. The particles are well recognized by dendritic cells and, upon internalization, trigger release of co-stimulatory molecules.[78] Figure 3 and Figure 4 show robust stimulation of humoral and cellular immune responses resulting from vaccination with antigen-loaded glucan particles.

The possible use of β-glucans for delivery of nanovaccines with nucleic acids (genes) as antigens was studied with synthetic cationic glucans originally isolated from reishi mushrooms (*Ganoderma lucidum*).[79] A positive therapeutic effect was reported when schizophyllan, combined with intraperitoneal-administered TNF-α oligonucleotides, was used for delivery to macrophages (through Dectin-1 targeting) for treatment of lipopolysaccharide-induced hepatic damage in mice.[80] A glucan-oligodeoxynucleotide complex increased antigen-specific T cell proliferation in both mouse and human models, in an IL-12–independent manner, making it able to overcome the species barrier for humanized CpG.[81]

Subsequent experiments (Figure 5) demonstrated that these complexes are well incorporated into the cells by Dectin-1–mediated endocytosis and hybridized with target mRNA in cytosol.[82] Another example is the combination of β-glucan and TNF-α oligonucleotides for direct delivery to macrophages. A study showed that these complexes were efficiently delivered via Dectin-1 receptor with satisfactory therapeutic effects.[80] Subsequent studies found that β-glucan–antigenic peptide complexes increased their immunogenicity and allowed the use of significantly lower doses of peptides, suggesting their potential role in the development of potent vaccines against infectious diseases and cancer.[83]

As the stability of oral vaccine in the stomach might be a problem, the inhibition of antigen degradation is imperative. A conjugation of glucan with glycine-arginine–glycine-aspartic acid-serine offered effective protection without interfering with M cell targeting. Subsequent in vivo tests demonstrated the superiority of this system, showing strongly elevated antibody concentrations in intestine, mucus and serum.[84]

6 | GLUCAN MICROPARTICLES AS A DELIVERY PLATFORM FOR ORAL VACCINATION

Among the numerous categories of particulate antigen delivery systems, such as immune-stimulating complexes, liposomes, micro- and nanoparticles, or virus-like particles, the *Saccharomyces cerevisiae*-derived β-glucan microparticles could be regarded as the most promising for an oral delivery platform.[85-88] Particulate nanocarriers may exert a high adjuvant potential and could increase the immune response to vaccination due to their size and structural similarity to natural pathogens. These preparations are particularly advantageous for nasal delivery of vaccines, which rapidly became favoured vaccines because of the efficient M cell uptake in the nasal-associated

lymphoid tissue. Various compositions of glucan-based materials for nasal deliveries are described by Cevher et al.[89]

The use of natural polymers in the preparation of antigen delivery systems is one of the contemporary tendencies on the development of innovative and more effective vaccines.[73] From a series of biopolymers, the β-glucans seem to be the most promising. β-glucans in the form of microparticles could serve not only as immunostimulants but also as receptor-targeted antigen carriers advantageously applied to mucosal vaccination.[90] Addition of antibodies to G protein-conjugated glucan particles further improved the specific targeting to enterocytes and dendritic cells.[91]

Oral vaccination by gavage is one of the most effective methods for administration of antigen compared with other routes of immunization (intravenous, subcutaneous, intramuscular) because both systemic and local mucosal immunity responses are induced. Other effective ways of antigen delivery from the point of view of systemic immune response induction occur only when antigens arrive at blood vessels after passing through the liver. Administration of vaccines through the oral route requires protecting the antigen from degradation prior to absorption in the gastrointestinal tract, where it directly elicits immune response within the gut-associated lymphoid tissue, which is the largest immune organ of the body.[92,93] During formulation of effective mucosal vaccines, limitations, such as the lowered immunogenicity of antigens used for vaccination, may be encountered.[94] A glucan-based encapsulation system has shown to be an optimal solution. This system protects antigens before their degradation, enhances their immunogenicity and increases their accumulation in the vicinity of mucosal tissue for better absorption.[35,95] Moreover, encapsulated antigen is selectively captured in the gut-associated lymphoid tissue.[96,97]

7 | CONCLUSION

Use of natural materials for the preparation of antigen delivery is currently trending in the field of vaccination. The low intrinsic immunogenicity, lack of toxicity, high- and well-documented immunomodulating properties, good biocompatibility, cost-effectiveness in large-scale manufacturing and relatively reasonable price make β-glucan a promising new candidate for novel vaccine design. More research is necessary to select the best adjuvant to challenge the monopoly of aluminium adjuvants in human and animal vaccines.

β-Glucans, particularly the insoluble version, might represent a suitable adjuvant as well as a valuable oral and systemic vaccine delivery platform. β-Glucan microparticles have a large antigen payload and offer the possibility to deliver not only a relevant antigen, but also provide a secondary adjuvant function.

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