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REVIEW PAPER

Liposome and polymer-based nanomaterials for vaccine applications

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ABSTRACT

Nanoparticles (NPs) are effective and safe adjuvants for antigen delivery in modern vaccinology. Biodegradable nanomaterials with suitable properties are frequently applied for conjugation or loading with antigens; they protect the antigens from degradation in vivo. NPs are applied as effective delivery system to facilitate antigen uptake by antigen presenting cells (APCs) and especially dendritic cells (DCs) both in vitro and in vivo. Using nanoparticles to target DCs is an effective method to deliver antigens and potent immunomodulators. Uptake of NPs by DCs enhances the intracellular process of antigens and the antigen presentation pathway by MHC class I and II molecules to induce both CD4+ and CD8+ T-cell responses. Liposome and polymer-based NPs are now extensively applied as effective adjuvants or immunomodulators in several types of vaccines. In this review, the nanomaterials for vaccine application are focused intensively in poly(lactic-co-glycolic) acid (PLGA), dendrimers, liposomes, nanogels and micelles which are the targeted antigen delivery system, and present high potential as a promising future strategy for DNA-based, bacterial and viral vaccines. Further advances in nanotechnology and molecular immunology techniques will enhance the success of targeting and lead to the next generation of nano-delivery systems.

Keywords: *Adjuvants, Dendritic cells, Liposome, Nanoparticles, Polymer*

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INTRODUCTION

Nanotechnology focuses specifically on immune-cell targeting strategies such as adjuvant activity and antigen delivery systems. Extensive research has targeted nanovaccines development, especially the use of biodegradable and biocompatible nanopolymers as antigen delivery systems to enhance humoral and cellular immunity [1]. Nanoparticles (NPs) are applied as immunomodulators for several types of vaccines due to their desired properties including slow release of antigens, increase of immune responses, protection of antigens and effective delivery to immune cells [1]. In particular, NP-based vaccines demonstrate a critical role in inducing CD8+ T-cell responses against viral infections by effective cross-presentation of antigenic peptides on major histocompatibility complex (MHC) class I and class II molecules [1]. Dendritic cells (DCs) are professional antigen presenting cells (APCs) intensively focused on vaccine strategies. Using nanoparticles to target DCs is an effective method to deliver antigens and potent immunomodulators. Uptake of NPs by DCs enhances the intracellular process of antigens and the antigen presentation pathway by MHC class I and II molecules to induce both CD4+ and CD8+T-cell responses [2]. This review discusses the application of nanoparticles for vaccines with particular focus on liposome and polymer-based NPs as safe vaccine adjuvants for antigen delivery and targeting systems.

Poly(lactic-co-glycolic) acid (PLGA)

PLGA is a copolymer which is widely used in nanotechnology as a biodegradable polymer which is safe for human and veterinary uses and approved by the American Food and Drug Administration (FDA) [3, 4]. PLGA particles have been applied for vaccine formulation [5]. PLGA NP application in vaccinology has focused extensively on use as a potential vaccine delivery system due

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to its slow rate of degradation and release before internalization in APCs [6, 7]. In general, APCs prefer to uptake particulate antigens rather than those in soluble form [7]. Nanoparticles protect antigen degradation from proteolytic enzymes [8] and facilitate antigen deposition after injection or oral delivery [9, 10]. PLGA NPs were effective in delivering antigens in vitro to DCs, suggesting that they are a practical, effective and useful medium for immunotherapy targeted DCs (Fig 1) [11]. Cruz *et al.* [8] confirmed success of DC-specific targeting antibodies on polyethylene glycol (PEG)-PLGA NPs. Moreover, a study of PLGA NPs by Newman *et al.* [12] suggested that they were able to induce and enhance immune responses for poor immunogens.

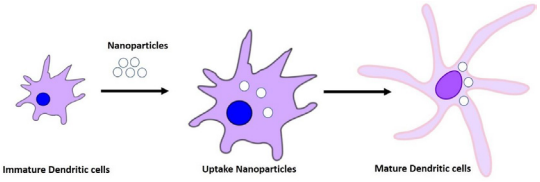

Fig 1. Maturation and activation of DCs by nanoparticles

Applications of PLGA NPs have been successful in many kinds of vaccines such as viral vaccine [13], bacterial vaccines particularly against tetanus [5, 14-20], and DNA vaccines [21, 22] Immunostimulators and antigens were encapsulated or adsorbed on PLGA particles including toll-like receptor (TLR) agonists such as LPS [23], MPLA [6, 24], CpG [25-28], β-glucan [29-31] and poly (I:C) [27].

PLGA NPs have been applied in viral vaccines to enhance cytosolic delivery of antigens to increase antigen-presentation via the MHC class I pathway (Fig 2) and induce cytokine release from T-lymphocytes [29, 30]. A very interesting study determined that HIV p24 protein adsorbed on the surface of surfactant-free anionic poly (D, L-lactide) or PLA NPs was efficiently taken up by mouse DCs, leading to DC maturation [31]. Polylactide acid (PLA) or PLGA has performed as a delivery system and adjuvant to increase both humoral and cellular immunity for HIV vaccines. Biodegradable NP vaccine carrying HIV antigens has proved a good strategy for HIV [31].

Moreover, PLGA microspheres have been successful for intranasal immunization as bacterial vaccines against *Toxoplasma gondii* in sheep [32], *Staphylococcus aureus* in cows [33], *Pasteurella multocida* antigen with cholera toxin in rabbits

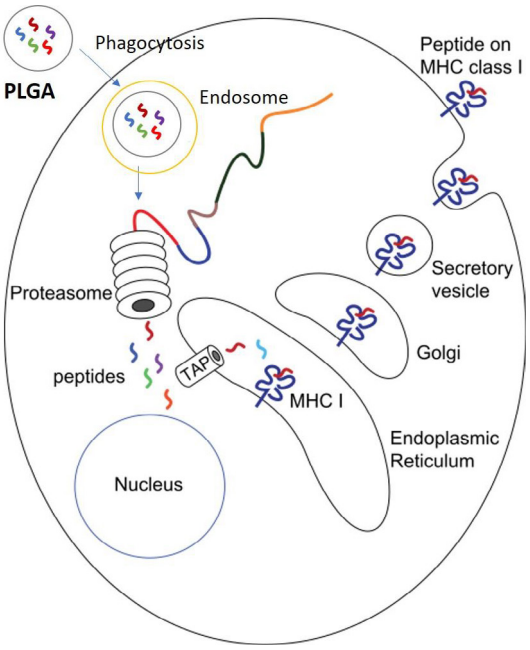

Fig 2. The proposed mechanism of cross-presentation by PLGA NPs. The NPs entered the APCs through phagocytosis, and then escaped or was released from an endosome. The released peptides are processed by a proteasome and presented by MHC class I molecules [30]

[34], and rhinitis or pleuropneumonia in swine to induce effective immune response without any adverse reactions [35]. This suggests that these methods are useful applications for veterinary vaccines. For DNA vaccine, chitosan-modified PLGA microspheres have induced both humoral and cellular immunity by intranasal route administration in rabbits. DNA-based vaccines, therefore, have potential to generate long-lasting immunity. Recently, DNA encoding hepatitis B surface antigen (HBsAg)-encapsulated PLGA NPs were shown to increase immunity in mice [36]. However, successful clinical trials with PLGA nanovaccines have never been reported, suggesting that extensive experimental work is still needed in this highly promising field.

Dendrimers

Dendrimers are a family of nano-sized polymers with symmetric spherical shape as several branches radiating from a central nucleus. Dendrimers are also well known as nontoxic agents targeting specific proteins that cross barriers such as cellular membranes or gut [37]. Recently, dendrimers have been used as potential carriers for drugs or immunogens due to their remarkable

nanostructure with interesting chemical and biological properties. Outstanding properties of dendrimers include the high degree of surface functionality and versatility to couple with related molecules. In particular, dendrimers are biologically biocompatible and have predictable biodistribution [38] with potential properties that can be applied as immunomodulating compounds or adjuvants to enhance the efficiency of vaccines. Polyamidoamine (PAMAM) dendrimers have been used as vaccine vehicles since their positive charge can protect DNA from nuclease and increase the efficacy of transfection [39, 40]. Vaccine efficacy was designed using the relevant peptides and particles as the adjuvant [41]. PAMAM dendrimers are conjugated and applied in the delivery of DNA to APCs to enhance the efficacy of DNA vaccines. Conjugation of PAMAM dendrimers with DNA on the surface of the targeted peptides effectively delivers APCs to enhance immune responses *in vitro*. Application of DNA-peptide-dendrimer complexes on subcutaneous administration could transfect DCs in the lymph nodes *in vivo*, leading to high-affinity of T-cells and rejection of tumors. Dendrimers are useful for conjugation of peptides, nucleotides, and antibodies for drug delivery and vaccines (Fig 3) [42].

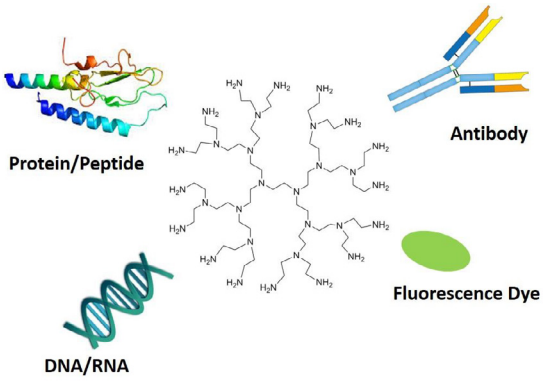

Fig 3. Dendrimer conjugated with peptides, antibody, nucleic acids for drug delivery and vaccines

Studies have shown that dendrimers can be used effectively as hepatitis B vaccines [43]. The commercial vaccine, VivaGel® which inhibits HIV transmission [44], contains an active component as dendrimeric polylysine tested in animals. Moreover, PAMAM dendrimers could be used as a multiantigenic vaccine candidates against malaria [45] to provide an effective remedy for this disease [46]. In particular, study of the T and B epitopes to define synthetic vaccines against malaria requires urgent and intensive investigation [47]. The possibility of tetra-branched peptide dendrimers as malaria vaccines has already been proved [45]. Thus, dendrimers are very useful for transfection and effective targeting of APCs *in vivo* by their essential properties to generate effective vaccines [48].

Liposomes

Liposomes were first reported as a delivery system for vaccines in 1974 [49]. Liposomes have advantageous properties for delivery of vaccines since they are composed of nonimmunogenic, nontoxic and biodegradable phospholipids from natural products [50]. Liposomes can be optimized in various sizes and lipid compositions or charges for relevant antigens [50-53]. Specific antigens can be encapsulated in a hydrophilic core, enclosed with a hydrophobic bilayer, adsorbed or anchored electrostatically on the surface. Several advantages of liposomes include prevention of enzymatic degradation of antigens and increasing absorption into biological cell membranes to enhance bioavailability [54]. These advantages are suitable for developing effective prophylactic and therapeutic vaccines and, because of an increase in the therapeutic window, they are more useful for therapeutic vaccines [55]. Liposomes can be targeted for a site-specific purpose [54, 56] especially skin for topical administration or releasing antigens targeted to endosomes, tumors and inflammatory tissues [57]. Applications of liposome-based vaccines are preferable in designing vaccines for administration by various routes such as oral, mucosal and topical [54]. Furthermore, application of liposomes as vaccine adjuvants has been extensively studied and results confirmed stimulation of the immune response by using peptide antigens. The first reported adjuvant activity of liposomes by Allison *et al.* showed an enhancement of humoral immune response against diphtheria toxoid in mice by liposome injection [49]. Liposome-based recombinant vaccines as human and veterinary vaccines registered in the market or clinical trials are very promising [58] with immune enhancing properties and good safety profiles. Some forms of liposomes, called virosomes, contain influenza hemagglutinin protein which facilitates binding with specific receptors on antigen presenting cells.

Anionic liposomes have been used for nasal

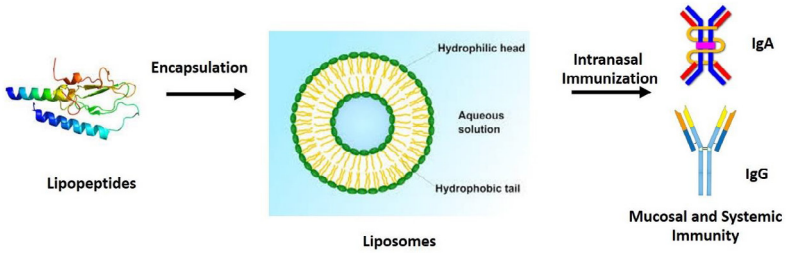

Fig 4. Liposome-based intranasal delivery of lipopeptide vaccine

vaccination immunization of Newcastle disease virus-induced enhanced levels of secretary-IgA and IgG antibodies more than cationic liposomes [59]. Administration of anionic liposomes to chickens induced higher levels of hemagglutination-inhibition antibodies when compared with cationic liposomes [59], while another report concerning liposome-based intranasal delivery conjugated lipopeptide with group A streptococcus (Fig 4) [60]. Liposomes could be a more effective antigen for delivery systems which are coupled or absorbed with TLR ligands [61, 62] and oligosaccharides to increase the adjuvant activity [63, 64]. Advantages of targeted liposomes include enhancing the immune system through boosting the amount of antigen delivered to a specific tissue, organ or APCs by increasing the ligands exposed on the surface of liposomes [50]. Liposomal NPs are suitable as immunomodulators or adjuvant molecules such as MPL A, CpG oligonucleotides and MDP [65].

Liposomal vaccine development faces challenges for effectiveness, safety, and expense. The effectiveness is based on several factors including liposomal sizes, surface charges and compositions, route of administration and adsorption or encapsulation efficiency [66]. Encapsulated antigens were more effective in inducing an immune response by using smaller vesicles [67-69]. Modification of the surface charge or coating highly affected the immune response, including the physiochemical properties and formula stabilization [70-74]. Taking all of the above into account, liposomes are effective as vehicles and/or adjuvants for vaccine delivery systems. Several reports have investigated the formulation effects of liposomes for antigen delivery and immunogenicity. Furthermore, studies regarding the formulation of adjuvants and mechanisms of action are important for stimulation of the desired immune response. Although several liposome-based vaccines are currently undergoing clinical trials, the storage is a problem for the stability of liposomes. Formulation development is focused on maintaining the intrinsic properties and cost-effectiveness of vaccines [75].

Nanogels

Nanogels are combinations of three kinds of polymeric materials which can be generated from both synthetic polymers such as PLGA and poly(ε-caprolactone) (PCL), or natural polymers as polysaccharides [76]. In particular, polysaccharide-based nanogels are very interesting due to their promising properties of biocompatibility and the variety of materials [77-79]. Recently, biodegradable cationic alginate-polyethylenimine or PEI nanogels have been demonstrated as a novel vaccine delivery system [80]. These nanogels contain high capacity for antigen-loading with minimal cytotoxicity. This not only facilitates antigen uptake by mouse bone marrow dendritic cells (BMDCs) but also the degradation of intracellular antigen and cytosolic releasing are enhanced which could increase cross-presentation in both MHC class I and class II antigens. Moreover, nanogels show higher potential stimulation of antibody production by vaccines and promote tumor cell lysis by CD8+ T cells. These results suggest that nanogels are efficient, effective and potent for the enhancement of vaccine-induced humoral and cellular immune responses (Fig 5) [80].

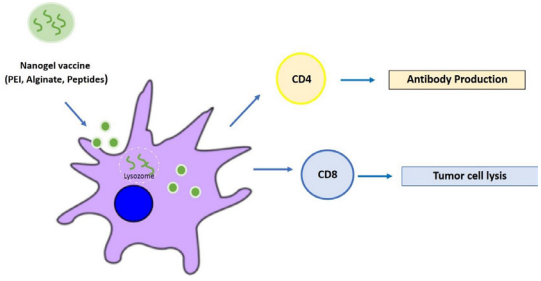

Fig 5. An alginate-PEI nanogels could enhance intracellular antigen degradation, cytosolic release, and increase antigen presentation to T lymphocytes which leads for antibody production and cytoxic T-cell responses

Cationic nanogels have been reported to provide an intranasal vaccine delivery system with significantly enhanced systemic vaccine and induced mucosal antibody production [81]. Chitosan nanogel-formulated vaccines have also been demonstrated as an effective intranasal or intraperitoneal immunization, with more efficient protection for mice from *Neospora caninum tachyzoites* infection [82]. Nanogels were applied for the treatment of brain diseases by intranasal administration and the results demonstrated rapid delivery of drug via the olfactory nerve pathway to the brain with high neuroprotection. These results suggest that nanogels are the effective delivery system for vaccines by their physicochemical properties. However, sizes, shapes, surface charges, and hydrophobicity of nanogels could affect the immunomodulating activity (Fig 6) [83 ,78]. Interestingly, a study by Hirosue *et al*. [84] has shown that poly(propylene sulfide) NPs conjugated with ovalbumin (OVA) peptides enhanced MHC class I presentation [85].

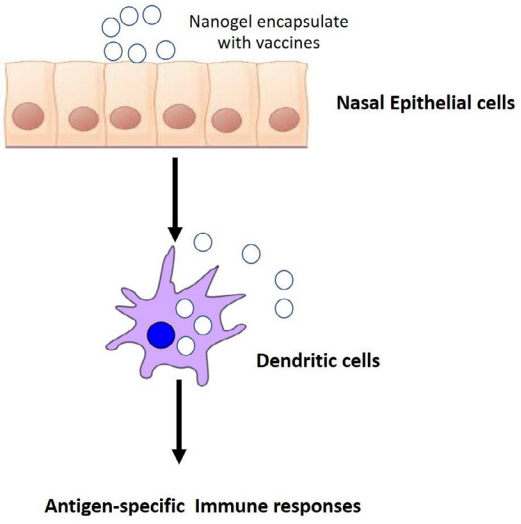

Fig 6. Intranasal administration of nanogels could enhance the immune response

However, the mechanism of nanogel vaccine effectiveness to induce humoral and cellular immunity remains unclear. Biodegradable nanogels could significantly enhance antibody production and MHC class I cross-presentation for cytotoxic T-cell mediated tumor cell lysis. Therefore, the nanogels are an effective strategy to trigger humoral and cellular immunity and enhance the development of nanogel-based vaccines.

Micelles

Polymeric micelles are made by self-assembly of individual amphiphilic polymeric molecules to generate core-shell NPs. Micelles have been investigated as adjuvant vaccines because of several characteristics. Firstly, amphiphilic (hydrophobic-hydrophilic) block copolymers are applied for self-assembly into micelles using an antigenic peptide for encapsulation or surface coupling (Fig 7A).

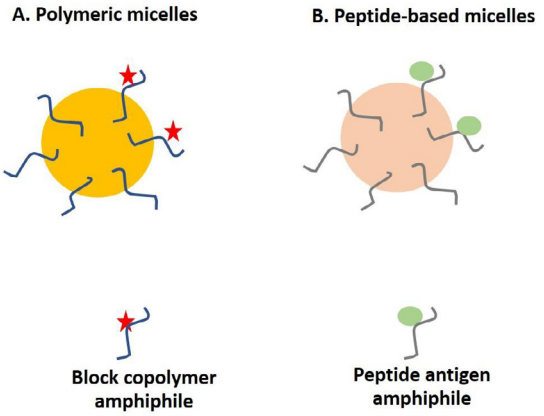

Fig 7. Micelle-based vaccines. (A) self-assembly of amphiphilic block copolymers polymeric micelles in water. The antigenic peptide is encapsulated or surface conjugated with the reactive groups (star symbols); (B) self-assembly of peptide antigen amphiphiles forming micelles in water

Secondly, the antigenic amphiphile peptide is a self-adjuvant with the hydrophilic head-group covalently bound with a hydrophobic moiety for self-assembly into micelles (Figure 7B) [86]. Polymeric micelles have been shown as effective delivery systems for drugs and genes due to their properties including high loading capacity, high stability and biocompatibility [87 ,88]. Recently, micelles were demonstrated to act as effective antigen delivery systems. Polymethacrylic acid-b-polyethylene oxide or poly-L-lysine (PLL) micelles were studied *in vitro* for antigen peptide delivery by Boudier *et al*. [89]. Their results suggested that polyion complex micelles are effective for antigen loading and facilitating the take up of antigens by DCs.

Significantly, these polyion micelles have shown immunostimulating effects by enhancing DC maturation [89, 90]. The immunogenicity of hepatitis B surface antigen (HBsAg) on PEG-PLA-

PEG micelles was studied by Jain *et al.* who showed that PEG-PLA-PEG micelles were substantially more potent than PLA NPs in the induction of mucosal antibody responses via intranasal and oral immunization by stimulating and prolonging HBsAg [91, 92]. Their results suggested that polymeric micelles behaved as potential vaccine adjuvants. PEG-PLL-PLLeu hybrid polypeptide micelles were effectively shown as vaccine delivery systems with a high capacity of antigen loading and enhanced stability with encapsulated ovalbumin (OVA). The polypeptide micelles showed immunoregulatory effects in both *in vitro* and *in vivo*, suggesting that they could enhance antigen uptake, antigen presentation, and DC maturation. In particular, the *in vivo* antibody production was also highly enhanced by polypeptide micelles and polypeptide micelles encapsulated with OVA and polyriboinosinic-polyribocytidylic acid (PIC) or a TLR3 agonist synergistically induced tumor-specific cytotoxic T-lymphocyte response [93].

Interestingly, mucosal vaccines are more advantageous than systemic vaccines; the former can be produced effectively for global health by inducing a protective immunity for mucosal infections such as *Mycobacterium tuberculosis*, HIV and other pathogens [94]. Development of an effective mucus delivery system by nanotechnology for the use of a viral antigen that can induce strong mucosal immunity is currently focusing on biosynthetic mucoadhesive polymer micelles to design and synthesize a mucosal vaccine delivery system [95]. However, micelle-formulated vaccines remain highly complicated to optimize for immunopotency [96]. Mucosal vaccine strategies must be improved to allow for individual delivery without the need of prior medical training, especially for the prevention of epidemic infections such as influenza virus disease [95].

Clinical Studies

Liposome-based vaccine is in clinical trial for HIV (AS01™) which has shown higher immune response induced by AS01™ [97]. In addition, the hepatitis A vaccine was the first licensed liposome-based vaccine for clinical use in humans (hepatitis A -HEPA, Epaxal). Moreover, the most advanced liposomal structures developed as nanovaccines is called virosome. A licensed virosome-based nanovaccine launched in the market for influenza is called InflexalVs [98]. The hemagglutinin (HA) and neuraminidase of influenza glycoproteins were integrated onto the surface of liposomal structures [99] to increase the antigenicity to APCs and enhance the cross presentation of MHC

Table 1. Liposome and polymer-based nanomaterials for vaccine formulation and antigen delivery

Nanomaterials	Vaccines	Antigen delivery	References
Poly(lactic-co-glycolic) acid (PLGA)	Bacterial vaccine	*Toxoplasma gondii, Staphylococcus aureus*	32, 33
		Pasteurella multocida	34
	DNA vaccine	HBsAg	36
	Viral vaccine	HIV	31
Dendrimers	DNA vaccine, DCs vaccine	Tumor	39,40
	Malarial vaccine	Malarial antigen	42
Liposomes	Bacterial vaccine	Diphtheria toxoid, Streptococcus	49, 60
	Viral vaccine	Hepatitis A virus, Influenza virus, HIV	97,98,99
		Avian paramyxovirus 1 (Newcastle disease)	59
Nanogels	Parasite vaccine	*Neospora caninum tachyzoites*	82
	DCs vaccine	Tumor	80, 85
Micelles	Viral vaccine	HBsAg	91,92
	DCs vaccine	Tumor	89,90

class I molecule. These promising features are useful for the therapeutic nanovaccines. However, the intensive research studies are still needed to improve the efficacy, stability and safety for the immunotherapeutic purpose.

Future perspectives

Biodegradable liposome and polymer-based nanomaterials are extensively investigated in vaccine formulation covering wide-ranging antigens (Table 1) to enhance the delivery of antigens in systemic or specific areas of the body. However, more research is required for NPs to advance to clinical trials and wider usage. Currently, there are several preclinical studies focusing on liposome and polymer-based nanovaccines. However, there are still a low number of clinical use bacause the majority of the studies are investigated in animal models. Therefore, more clinical trials are still needed to confirm the safety, efficacy and stability of nanovaccines for human use. More effective human nanovaccines will be increasingly used in the near future, as this exciting research field progresses forward and successfully overcomes the restrictive barriers that are currently hindering advancement.

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