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REVIEW

Natural vaccine adjuvants from traditional Chinese medicines: Mechanisms to applications

Xiaoyuan Fan[a,†], Fengxiang Liu[b,†], Fei Sun[b], Yiyang Wang[b], Wenwen Shen[d], Shujun Wang[a,*], Jin Sun[b,c,*], Kaiyuan Wang[b,c,*]

[a]*Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China*
[b]*Department of Pharmaceutics, Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang 110016, China*
[c]*Joint International Research Laboratory of Intelligent Drug Delivery Systems, Ministry of Education, Shenyang 110016, China*
[d]*Department of Pharmacy, the First Affiliated Hospital of Jinzhou Medical University, Jinzhou 121000, China*

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Abstract With the rapid advancement of vaccines, the research and application of vaccine adjuvants have garnered significant attention. Despite the development of numerous vaccine adjuvants, their applications in human vaccines remain limited due to either insufficient efficacy or severe side effects. Consequently, there is growing interest in developing bioactive compounds derived from traditional Chinese medicines (TCMs) as vaccine adjuvants, owing to their natural biocompatibility, diversity, and safety. Here, we systematically review the current application status and potential value of TCM-based bioactive compounds in vaccine adjuvants. Firstly, we elaborate on the types and characteristics of active ingredients, such as polysaccharides, saponins, flavonoids, acids, and alkaloids. The mechanisms by which these compounds function as vaccine adjuvants are then discussed, including their roles in enhancing humoral immunity, cellular immunity, and relieving the immune suppression in the microenvironment. Additionally, we summarize the current strategies for structural modification and platform optimization to adapt to different application scenarios. Finally, we offer insights into the future development directions for these potential adjuvants, highlighting research priorities, technical approaches, and application prospects. In conclusion, natural vaccine adjuvants derived from TCMs present broad application prospects and hold promise for future vaccine development.

*Corresponding authors.
E-mail addresses: 101030124@syphu.edu.cn (Shujun Wang), sunjin@syphu.edu.cn (Jin Sun), wangkaiyuan@hotmail.com (Kaiyuan Wang).
[†]These authors made equal contributions to this work.
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1. Introduction

The immune system functions as a complex and sophisticated defense mechanism within the human body, responsible for safeguarding against external pathogens such as bacteria, viruses, fungi, and parasites, while also maintaining internal homeostasis and establishing long-term immune memory[1−5]. Consisting of a diverse array of immune cells, lymphoid organs, and signaling molecules, the immune system operates collaboratively to construct a robust defense against various diseases and infections[6]. Despite the immune system serving as a critical shield in preserving human health, the body remains vulnerable to foreign pathogens and sometimes fails to prevent their intrusion. Fortunately, the emergence of vaccines significantly overcome this dilemma. Since the end of the 18th century, the discovery of the cowpox vaccine has laid the foundation for modern vaccine research[7]. The growing understanding of diseases and the rapid advancement of biotechnology have led to a diverse array of vaccine types, ranging from attenuated live vaccines and inactivated vaccines to subunit vaccines and nucleic acid vaccines, which have markedly reduced or even eradicated the spread of numerous infectious diseases[8,9]. Notably, the COVID-19 vaccines have played a pivotal role in controlling the recent pandemic, underscoring the vital importance of vaccines[10].

Vaccine adjuvants are substances that serve to enhance the immune response to foreign antigens. They have the capability to modify the type of immune response or stimulate a sustained and effective immune response by providing an antigen reservoir, enhancing targeted antigen delivery, increasing antigen uptake by immune cells, and promoting immune cell activation. These functions are crucial for enhancing the protective efficacy of vaccines, minimizing the required dosage, and reducing production costs, ultimately improving both the effectiveness and safety of vaccines[11]. Certainly, the discovery of new adjuvants has consistently served as a significant force motivating the revolution of immune vaccines[12,13]. Through ongoing exploration, multiple types of adjuvants have been discovered and utilized in clinical practice. Currently, adjuvants can be divided into human vaccine adjuvants and veterinary vaccine adjuvants[14,15]. Human vaccine adjuvants include aluminum-based compounds, virosomes, and oil-in-water emulsions, while veterinary vaccine adjuvants encompass Freund's adjuvants, propolis, and aluminum hydroxide, among others[16]. Despite the significant efficacy of vaccines promoted by these adjuvants, insufficient cellular immune responses, reduced efficacy in elderly populations, and the short durability of protection still limit their applications. Consequently, there is a pressing need to develop more advanced and comprehensive vaccine adjuvants.

Traditional Chinese medicines (TCMs), derived from plants, animals, and minerals, have guarded the health of Chinese people for thousands of years[17,18]. Compared to chemically synthesized drugs, TCMs offer unique advantages, including a wide range of sources, diverse bioactive ingredients, and fewer side effects[19]. Additionally, numerous studies have reported that TCMs can enhance immunity[20−22]. The active ingredients in TCMs, such as polysaccharides, saponins, flavonoids, acids, and alkaloids, are widely used in the treatment of immunological diseases, including autoimmune disorders and tumors. These properties suggest that TCM-derived compounds hold potential for application in immunotherapy and vaccine development. Compared to the limitations of existing adjuvants, TCM-based adjuvants offer effective solutions. They not only promote the maturation of dendritic cells (DCs) but also increase the secretion of cytokines such as IL-12, thereby activating $CD8^+$ T cells and enhancing Th1/CTL immune responses[23]. Moreover, TCM-based adjuvants improve antigen presentation, which is impaired by immunosenescence, by boosting the activity of antigen-presenting cells (APCs)[24]. Additionally, they restore immune function by modulating immunosuppressive cells, including tumor-associated macrophages (TAMs) and regulatory T cells (T_{reg})[25]. Importantly, TCM-based adjuvants facilitate the generation of central memory T cells (T_{CM}) and effector memory T cells (T_{EM}), thereby prolonging immune memory[26−28].

In this article, we provide a comprehensive overview of reported and potential TCM-derived active ingredients that serve as immune adjuvants. We systematically outline their application scenarios, underlying mechanisms, and optimization strategies, focusing on four key aspects: (i) the diverse range of active ingredients, (ii) mechanisms for modulating immune responses, (iii) rational structural modifications to enhance immune response, and (iv) suitable carriers for efficient delivery (Fig. 1). At the end of the article, potential future directions of the TCM-derived adjuvants were discussed. This review systematically summarizes current findings on natural adjuvants, aiming to offer valuable insights for the further exploration and development of safe and effective adjuvants for human applications.

2. Potential adjuvants from TCM-derived bioactive ingredients

The active ingredients abundant in TCMs, including saponins, polysaccharides, flavonoids, and alkaloids, form the pharmacological foundation of TCMs and are increasingly supported by significant scientific evidence (Fig. 2)[29]. These compounds exert diverse therapeutic effects, including anti-inflammatory, antimicrobial, and antioxidative properties, with a particular emphasis on their immunoregulatory functions. Therefore, understanding these active ingredients is essential for the discovery of new natural adjuvants. Here, we briefly introduce some reported TCM-derived active ingredients and their therapeutic effects.

2.1. *Polysaccharides*

Polysaccharides, together with proteins and nucleotides, are considered the triumvirate of essential biological substances, playing pivotal roles in biological processes and exhibiting diversepharmacological activities[30,31]. Since the discovery of the anti-tumor activity of mushroom polysaccharides in 1968, there has been a substantial increase in interest in natural active polysaccharides[32]. In recent years, numerous TCM-derived

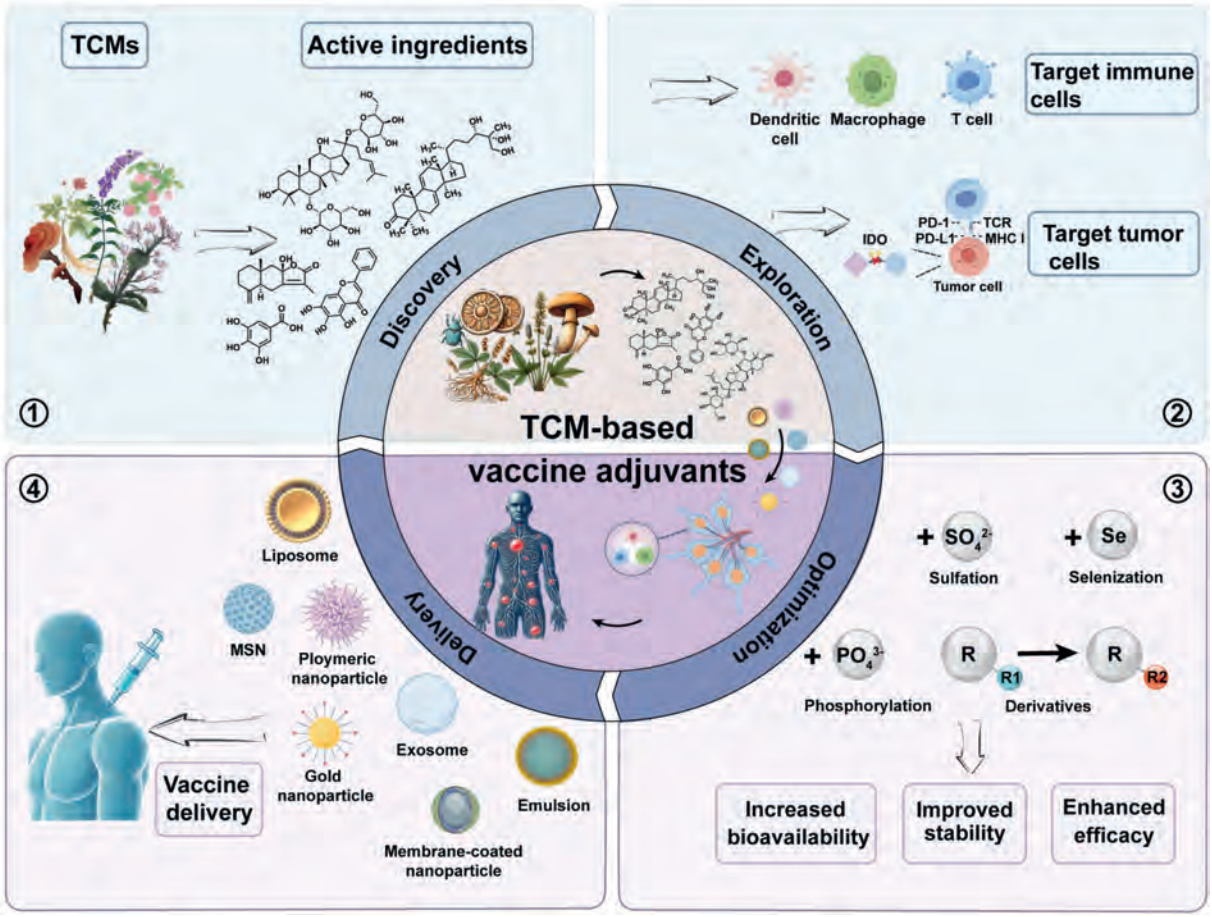

Figure 1 Schematic illustration of the TCM-based bioactive ingredients for vaccine adjuvants. The review covers the following key aspects: (1) classification of natural adjuvant categories; (2) mechanisms of interaction with the immune system; (3) strategies for structural modification; and (4) options for delivery carriers.

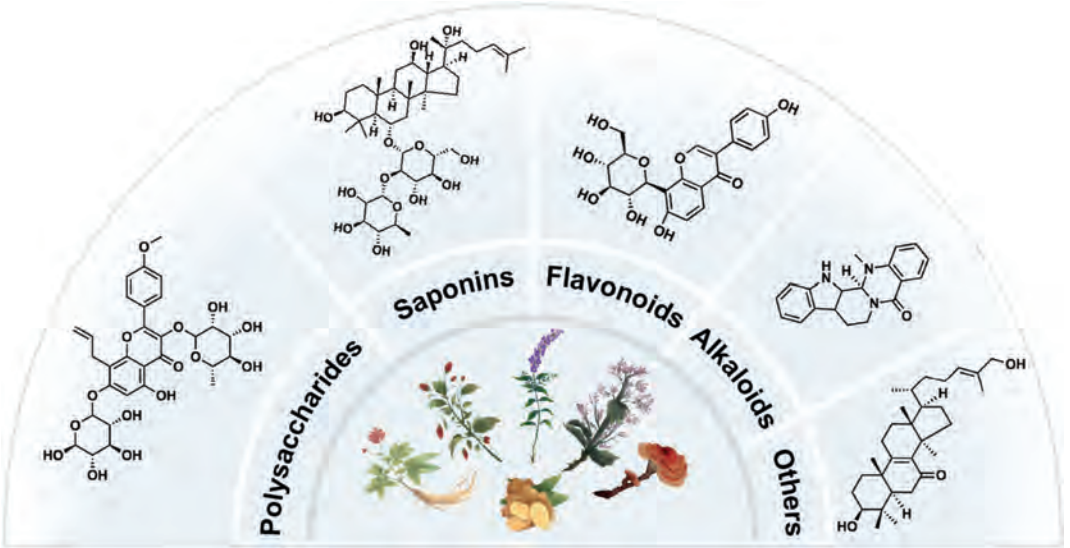

Figure 2 Structural categories and various sources of TCM-derived adjuvants.

polysaccharides have been reported to possess immunomodulatory effects, along with excellent biocompatibility and biodegradability. Furthermore, polysaccharides not only promote the development of immune organs and the secretion of immune-related molecules but also enhance innate immune function and antigen-specific immune responses[33]. TCM-derived polysaccharides can activate diverse downstream signaling pathways, including NF-κB, MAPK, and PI3K/Akt, by interacting with

various pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), Dectin-1, and mannose receptors. These multi-targeted actions enable polysaccharides to exert systemic immunomodulatory effects.

Astragalus polysaccharide (APS), derived from *Astragalus membranaceus*, exhibits antitumor, anti-aging, and immunomodulatory effects[34,35]. APS has been proven to enhance immune organ function, stimulate immune cell proliferation, trigger cytokine release, and influence immunoglobulin secretion[36]. Zhu et al.[37] demonstrated that maturation markers of dendritic cells (DCs) such as CD40, CD80, and CD86 were upregulated after incubation with the pure polysaccharide APSII. In C57BL/6 mice, APS not only promoted the maturation of splenic DCs and the secretion of pro-inflammatory cytokines (IL-6, IL-12, and TNF-α), but also enhanced the proliferation of T lymphocytes (T cells) and B lymphocytes (B cells), leading to the production of various immune antibodies (IgA, IgG, and IgM) and complements (complement 3 and complement 4)[38,39]. Hence, APS holds significant promise as a vaccine adjuvant, demonstrating efficacy in enhancing the effectiveness of vaccines against various viruses, including Newcastle disease virus (NDV), hepatitis B virus (HBV), COVID-19, and foot-and-mouth disease virus (FMDV)[40−43].

Ganoderma lucidum polysaccharide (GLP) is the principal bioactive component derived from *Ganoderma lucidum* (*G. lucidum*)[44], the addition of GLP to enterovirus vaccines significantly enhances the production of neutralizing antibodies and induces antiviral-related immune responses[45,46]. Compared to vaccines alone, those supplemented with GLP can stimulate greater generation of specific IgG and IgA antibodies. GLP also exhibits notable adjuvant activity in the NDV vaccines by improving DC activation and DC maturation, thereby substantially enhancing the vaccine's antiviral efficacy[47,48]. Furthermore, *G. lucidum* spore polysaccharide (GLSP) could polarize primary macrophages towards the tumor-inhibitory M1 type, thereby enhancing tumor suppression[49].

As a natural immune booster, *Lycium barbarum* polysaccharide (LBP) shows substantial potential for broad applications. LBP was reported to enhance macrophage phagocytosis, increase the cytotoxic activity of natural killer (NK) cells, and promote the proliferation and differentiation of T cells[50,51]. Additionally, LBP facilitated DC maturation and stimulated the secretion of IL-6, TNF-α, and IL-12 by activating the TLR4-Erk1/2-Blimp1, TLR4−MyD88−NF-κB, and Notch signaling pathways[52−54].

GPS-1 is a water-soluble neutral polysaccharide isolated from *Glycyrrhiza* polysaccharide (GPS), which has been shown to enhance the maturation, antigen presentation, and phagocytic capacity of DCs, primarily via the TLRs/NF-κB signaling pathway[55]. Wu and co-workers[56] also discovered the co-administration of GPS-1 with the Newcastle disease vaccine in chicken resulted in increased secretion of IL-2, IL-4, IL-17, and IFN-γ. The addition of GPS-1 not only expanded the proportions of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells in the spleen and peripheral blood, but also elevated the concentrations of IgG and IgA, demonstrating that GPS-1 enhanced both cellular and humoral immunity.

In addition to the polysaccharides mentioned above, polysaccharides derived from *Codonopsis pilosula*, *Taraxacum officinale*, *Angelica sinensis*, *Isatis indigotica, Achyranthes bidentata, Rehmannia glutinosa,* and *Epimedium* can also serve as adjuvants to regulate immune responses and improve vaccine efficacy[57−64]. Compared to other bioactive compounds, TCM-derived polysaccharides exhibit distinct advantages, including high safety profiles and significant structural modifiability. These polysaccharides are typically characterized by low toxicity, excellent biocompatibility, and minimal adverse effects on the immune system, even with prolonged use. Nevertheless, the poor water solubility of certain polysaccharides limits their bioavailability and *in vivo* effectiveness[65]. Functional modifications, such as sulfation or phosphorylation, have been shown to markedly enhance their water solubility and bioactivity. Therefore, focusing on the structure of these polysaccharides and making appropriate modifications are crucial for enhancing their immunomodulatory functions and broadening their potential applications. Notably, the intricate chemical structures and the broad molecular weight distribution of polysaccharides remain substantial challenges for both their research and large-scale industrial production, representing key issues that need to be resolved.

2.2. *Saponins*

Saponins are triterpenoid glycosides widely present in various plants. Since they were extracted from *Quillaja saponaria Molina*, their potential as adjuvants has been discovered and applied in vaccines. The human shingles vaccine (Shingrix) and the malaria vaccine (Mosquirix), have both been approved for use with the first saponin-based adjuvant AS01[66]. Furthermore, AS01 is gaining recognition as a potential adjuvant for the tuberculosis vaccine. The adjuvant component QS-21 in AS01 is derived from the *Quillaja saponaria*, which can induce a strong antibody immune response and direct killing of foreign substances by $CD8^+$ T cells, making it widely used in clinical trials for vaccines treating cancer and infectious disease[67]. Saponins not only reduce the number of vaccine doses and prolong the duration of action but also improve the safety of vaccines by reducing antigen dosage, which contributes to their low toxicity and high efficiency. However, the complex chemical composition of QS saponins, which are only obtainable from the bark of soapbark trees, significantly impacts their commercial viability[68]. Currently, only a few saponin adjuvants have been approved for marketing. Therefore, exploring environmentally sustainable "tree-free" strategies to obtain these compounds and discovering more saponin adjuvants has garnered increasing interest.

With the growing understanding of anti-tumor immunity, the immunomodulatory functions of ginsenosides have garnered increasing attention[69]. For instance, Wang et al.[70] found that ginsenoside Rh2 not only promoted the polarization of TAMs from the M2 to M1 macrophages but also reduced the expression of various pro-tumorigenic factors, such as vascular endothelial growth factor (VEGF), thereby inhibiting the proliferation and migration of lung cancer cells. This suggested that Rh2 improved the tumor microenvironment (TME) by modulating the phenotype of TAMs. Additionally, ginsenoside Rh2 significantly increased T cell infiltration into tumor tissue and enhanced the anti-tumor immune response. As a result, it notably inhibited melanoma growth and extended the survival time of B16-F10-bearing mice. Lee et al.[71] also found that ginsenosides Rg3 can promote NK cell activity by activating the MAPK/ERK pathway, indicating that ginsenosides Rg3 can enhance immune function by increasing NK cell activity.

In addition to ginsenosides, various natural saponins have been identified as playing significant roles in enhancing immune responses. *Panax notoginseng* saponins (PNS) have been

demonstrated to reduce the number of TAMs in the TME and decrease the expression levels of pro-tumor cytokines such as IL-6, IL-10, and TNF-α[72]. Furthermore, PNS can downregulate the expression of genes involved in tumor angiogenesis, migration, and adhesion[72]. *Polygala tenuifolia* saponin or *Chenopodium quinoa* saponin have shown significant therapeutic effects on mice infected with influenza virus a/PR/8/34, while also increasing serum hemagglutination inhibition (HI) antibody levels and nasal anti-influenza virus IgA and IgG antibody titers[73,74].

Saponins significantly enhance immune responses by directly stimulating immune cell receptors, particularly promoting cellular immunity and humoral immunity, demonstrating considerable immunomodulatory potential. Concurrently, saponins help maintain immune system homeostasis by preventing both excessive activation and suppression, thus offering therapeutic potential for immune-related disorders such as allergic and autoimmune diseases. However, certain saponin components (*e.g.*, ginsenosides, glycyrrhizin) may possess toxicity, and excessive use can lead to adverse effects. Furthermore, the extraction and purification of saponins are complex, posing challenges for industrial-scale production and limiting their broad application in certain fields. Additionally, the immunomodulatory effects of saponins are highly dose-dependent, necessitating precise dosage control to optimize therapeutic efficacy and avoid potential side effects.

2.3. Flavonoids

Flavonoids are widely present in most TCMs, with notable examples such as *Scutellaria baicalensis, Phellodendron amurense,* and *Sophora flavescens*. They are widely distributed natural organic compounds, with complex structural types, mainly including flavonoids, flavonols, dihydroflavonols, isoflavones, dihydroisoflavones, anthocyanins, and bifidoflavonoids. Flavonoids are renowned for their potent anti-inflammatory, antibacterial, and anti-tumor effects[75–77]. Recently, their roles in immune regulation have gained increasing attention. Hesperidin, a dihydroflavonoid derivative from citrus fruits, has emerged as a promising therapeutic agent. It was found that oral administration of hesperidin can increase NK cell cytotoxicity in rats after vigorous exercise and the proportion of lymphocytes in the thymus, blood, and spleen[78]. Camps Bossacoma et al.[79] reported that a 5% hesperidin diet altered the lymphocyte composition in the intestinal epithelium and lamina propria of rats sensitized by oral ovalbumin (OVA) and cholera toxin.

Puerarin, a naturally occurring active ingredient derived from *Pueraria lobata,* has been identified as a potential therapeutic agent for viral infections, demonstrating significant antiviral activity. Liang et al.[80] indicated that puerarin may be effective in treating EHF/COVID-19 patients through anti-inflammatory, immune regulation, and antiviral mechanisms. Studies have demonstrated that the natural flavonoid compound baicalin enhances anti-tumor immune responses by promoting the autophagic degradation of CD274/Programmed Death-Ligand 1 (PD-L1) on non-small-cell lung cancer (NSCLC) cells[81]. This process restores the ability of T cells to effectively target and eliminate tumor cells, thereby strengthening the overall anti-tumor immune response.

Flavonoids exert nuanced effects on immune regulation through multiple mechanisms, including the modulation of immune cell activity and cytokine secretion, thereby enhancing immune function. Moreover, flavonoids are characterized by low toxicity and minimal adverse effects, rendering them suitable for long-term use in managing chronic conditions. Their relatively mild immunomodulatory effects further underscore their safety profile. In addition, flavonoids possess potent antioxidant properties that mitigate free radical production and protect immune cells from oxidative damage, thus preserving the normal function of the immune system. However, the poor bioavailability, the complexity of immune regulatory mechanisms, and challenges associated with precise dosage control limit their broader application in certain therapeutic contexts.

2.4. Alkaloids and others

Alkaloids are critical active components in TCMs, known for their diverse therapeutic functions, including pain relief, anti-inflammatory, anti-tumor, and antiviral effects[82]. Over 60 distinct alkaloids with varying structures have been identified, encompassing organic amines (*e.g.*, ephedrine, motherwort alkaloids, colchicine), pyridines (*e.g.*, arecoline, hemiphylline), and indoles (*e.g.*, camptothecin, ergoline). Alkaloids have also attracted significant interest for their immunomodulatory effects. Matrine, an alkaloid derived from *Sophora flavescens* Aiton, enhanced anti-tumor immunity by promoting cytokine release through the regulation of TLR7/8 signaling pathways[83]. Researchers proved that matrine stimulated immune cells to secrete higher levels of pro-inflammatory cytokines including IL-6, TNF-α, and IL-12, while reducing IL-10 secretion. After administration, the proportion of $CD80^{+}CD86^{+}$ DCs was also elevated[83]. Furthermore, matrine regulated the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (PKB) signaling pathway to inhibit the differentiation of TAMs into the M2 type, thereby improving the immunosuppressive microenvironment[84]. Sanguinarine is an important benzylisoquinoline alkaloid (BIA) extracted from the roots of *Sanguinaria canadensis*, possessing significant anti-tumor potential[85]. Myeloid-derived suppressor cells (MDSCs) exert immunosuppressive ability by suppressing T cell function, promoting Treg development, inhibiting NK cell function, interfering with APCs, and aggravating the immunosuppressive condition in the TME[86]. Sanguinarine could promote the differentiation of MDSCs into macrophages and DCs, thereby weakening their immunosuppressive capabilities and enhancing anti-tumor immune responses[87].

Furthermore, other components in TCMs, such as polyphenols, quinones, and organic acids, also play important roles in immunomodulation[88]. Gallic acid, which can be extracted from natural plants like bananas, lemons, grapes, and tea leaves, as well as produced by *Bacillus subtilis* and *Lactobacillus plantarum* in the human body, has been closely linked to the development of colorectal cancer (CRC). Deng et al.[89] discovered that gallic acid inhibited the expression of Usp21, thereby downregulating Foxp3 protein levels, which induced the generation of T_{reg} and weakened their immunosuppressive function. In addition, gallic acid downregulated the expression of PD-L1 in Tregs, thereby enhancing the anti-tumor immune effects of anti-Programmed Death-1 (aPD-1) therapy.

Compared to flavonoids, alkaloids exhibit more direct and rapid immunomodulatory effects, significantly enhancing immune responses within a short time frame. However, alkaloids are typically characterized by low bioavailability, which results in relatively slow absorption, distribution, and metabolism in the body, potentially affecting their immunomodulatory efficacy. Furthermore, the immunological effects of alkaloids are highly dose-dependent, and the suboptimal doses may fail to effectively activate immune responses, while excessive doses can induce

toxic side effects. At high concentrations, certain alkaloids may cause toxic damage to organs such as the liver and kidneys, thereby limiting their broader therapeutic application. Additionally, the extraction and purification processes of alkaloids are complex, particularly for alkaloids such as colchicine, which are sensitive to environmental conditions. This necessitates strict control over extraction conditions, increasing production costs and limiting their industrial application in some areas.

3. Immune regulatory mechanisms of TCM-derived active ingredients

Currently, immunotherapy is playing a crucial role in treating stubborn diseases such as cancer, influenza, and hepatitis B. Several treatment modalities, including CAR-T, TCR-T, vaccines, and immune checkpoint inhibitors, have been developed. Unlike other treatment methods, vaccines function by introducing specific antigens to elicit targeted immune responses, mobilizing the immune system to destroy target cells. Vaccines can rapidly activate the immune system, inducing sustained humoral and cellular immunity, as well as triggering long-term immunity. Adjuvants, as essential components of vaccines, ensure their effectiveness[90]. In recent years, various active ingredients derived from TCMs, such as polysaccharides, flavonoids, saponins, and alkaloids, have been identified and used as vaccine adjuvants, exhibiting strong immunological activity. Understanding the mechanisms of these active ingredients aids in the discovery of more potential adjuvants, determining their application scenarios, and achieving structural modifications. In the following sections, we will introduce the adjuvant functions of TCM-based adjuvants from three perspectives: regulating immune cells, influencing target cells, and modulating immune pathways (Fig. 3).

3.1. *Directly modulate immune cells*

3.1.1. *Modulating DCs*

DCs, as the most specialized APCs, are essential for linking innate and adaptive immunity. They achieve this by capturing, processing, and presenting antigens, thereby triggering both humoral and cell-mediated immune responses[91]. Immature DCs have limited proficiency in antigen presentation to T cells and possess a restricted capacity to initiate immune responses. Upon antigen stimulation, the expression of major histocompatibility complex (MHC) molecules and costimulatory molecules (such as CD80 and CD86) on the surface of mature dendritic cells is upregulated, enabling their interaction with recognition receptors on naïve T cells, thereby activating T cells and ultimately initiating a specific immune response[92,93]. DCs serve as central regulators of vaccine efficacy, owing to their robust antigen-presenting capabilities and diverse immunomodulatory functions, which are essential for effective vaccination outcomes. Feng et al.[94] isolated and purified a new 62,722 Da polysaccharide (GSPA-0.3) from ginseng roots. GSPA-0.3 enhanced the uptake of FITC-OVA antigen by DC2.4 cells and promoted the maturation of DCs by activating the TLR4 receptor, demonstrating its potential as an immune adjuvant. Other polysaccharides have been reported to promote the maturation of DCs modulating signaling pathways such as TLRs, NF-κB, and MAPK[53,95–99]. This activation led to the secretion of pro-

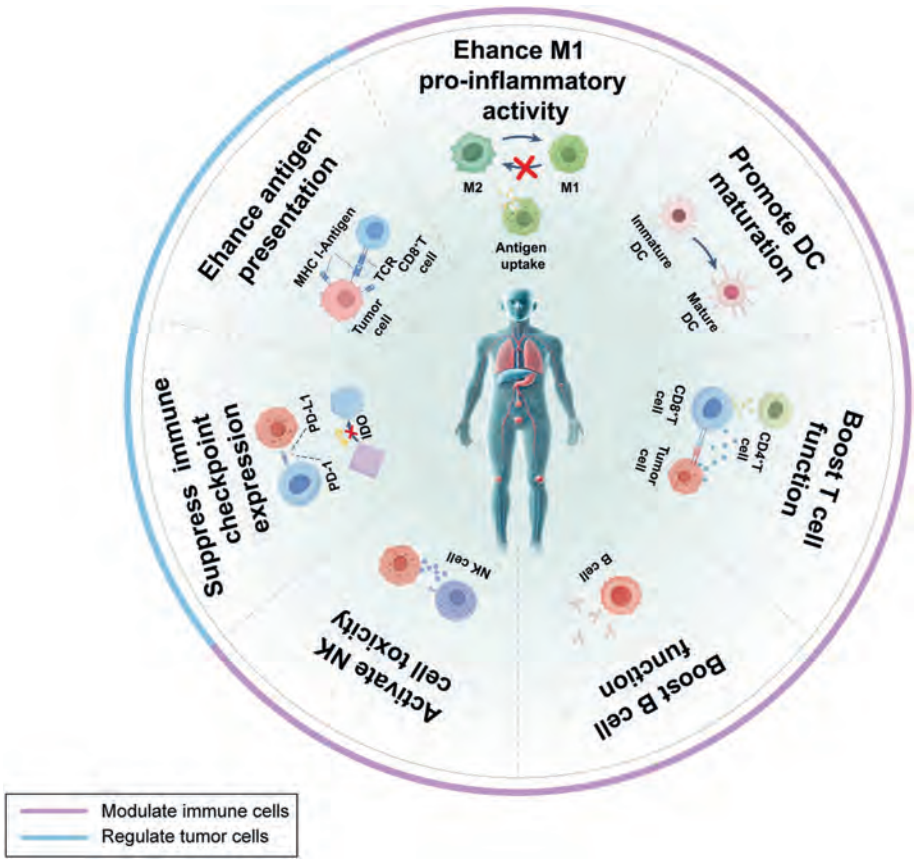

Figure 3 Overview of immunological mechanisms by which natural adjuvants engage and mobilize the immune system.

inflammatory cytokines, thereby contributing to enhanced antitumor efficacy. These findings highlight the significant potential of polysaccharides as adjuvants for promoting DC maturation and improving the efficacy of vaccine therapies.

3.1.2. Modulating macrophages

Macrophages play multiple roles in the immune system, functioning as both primary agents of innate immunity and critical regulators of adaptive immunity[100]. As the first guard in the innate immune system, macrophages can swiftly recognize and engulf invading pathogens, initiate local inflammatory responses by secreting inflammatory mediators, and attract other immune cells to limit their spread. Macrophages exhibit varying functional states depending on the microenvironment and are generally classified into M1 and M2 phenotypes[101]. In anti-tumor immunotherapy, the quantity and proportion of M1 macrophages play a crucial role in determining the efficacy of vaccines[102,103]. Deng et al.[104] found that icariin II can suppress M2 macrophage polarization *via* the PI3K/Akt/β-catenin pathway. Additionally, ganodermanontriol could modulate the M2 polarization of RAW264.7 macrophages by binding to STAT6 and inhibiting its phosphorylation, suggesting the improved treatment of gastric cancer in mice[105]. The expression of Arg-1, IL-10, and CD206 in macrophages was decreased, which were classic markers on M2 macrophages. Chen et al. reported that Vitexin could promote the polarization of TAMs to M1 type through the vitamin D receptor (VDR)/phenazine biosynthesis-like domain protein (PBLD) pathway[106]. In Hepa1-6 tumor-bearing mice, oral administration of Dendrobium officinale polysaccharide significantly reduced the number of M2 macrophages in the TME, while enhancing the M1 polarization of TAMs *via* targeting the TLR2[107].

3.1.3. Modulating NK cells

NK cells, with the ability to quickly identify and eliminate infected cells and tumor cells, contribute to antiviral, anti-tumor, and immune response regulation through mechanisms including direct cytotoxicity (*via* perforin and granzyme release), antibody-dependent cell-mediated cytotoxicity (ADCC), and cytokine secretion (*e.g.*, IFN-γ, TNF-α, and GM-CSF)[108]. Therefore, modulating the activity of NK cells by TCM-based adjuvants is crucial for enhancing immune responses. Oridonin, a diterpenoid compound extracted from *Rabdosia rubescens*, shows promise in treating inflammation, infections, and cancer[109−112]. Chang and coworkers demonstrated that oridonin treatment upregulated the expression of activating receptors (DAP10, NKp30, NKp46) in NK-92MI cells and increased the gene expression levels of perforin, IFN-γ, and NKG2D ligand, all associated with NK cell cytotoxicity[113]. The synergistic effect of oridonin and NK-92MI cells killing tumor cells may be mediated through inhibition of STAT3 phosphorylation, a key regulator of NK cell-mediated tumor surveillance function.

3.1.4. Modulating B cells and T cells

The main functions of B cells include antigen recognition, antibody production, and immune memory formation. The antibodies they produce, such as IgA, IgD, IgE, IgM, and IgG, bind to key epitopes on the surface of pathogens, reducing their aggressiveness and increasing their recognition by the immune system. Additionally, these antibodies can activate the complement system and trigger ADCC, thereby enhancing the destruction of infected cells[114]. Xie et al.[115] found that alfalfa polysaccharides enhanced the immune function of splenic B cells *via* the TLR4 pathway.

T cells can be classified into different subtypes based on their specific roles in immune regulation: cytotoxic T cells ($CD8^+$ T cells), helper T cells ($CD4^+$ T cells), T_{reg} ($CD4^+Foxp3^+$ T cells), and memory T cells (T_M). $CD8^+$ T cells are a specific subset of killer T cells capable of inducing apoptosis in target cells *via* the perforin-granzyme and the Fas-FasL pathway. These cells are key components of the adaptive immune system, and their regulation by TCMs directly influences the strength of immune responses. For example, evodiamine could enhance the recognition and infiltration of $CD8^+$ T cells into NSCLC tumors, thereby increasing the efficacy of PD-1 therapy[116]. Taurine plays a vital role in maintaining leukocyte function and immune activity[117]. Mechanistically, tumor cells upregulate SLC6A6 expression to compete for taurine, thereby suppressing $CD8^+$ T cell immune responses. Cao et al.[118] found that taurine supplementation not only reversed the T cell functional defects caused by taurine deficiency mediated by SLC6A6 in tumor cells but also reduced chemotherapy-induced apoptosis of $CD8^+$ T cells, enhancing the antitumor efficacy of combined treatment with fluorouracil. $CD4^+$ T cells play crucial regulatory and auxiliary roles in the immune system. By secreting cytokines and interacting with other immune cells, they coordinate and enhance immune responses. Among them, Tregs play a dual role in maintaining immune homeostasis while suppressing anti-tumor immunity, making them key regulators of vaccine-induced immune responses. GLPs have been found to increase promote Th1 cell infiltration in the spleen and tumors while reducing T_{reg} populations[119]. Yu et al.[120] demonstrated that ailanthone inhibited T_{reg} differentiation by suppressing the c-JUN/PD-L1 axis in melanoma cells, which significantly enhanced the efficacy of anti-PD-L1 therapy. Tms are a subset of T cells that differentiate after encountering specific antigens and possess the ability to provide long-term immune protection. Vaccines enhance this protection by increasing both the number and functionality of Tms, ensuring a sustained and effective immune response against previously encountered pathogens[121]. It was found that the berberine-based vaccine increased the proportion of $CD8^+$ Tcms by modulating the AMPK and JAK3/STAT5 pathways and triggered a stronger antitumor immune response[26].

3.2. Targeting tumor cells

Tumor cells can evade immune surveillance through mechanisms such as self-camouflage or by expressing specific surface proteins that diminish the effectiveness of immune cell-mediated destruction[122]. As a potential adjuvant, TCMs not only enhance immune responses by modulating immune cells but also directly influence target cells. For example, Rocaglamide A (RocA) could enhance the sensitivity of NLSLC cells to NK cell-mediated killing by inhibiting autophagy and promoting NK cell infiltration and antitumor immunity through activation of the cGAS−STING signaling pathway[123]. Moreover, TCMs can improve the immune sensitivity of target cells and enchance the capability of immune cells to recognize and eliminate them by downregulating immune checkpoints and enhancing recognition signals.

3.2.1. Blocking the immune checkpoint

Immune checkpoints play a key role in regulating the immune system's response to tumor cells, primarily by modulating T cell activity and preserving immune homeostasis. These regulatory mechanisms are essential for preventing excessive T cell activation, which can lead to autoimmune reactions. However, within

the TME, malignant cells exploit them to evade immune surveillance, thereby reducing the effectiveness of anti-tumor immune response[124].

Tumor cells often upregulate the expression of PD-L1, which binds PD-1 on the surface of T cells, to diminish anti-tumor immunity. The interaction results in the inhibition of T cell function, compromising their capability to effectively recognize and destroy tumor cells[125]. This immune evasion mechanism employed by tumor cells facilitates their persistence, proliferation, and eventual metastasis[126]. Interestingly, many TCM-derived bioactive compounds have been found to regulate the PD-1/PD-L1 axis to enhance anti-tumor immune responses[127−131]. For example, berberine was demonstrated to reduce PD-L1 expression on various tumor cells, such as non-small cell lung cancer cell lines (H460, H1975, H358, HCC827) and PD-L1-overexpressing A549 and H1299 cells[132]. The ubiquitin−proteasome pathway is a key mechanism for the self-degradation of PD-L1 in tumor cells[133]. However, tumor-induced TNF-α triggers the binding of COP9 signalosome subunit 5 (CSN5) to PD-L1, leading to its deubiquitination and subsequent stabilization of PD-L1[134]. Further research revealed that berberine inhibited CSN5 activity by directly binding to it, thereby affecting the ubiquitin−proteasome-mediated PD-L1 degradation and promoting its breakdown. Zhang and coworkers[135] found that the natural small molecule Benzosceptrin C from Pacific sponges could bind to the palmitoyltransferase DHHC3 enhancing PD-L1 ubiquitination, which promoted the encapsulation of PD-L1 by endosomal membranes, facilitating its transport to lysosomes for subsequent degradation. They also discovered that *Kaempferia parviflora*-derived 5,7,4′-trimethoxyflavone (TF) upregulated the expression of HRD1, an E3 ubiquitin ligase, in CRC cells. This increase in HRD1 expression promoted the ubiquitination and subsequent degradation of PD-L1, significantly enhancing the anti-tumor immune response. Compared to anti-CTLA-4 or anti-PD-1 therapy alone, the combined use of TF exhibited anti-tumor efficacy comparable to that of these therapies, underscoring the potent immune-enhancing capabilities of TCMs[136].

In addition to PD-1/PD-L1, indoleamine 2,3-dioxygenase (IDO) is highly expressed in various tumors and facilitates tumor immune evasion. IDO can catalyze the breakdown of tryptophan into kynurenine and its metabolites, which inhibits T cell activity and proliferation while promoting the function of immunosuppressive cells, thereby enabling tumor cells to evade immune surveillance[137]. Therefore, inhibiting IDO activity is important for restoring T cell function. Myricetin has been shown to significantly inhibit the proliferation of lung cancer cells (A549, NCI-H1650, and NCI-H460)[127]. Furthermore, treatment with myricetin inhibited IDO1 expression in these lung cancer cell lines, which might contribute to alleviating T cell immunosuppression. Atractylenolide III, a natural compound extracted from *Atractylodes macrocephala*, has demonstrated potential in downregulating IDO expression in mouse lung cancer cells by targeting the JAK3/STAT3 pathway[138].

3.2.2. *Enhancing the presentation of tumor antigens*

One of the key steps in the antigen presentation process is the processing and degradation of proteins within tumor cells into smaller peptides, which are subsequently loaded onto MHC-I molecules[139]. The proteasome, a multisubunit enzyme complex, is responsible for the degradation of most intracellular proteins[140]. It plays a crucial role in antigen presentation, particularly in the MHC-I-mediated antigen presentation pathway[141]. The proteasome degrades large proteins into smaller peptide fragments, which are then transported to the endoplasmic reticulum, where they bind to MHC-I molecules[142,143]. Upon being primed by DCs, activated $CD8^+$ T cells recognize and eliminate target cells *via* the TCR/CD3/MHC-I signaling pathway. In response, tumor cells can evade immune surveillance by disrupting antigen processing pathways, thereby impairing the recognization of $CD8^+$ T cells to them[144]. Xu et al.[145] discovered that atractylenolide I (ATT-I) specifically targeted and bound to the proteasome 26S subunit non-ATPase 4 (PSMD4), which was a crucial component of the immunoproteasome complex. The interaction between ATT-I and PSMD4 enhanced the antigen processing activity of the immunoproteasome, thereby improving the presentation of MHC-I-mediated antigens on both human and mouse colorectal cancer cells.

Studies have shown that tumor cells can degrade MHC-I molecules *via* the autophagic pathway by transporting them to the lysosomes, resulting in a reduction in the availability of MHC-I for antigen presentation on the cell surface, thereby leading to immune evasion[146−148]. Deng et al.[149] demonstrated that treatment with cycloastragenol significantly increased the expression levels of antigen presentation-related genes, including H2-K1, B2m, Anxa1, and CD74, in MC38 tumor tissues of treated mice. Additionally, cycloastragenol upregulated the expression of transcription factors associated with antigen presentation, thereby enhancing the process of antigen presentation. The authors also observed a negative correlation between cathepsin B and MHC-I expression after cycloastragenol treatment. As a result, cycloastragenol could inhibit the binding of cathepsin B to MHC-I, resulting in increased accumulation of MHC-I on the cell membrane and enhanced $CD8^+$ T cell-mediated cytotoxicity (Table 1).

4. Structural modification to enhance activity

The chemical structure of a compound is intricately linked to its biological function, with specific functional groups, molecular configurations, and stereochemical features playing a crucial role in determining its bioactivity. Although active components of TCMs have demonstrated strong immunomodulatory effects, to meet the stringent criteria for effective immune adjuvants requires further enhancements in activity, selectivity, stability, and bioavailability. Consequently, targeted chemical modifications of these natural compounds can pave the way for the development of more potent and safer natural adjuvants, thereby increasing their potential for clinical application. Through continuous research, scientists have optimized these natural adjuvants by implementing precise chemical modifications, such as sulfation and selenization, or by synthesizing derivatives from the original compounds.

4.1. *Sulfation*

Sulfation of natural products involves introducing negatively charged sulfate groups into these compounds, which can profoundly influence their physicochemical properties and biological activities. This chemical modification enhances the potential of natural products in various therapeutic areas, including drug development, immunomodulation, antiviral treatments, and anticancer therapies. Sulfation is particularly common in natural polysaccharides, which exhibit improved bioavailability, antibacterial activity, and effectiveness in the prevention and treatment of thrombosis once sulfated. For example, sulfated

Table 1 List of active compounds with adjuvant properties from TCMs.

Structure	Adjuvant component	Solubility	Dosage	Source	Key aspects of enhancing immunity	Ref.
Polysaccharides	*Astragalus* polysaccharide	Soluble in water (10 mg/mL)	45 μg/mL *in vitro* 80 mg/kg *p.o.* in mice	*Astragalus membranaceus*	Promote DC maturation Enhance antibody secretion Boost T cell and B cell function	37–39
	Ganoderma lucidum polysaccharide	Soluble in water	800 μg/mL *in vitro* 10 μg/mouse i.n.	*G. lucidum*	Enhance antibody secretion Promote DC maturation Regulate macrophage polarization	45,46,49
	Lycium barbarum polysaccharide	Soluble in water	125 μg/mL *in vitro* 250 mg/kg i.g. in mice	*Lycium barbarum*	Promote DC maturation Activate NK cells Enhance macrophage phagocytosis Boost T cell function	50–54
	Glycyrrhiza polysaccharide	Soluble in water	600 mg/kg i.g. in chickens	*Glycyrrhiza*	Promote DC maturation Boost T cell function Regulate Th1/Th2 Reduce Tregs	55,56,119
	Codonopsis pilosula polysaccharide	Soluble in water	0.782 μg/mL *in vitro* 20 μg/mouse s.c.	*Codonopsis pilosula*	Boost T cell function Enhance antibody secretion	57
	Dandelion polysaccharide	Soluble in water	200 mg/kg i.p. in mice	*Dandelion*	Boost $CD8^+$ T cell function	58
	Angelica sinensis polysaccharide	Soluble in water	15.625 μg/mL *in vitro*	*Angelica sinensis*	Boost T cell function Activate NK cells	59
	Epimedium polysaccharides-1 (EPS-1)	Soluble in water	19.53 μg/mL *in vitro*	*Epimedium acumnatum*	Boost T cell function Promote DC maturation	60
	IIP-A-1 and IIP-2	Soluble in water	200 μg/mouse i.m.	*Isatis indigotica*	Boost T cell function	61
	Achyranthes bidentata polysaccharide	Soluble in water	12.5 μg/mL *in vitro* 200 μg/mouse i.m.	*Achyranthes bidentata*	Boost T cell function Promote DC maturation	62
	Rehmannia glutinosa polysaccharide	Soluble in water	50 mg/kg i.n. in mice	*Rehmannia glutinosa*	Promote DC maturation	63
	GSPA-0.3	Soluble in water	50 μg/mL *in vitro* 100 μg/mouse i.m.	*Panax ginseng*	Promote DC maturation	94
	Alfalfa polysaccharide	Soluble in water	19.53 μg/mL *in vitro*	Alfalfa	Boost B cell function	115
	Dendrobium officinale polysaccharide	Soluble in water	200 mg/kg *p.o.* in mice	*Dendrobium officinale*	Regulate macrophage polarization	107
Saponins	Ginsenoside Rh2	Water-insoluble/ Soluble in ethanol	0.2 mg/kg s.c. in mice	*Panax ginseng*	Regulate macrophage polarization Boost T cell function	70
	Ginsenoside Rg3	Soluble in ethanol (≥50 mg/mL)	100 μmol/L *in vitro*	*Panax ginseng*	Activate NK cells	71
	Panax notoginseng saponin	Soluble in water (1 mg/mL)	160 mg/kg *p.o.* in mice	*Panax notoginseng*	Regulate macrophage polarization	72
	Chenopodium quinoa saponin	Soluble in water	133 μg/mouse s.c.	*Chenopodium quinoa*	Boost T cell function Enhance antibody secretion	73
	Icariin II	Soluble in DMSO	10 mg/kg i.g. in mice	*Epimedium brevicornum*	Regulate macrophage polarization	104
Flavonoids	Hesperidin	Slightly soluble in water (0.02 mg/mL)	200 mg/kg *p.o.* in rats	Citrus fruits	Change lymphocyte composition Activate NK cells	78,79
	Rocaglamide A	Soluble in DMSO (100 mg/mL)	1 mg/kg i.p. in mice	*Aglaia odorata*	Activate NK cells	123

	5,7,4′-trimethoxyflavone	Soluble in DMSO	20 μmol/L *in vitro* 25 mg/kg i.p. in mice	*Kaempferia parviflora*	Reduce PD-L1 expression	136
	Myricetin	Soluble in water (54.9 mg/mL)	20 μmol/L *in vitro*	*Myrica rubra*	Reduce IDO expression	127
	Vitexin	Soluble in DMSO	20 μmol/L *in vitro*	Hawthorn	Regulate macrophage polarization	106
Alkaloids	Matrine	Soluble in water	20 μmol/L *in vitro* 40 mg/kg i.g. in mice	*Sophora flavescens*	Promote DC maturation Regulate macrophage polarization	83,84
	Sanguinarine	Slightly soluble in DMSO	5 mg/kg i.p. in mice	*Sanguinaria canadensis*	Reduce MDSCs	87
	Berberine	Soluble in DMSO (10 mmol/L)	10 μmol/L *in vitro* 8 mg/kg i.p. in mice 100 mg/kg *p.o.* in mice	*Coptis chinensis* et al.	Boost $CD8^+$ Tcms function Reduce PD-L1 expression	26,132
	Evodiamine	Soluble in DMSO	30 mg/kg i.g. in mice	*Evodia rutaecarpa*	Boost $CD8^+$ T cell function Reduce PD-L1 expression	116
Others	Gallic acid	Soluble in ethanol	5 mg/kg i.p. in mice	Various plants	Reduce Tregs	89
	Ganodermanontriol	Soluble in DMSO	20 μmol/L *in vitro* 20 mg/kg i.g. in mice	*Ganoderma lucidum*	Regulate macrophage polarization	105
	Oridonin	Soluble in DMSO (≥150 mg/mL)	10 mg/kg i.p. in mice	*Isodon rubescens*	Activate NK cells	113
	Taurine	Soluble in water (50 −100 mg/mL)	60 μmol/L *in vitro* 40 mg/kg i.m. in mice	Animals	Boost $CD8^+$ T cell function	118
	Ailanthone	Soluble in DMSO (40 mg/mL)	0.5 mg/kg i.p. in mice	*Ailanthus altissima*	Reduce Tregs	120
	Benzosceptrin C	Soluble in ethanol	10 μmol/L *in vitro* 50 mg/kg i.p. in mice	Pacific sponges	Reduce PD-L1 expression	135
	Atractylenolide III	Soluble in DMSO (≥40 mg/mL)	32 μmol/L *in vitro* 100 mg/kg i.g. in mice	*Atractylodes macrocephala*	Reduce IDO expression	138
	Atractylenolide I	Soluble in DMSO	50 mg/kg i.p. in mice	*Atractylodes macrocephala*	Promote antigen presentation	145
	Cycloastragenol	Soluble in DMSO	50 mg/kg i.g. in mice	*Astragalus membranaceus*	Promote antigen presentation	149

glycosaminoglycans (sGAGs), found in TCMs such as animal skin, bone, horn, and scales, display a broad spectrum of pharmacological activities. These sGAGs are widely present in the extracellular matrix, on cell surfaces and within intracellular vesicles, where they interact with positively charged amino acids or polar residues on target proteins. This interaction can affect protein−protein interactions or induce the oligomerization of target proteins[150,151]. Fang et al.[152] identified that the interaction between sGAGs and STING is a key factor in determining STING activation and its strength, highlighting the close relationship between sulfated polysaccharides and their immunomodulatory functions.

Yu et al.[153] found that sulfated *Cyclocarya paliurus* polysaccharides (SCP3) not only enhanced the phagocytic activity of macrophages, leading to an improved innate immune response against exogenous pathogens, but also activated the MAPKs and NF-κB signaling pathways in macrophages through interaction with TLR4. This activation subsequently regulated the secretion levels of nitric oxide (NO) and cytokines in macrophages, demonstrating its potential for immune modulation (Fig. 4A). Yang et al.[154] isolated a sulfated polysaccharide named DCS1 from *Dictyosphaeria cavernosa*, which was found to restore immune function in immunosuppressed mice. Specifically, DCS1 significantly increased the spleen and thymus indices, reversed the $CD4^+$ T cell reduction, and enhanced the secretion of IL-2 and TNF-α. Liu et al.[155] reported that sulfated Chinese yam polysaccharide (CYP), in contrast to traditional CYP, significantly enhanced the production of NO, TNF-α, and IL-6 by

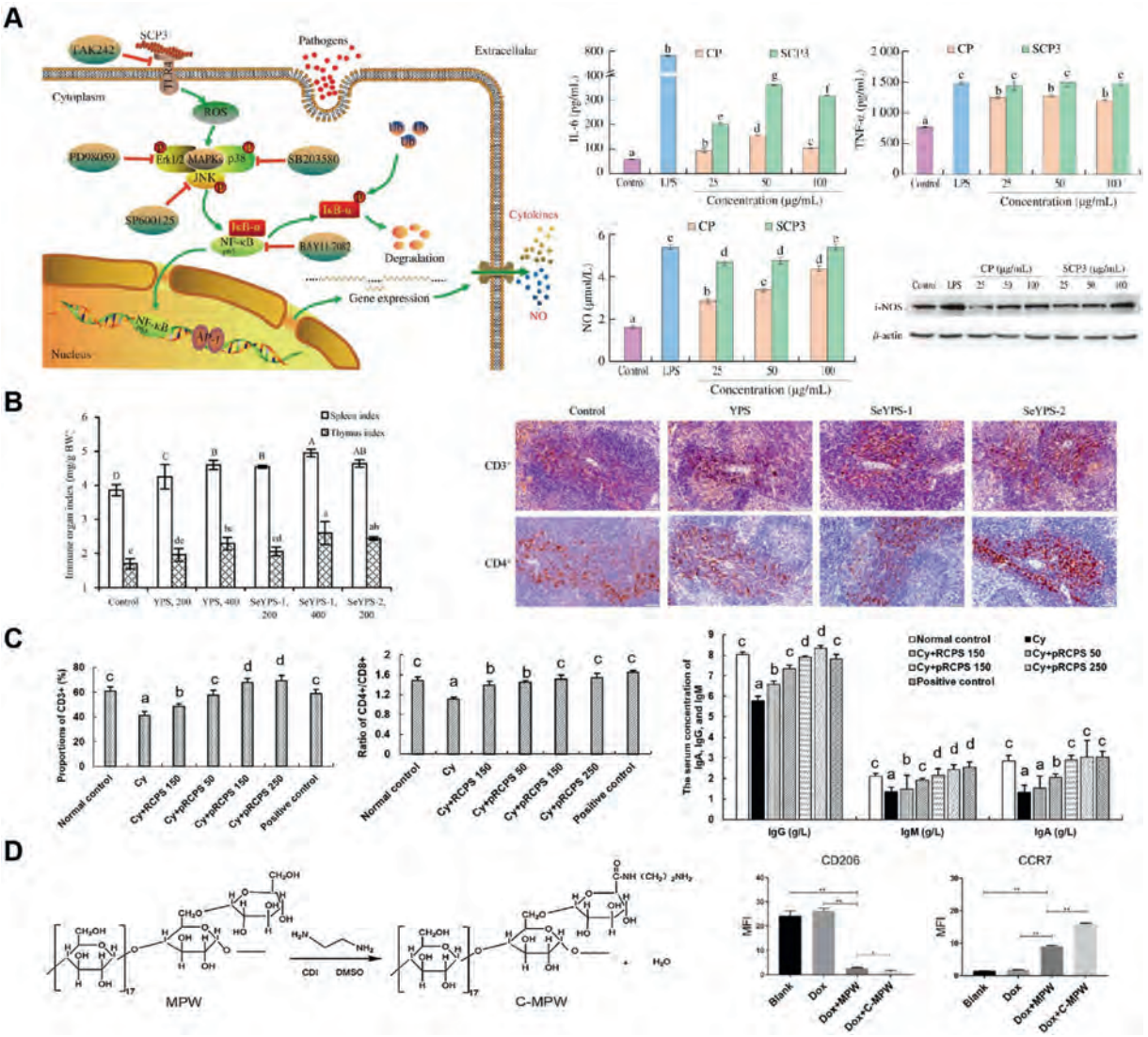

Figure 4 Strategies for the structural improvement of natural adjuvants. (A) Schematic diagram of the immunoregulatory mechanism of SCP3 on macrophages and the resulting release of pro-inflammatory factors. Reprinted with permission from Ref. 153. Copyright 2024, Elsevier. (B) Assessment of immune organ indices and immunohistochemical analysis of $CD3^+$ and $CD4^+$ T cells in the spleen following oral administration of selenylated YSP. Reprinted with permission from Ref. 160. Copyright 2022, Elsevier. (C) Evaluation of the reversal effects of RCPS and pRCPS on cyclophosphamide-induced immunosuppression. Reprinted with permission from Ref. 167. Copyright 2019, MDPI. (D) The synthesis strategy of C-MPW and the effect of MPW and C-MPW on macrophage polarization in 4T1 tumor tissues. Reprinted with permission from Ref. 177. Copyright 2020, Elsevier.

macrophages, highlighting the critical role of sulfation modification of TCM-derived active ingredients in augmenting immunological activity.

4.2. Selenization

Selenium, as an essential trace element, plays a vital role in maintaining immune function. Selenium-modified compounds have been shown to regulate the activity and function of various immune cells. Specifically, selenium compounds can enhance macrophage phagocytosis, increase NK cell cytotoxicity, and modulate the proliferation and differentiation of B and T cells, thereby boosting the overall immune response[156,157]. Additionally, selenium modification promotes immune cells to secrete key cytokines such as IFN-γ, TNF-α, and IL-2 while also modulating their activation and differentiation to strengthen the immune memory[158]. Bo et al.[159] prepared selenized garlic polysaccharides (sGPS) by selenization with HNO_3-Na_2SeO_3, which increased the secretion of IgA, IFN-γ, and IL-2 in chicken models, thereby enhancing their immune function. Guan et al.[160] found that while neither selenized yam polysaccharides (sYPS) nor native yam polysaccharides significantly affected body weight changes in BALB/c mice, sYPS exhibited a more pronounced dose-dependent effect in increasing thymus and spleen indices. In addition, sYPS elevated serum immunoglobulin levels, such as IgG, IgA, and IgM. Importantly, sYPSs were more effective in expanding the white pulp area of the spleen, promoting the formation of germinal centers, and elevating levels of $CD4^+$ T cells in the spleen of BALB/c mice (Fig. 4B). Qiao and colleagues[161] compared the immunomodulatory effects of selenized Chinese angelica polysaccharide (sCAP) with native CAP on bone marrow-derived dendritic cells (BMDCs). They found that sCAP significantly enhanced the phagocytic activity and upregulated the CD40 and MHC-II expression of BMDCs compared to native CAP. These results indicated that selenization not only promoted the maturation of BMDCs but also boosted cellular immunity.

4.3. Phosphorylation

Phosphorylation is also a crucial strategy to enhance the bioactivity of active compounds[162,163]. Traditional active ingredients often suffer from poor water solubility, significantly limiting their absorption and pharmaceutical potential[164]. By introducing phosphate groups, the water solubility and bioavailability of these compounds can be enhanced, which facilitates their better absorption and distribution within the body. Several techniques for phosphorylation have been developed, including the use of phosphorus oxychloride, phosphate, phosphorus pentoxide, as well as phosphoric acid and its anhydride[165]. Phosphorylation not only expands the application scope of these active compounds but also enhances their potential as viable drug carriers or adjuvant therapies. Additionally, phosphorylation can augment the immunomodulatory properties of natural compounds[33]. For example, continuous administration of phosphorylated *Radix Cyathulae officinalis* polysaccharide (pRCPS) to Newcastle disease-infected chickens significantly elevated serum antibody levels compared to the native CPS[166]. In immunosuppressed mice, pRCPS substantially increased serum immunoglobulin levels (IgG, IgA, IgM) and improved thymus and spleen indices, thereby enhancing both cellular and humoral immune responses in immunosuppressed models (Fig. 4C)[167]. These findings emphasize the potential of phosphorylated polysaccharides to elicit stronger humoral and cellular immune responses.

4.4. Synthetic derivatives

In addition to conventional modifications, multiple studies have focused on synthesizing derivatives with optimized functional groups to meet broader application needs[168]. These synthesized derivatives exhibit enhanced specificity in immune responses, reduced toxicity and side effects, and the ability to target specific immune pathways[169]. As multifunctional immunomodulators, they hold significant potential in cancer immunotherapy, vaccine development, and the treatment of autoimmune diseases[170–173]. For example, Naamidine J, initially isolated from the sponge *Pericharax heteroraphis*[174], has been found to selectively inhibit tumor cell lines, including K562, HepG2, and HeLa[175]. Fu et al.[176] synthesized a series of derivatives by substituting the imidazole ring of Naamidine J and discovered that compound 11c, in which the imidazole ring was replaced by a carbamate group (NHBoc), significantly inhibited PD-L1 expression in RKO tumor cells, thereby enhancing antitumor immune responses. Guo et al.[177] developed a cationic polysaccharide derived from *Lepidium meyenii* and conjugated with ethylenediamine (C-MPW), which significantly enhanced the ability of MPW to reprogram M2-like TAMs into the M1 phenotype (Fig. 4D). Hu et al.[169] developed a berberine derivative B68 that specifically targeted CSN5 to enhance the degradation of PD-L1 in CRC cells, thereby inhibiting the PD-1/PD-L1 pathway and facilitating the rapid elimination of tumor cells. Zdioruk et al.[178] synthesized 6′-bromoindirubin-3′-acetoxime (BiA), using the natural indirubin as a template, which could effectively block the IDO1 protein expression in GBM cells (Table 2).

5. Carriers for efficient delivery of TCM-derived adjuvants

Adjuvant delivery platforms have undergone rapid evolution, significantly advancing the clinical application of various immunoactive components derived from TCMs. Natural adjuvants, such as polysaccharides and saponins, typically contain multiple reactive sites and exhibit high reactivity, leading to their inherent instability, short half-lives, and low bioavailability[95,179]. These limitations are significant factors in the insufficient immune activation often observed with natural adjuvants, thereby restricting their broader clinical use. However, recent advancements in delivery technologies and the development of innovative drug carriers have begun to address these challenges. Protective carriers are instrumental in preventing the premature degradation of natural adjuvants within complex administration environments, thereby enhancing their stability and bioavailability[180,181]. Furthermore, appropriate delivery platforms can integrate multiple immunological cues, target APCs, and enhance the exposure of adjuvant and antigens in lymph nodes, thereby significantly boosting immune responses[182–185]. Simultaneously, the innovation and optimization of various delivery forms have greatly expanded the application scenarios of natural adjuvants (Fig. 5). However, the distinct physicochemical properties and mechanisms of action inherent to different carriers can significantly impact the efficacy of the adjuvants[186]. Here, we summarize the mechanisms and applications of various carriers, with a particular emphasis on both organic and inorganic carriers.

Table 2 List of strategies for modifying and optimizing the structures of natural adjuvants from TCMs.

Structural mortification	Adjuvant component	Enhanced immune function	Ref.
Sulfation	Glycosaminoglycans	STING activation	152
	Cyclocarya paliurus polysaccharides	Macrophages activation	153
	Dictyosphaeria cavernosa polysaccharide	T cell function	154
	Chinese yam polysaccharide	Macrophages activation	155
Selenization	Garlic polysaccharides	Antibody and cytokine secretion	159
	Yam polysaccharide	T cell function Antibody secretion	160
	Chinese angelica polysaccharide	DC maturation	161
Phosphorylation	*Radix Cyathulae officinalis* polysaccharide	T cell function Antibody secretion	166
Synthetic derivatives	Naamidine J	PD-L1 downregulation	175
	Lepidium meyenii Walpers polysaccharide	Macrophages polarization	177
	B68	Reduce PD-L1 expression	169
	6′-Bromoindirubin-3′-acetoxime	Reduce IDO expression	178

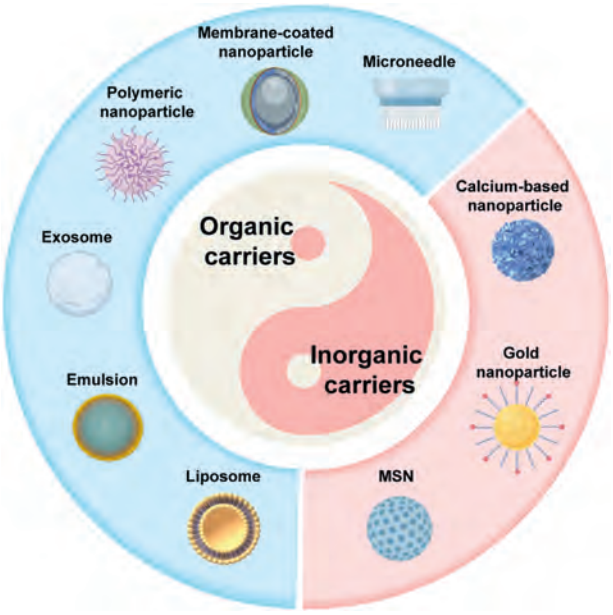

Figure 5 TCM-derived adjuvants equipped with various delivery carriers for enhancing vaccine efficacy.

5.1. *Organic carriers*

Organic carriers represent a broad and multifaceted category that encompasses lipid-based carriers, polymer carriers, and bio-derived carriers, covering most current and potential drug delivery platforms. These carriers are typically composed of biocompatible and biodegradable materials, ensuring safe and efficient transport of therapeutic agents. Additionally, they offer high plasticity and modifiability, facilitating the design of personalized and multifunctional drug delivery systems. In the context of vaccines, organic carriers not only enhance the bioavailability of adjuvants and antigens but also prolong the exposure time of adjuvants, facilitating sustained immune responses and improving long-term immunity. In this section, we review the research and application of organic vaccine carriers in the delivery of natural adjuvants, focusing on liposomes, polymeric carriers, emulsions, biomimetic nanocarriers, and microneedles.

5.1.1. *Liposomes*

Liposomes, driven by hydrophobic interactions, are enclosed bilayer lipid spheres formed through the self-assembly of amphiphilic phospholipids[187]. Their unique structure, comprising a hydrophilic core and a lipid bilayer wall, allows for the effective loading of adjuvants and antigens with varying solubilities[188]. Specifically, hydrophilic components are confined within the aqueous chamber, while hydrophobic components are embedded in the lipid bilayer, enabling the simultaneous delivery of multiple compounds. Antigen-adjuvant co-delivery systems utilizing liposomes have attracted considerable attention for their capacity to safeguard encapsulated components and deliver them to immune cells, which have been recognized as the preferred nanocarrier for delivering natural adjuvants[189,190].

Building on these benefits, Liu et al.[191] reported a liposome co-loaded with GLP and OVA (GLPL/OVA) that elicited robust antigen-specific immune activation, benefiting from the immunostimulatory effects of GLP and the transporting properties of liposomes. Compared to free single- and dual-component formulations, GLPL/OVA upregulated the expression of co-stimulatory molecules on DCs in lymph nodes and significantly increased the levels of $CD4^+$ and $CD8^+$ T cells in the spleen of BALB/c mice. Additionally, GLPL/OVA induced a Th1-biased Th1/Th2 mixed immune response, as evidenced by elevated cytokine levels, indicating a predominance of cell-mediated immune mechanisms. In another study, GLP and inactivated porcine circovirus type II (PCV2) co-loaded liposome (Lip-PS) could induce substantial cellular and humoral immune responses, presenting a novel strategy for PCV2 vaccine development[192]. Compared to free LBP, liposomes loaded with LBP (LBPL) enhanced antigen phagocytosis by DCs and facilitated their maturation through the upregulation of the TLR4−MyD88−NF-κB pathway. The liposomal delivery platform significantly improved the capacity of the adjuvant to activate DCs, thereby enhancing the initiation of the immune response[53]. In addition to the aforementioned examples, *Astragalus* polysaccharide, *Rehmannia glutinosa* polysaccharide, *Astragalus* saponin, and berberine have also emerged as successful cases of liposome delivery of natural adjuvants, providing more options for the rational design of vaccines to enhance efficacy(Fig. 6A)[193−196].

Active ingredients derived from TCMs, such as polysaccharides, flavonoids, and alkaloids, often encounter challenges including poor solubility, susceptibility to enzymatic degradation,

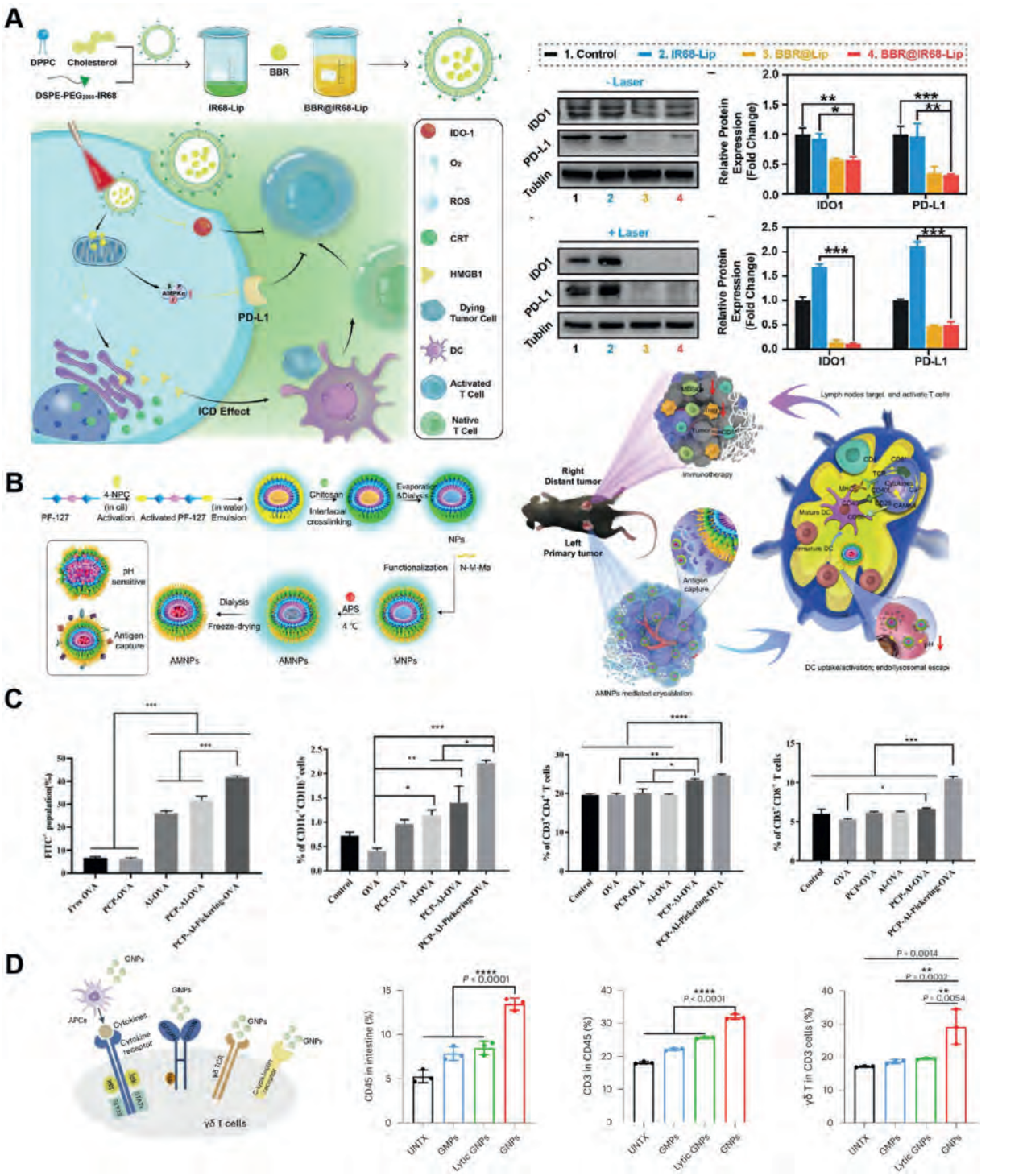

Figure 6 Organic carriers mediated delivery of natural adjuvants for enhanced the immune response. (A) Liposomes encapsulating berberine enhanced immune response in photodynamic therapy. Reprinted with permission from Ref. 196. Copyright 2023, Elsevier. (B) Polymer nanoparticles loaded with APS employed maleimide handles to capture tumor antigens from cryoablation for enhanced DC activation. Reprinted with permission from Ref. 24. Copyright 2023, The Royal Society of Chemistry. (C) Pickering emulsion stabilized by PCP-loaded particles activated immune system both *in vivo* and *in vitro*. Reprinted with permission from Ref. 207. Copyright 2024, Elsevier. (D) Oral administration of garlic-derived nanoparticles promoted increased levels of $\gamma\delta$ T cells. Reprinted with permission from Ref. 221. Copyright 2024, Springer Nature.

and low absorption efficiency, which limit their potential applications in immune modulation. Liposomes, as an advanced drug delivery system, leverage their phospholipid bilayer structure to encapsulate TCM-derived adjuvants, thereby significantly improving drug stability and bioavailability. Furthermore, liposomes can be functionalized with targeting molecules, such as antibodies, ligands, or sugar moieties, enabling precise delivery of adjuvants to those immune cells. This targeted approach not only

enhances immune efficacy but also minimizes systemic toxicity. Moreover, their sustained-release properties allow for gradual and controlled release of encapsulated drugs, maintaining therapeutic concentrations *in vivo* over extended periods and thus supporting prolonged immune regulation. To promote the application of liposomes in the delivery of TCM-based adjuvants, stimuli-responsive liposomes are tailored to specific disease and tissue characteristics. For instance, pH-, temperature-, or enzyme-sensitive liposomes can ensure the targeted release of adjuvants at pathological sites, enhancing therapeutic efficacy while minimizing systemic adverse effects.

5.1.2. Polymeric nanoparticles

Polymeric nanoparticles are nanoscale carriers constructed from natural or synthetic polymers, commonly utilizing biodegradable materials such as polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA), chitosan, and gelatin[197,198]. These polymer nanoparticles have garnered significant attention and are extensively utilized in the field of adjuvant delivery due to their excellent biocompatibility, biodegradability, and capacity for multiple modifications[199]. For example, ASP−PLGA−PEI, designed to deliver *Angelica sinensis*, could effectively adsorb antigens due to the incorporation of PEI molecules[200]. Compared to free ASP, ASP−PLGA−PEI exhibited superior phagocytic activity and significantly upregulated the expression of MHC-II and CD86 on macrophages. Zhong et al.[24] developed maleimide-modified Pluronic F127 and chitosan self-assembled nanoparticles (AMNPs) for *in situ* tumor vaccines and targeted delivery of APS (Fig. 6B). When combined with cryoablation, the maleimide groups on the surface of AMNPs captured tumor antigens (from Lewis lung cancer) induced by cryoablation, thereby promoting the activation of tumor-specific immunity. APS-loaded polymer particles exhibited more effective lymph node targeting owing to their nanoscale size, promoting DC maturation through synergistic utilization of tumor antigens. In murine experiments, this combined strategy demonstrated potent DC maturation and elicited tumor-specific $CD8^+$ T cell responses.

Polymeric nanoparticles not only enhance the stability, bioavailability, and targeted delivery of active components in TCMs but also enable the co-loading of multiple therapeutic agents, facilitating combined therapeutic and diagnostic applications. Furthermore, compared to other delivery systems, polymeric nanomaterials offer advantages such as lower production costs and well-established preparation methods, making them highly suitable for the commercialization and large-scale production of TCM-based adjuvant formulations. This combination of multifunctionality and cost-efficiency establishes polymeric nanoparticles as a promising platform for advancing the application of these natural adjuvants.

5.1.3. Emulsions

Emulsions are mixtures of two or more immiscible liquids, stabilized by the addition of surfactants. Emulsions were first introduced as key vaccine components in 1997 with the development of MF59, which marked the beginning of emulsion adjuvants. Since then, other emulsion-based vaccine delivery platforms, such as AS03, have been developed. Due to their proven efficacy and safety in clinical applications, as well as their ease of production, emulsions have become a leading platform in vaccine development[201]. Notably, compared to aluminum adjuvants, which primarily induce humoral immunity, emulsions offer greater advantages in initiating cellular immunity[202]. Additionally, they enhance antigen immunogenicity and immune responses by slowly releasing antigens and promoting immune cell recruitment[203−205]. Building on these properties, Liu et al.[206] developed a novel immune booster (PF3) by combining the nanoemulsion MF59 with ginsenoside Rg1. PF3 not only elevated the levels of IgG1 and IgG2a in mouse serum, but also increased the concentration of IFN-γ and IL-2 in the spleen. Furthermore, following PF3 administration, the proportion of splenic $CD8^+$ T cells secreting IFN-γ and IL-2 was significantly increased, suggesting that PF3 induced a more robust and balanced cellular and humoral immune response. In a recent study, Pickering emulsions stabilized by solid particles were utilized to enhance the adjuvant activity of the *Poria cocos* polysaccharide (PCP). Alum particles loaded with PCP positioned at the oil-water interface ensured both emulsion stability and high loading capacity for the active components. This adjuvant delivery system effectively promoted the maturation of DCs, leading to sustained antibody secretion and enhanced T cell activation (Fig. 6C)[207].

Emulsions can efficiently disperse the active components of TCMs within both lipid and aqueous phases, significantly enhancing the drug's bioavailability, stability, and absorption efficiency. This makes them particularly suitable for those components with variable solubility. The reduced particle size facilitates the penetration of biological barriers, thereby increasing drug accumulation at the target site and further enhancing the efficacy of immunotherapy. Additionally, emulsions offer controlled release function, enabling the gradual release of antigens and adjuvants at the injection site, thereby prolonging immune stimulation and improving the persistence of immune effects. For TCM-derived components that exhibit significant side effects or require high effective concentrations, controlled release helps mitigate toxicity risks and side effects, ensuring both safety and therapeutic efficacy. In clinical applications, emulsions are especially valuable for diseases requiring prolonged immune stimulation or modulation, such as cancer immunotherapy and chronic viral infections. By precisely controlling adjuvant release and immune responses, emulsions not only enhance therapeutic outcomes but also enhance patient compliance and treatment experience, guiding TCM-based immunotherapy towards a more personalized and safer approach.

5.1.4. Biomimetic nanocarriers

Drawing inspiration from the complex interactions between organisms, researchers have developed biomimetic nanocarriers derived from cells, bacteria, and viruses. Among these, cell membrane-based biomimetic delivery systems (CMDSs) have become a focal point of research due to their capacity to preserve the biological functions and immunogenicity of their parent cells[208,209]. Cell membranes their inherent immunogenic properties to enhance vaccine efficacy and induce targeted immune responses[210]. Depending on the type of cells from which they are derived, CMDSs can achieve precise targeting of multiple sites while overcoming the limitations of transmembrane transport. For instance, nanoparticles coated with tumor cell membranes exhibit enhanced tumor localization due to the expression of specific membrane receptors[211,212]. Similarly, immune cell membranes direct drugs to inflammatory sites and immune organs due to their inherent tropism[213]. In a study, *Dendrobium devonianum* Polygonatum (DP) nanoparticles encapsulated within macrophage membranes were shown to enhance macrophage uptake and

promote the expression of CD80 and CD86 by mimicking homologous targeting mechanisms[214]. Exosomes are also commonly used for the delivery of active components derived from TCMs[215,216]. Their nanoscale size (typically 30−150 nm) and flexible membrane structure enable efficient penetration of biological barriers, including the blood−brain barrier and tumor microenvironment. Such unique capability provides a promising strategy for delivering TCMs to otherwise inaccessible pathological sites, thereby expanding their therapeutic potential. Li et al.[217] developed a ginsenoside Rg3-engineered exosome delivery system (Rg3-Exo/ATO@Ce6) for glioblastoma therapy. By incorporating Rg3 onto the surface of exosomes, Rg3-Exo/ATO@Ce6 demonstrated enhanced capability to penetrate the blood−brain barrier and effectively modulate the immunosuppressive tumor microenvironment.

Notably, plant-derived extracellular vehicles (EVs) present a highly biocompatible approach by mimicking the characteristics of natural materials while retaining bioactive molecules[218]. These features render them inherently suitable as carriers for delivering active ingredients. Recent studies have highlighted the potential of ginseng-derived EVs, which exploited ceramide lipids and proteins to activate the TLR-4 and MyD88 pathways[219,220]. This activation is crucial for in modulating macrophage polarization towards the pro-inflammatory M1 phenotype. The resulting immune modulation effectively targeted and modified the tumor microenvironment, thereby synergistically amplifing the antitumor efficacy of anti-PD-1 mAb. Recently, Xu et al.[221] explored the health benefits of garlic and discovered that nanoparticle-like structures extracted from garlic (GNPs) exhibited potent immunomodulatory potential. The distinctiveness of GNPs lay in their capacity to increase the proportion of $\gamma\delta$ T cells among T lymphocytes and to stimulate the production of IFN-γ through the C-type lectin pathway (Fig. 6D). Compared to microparticles derived from the same source (GMPs), GNPs demonstrated a significantly longer retention time in the intestine and exhibited a stronger affinity for intestinal immune cells.

Cell membranes and exosomes, both derived from biological cells, retain components analogous to their source cells, which exhibit low immunogenicity and minimize undesired immune responses typically associated with conventional nanocarriers, such as polymeric or metallic nanoparticles. Leveraging their intrinsic receptor recognition capabilities, these vesicles and exosomes can precisely target and bind specific receptors, thereby facilitating targeted delivery and improving therapeutic efficacy while significantly reducing off-target side effects. Additionally, active components, such as saponins, can be directly incorporated into the cell or exosome membranes by embedding them into the lipid bilayer. This strategy not only increases drug-loading efficiency but also preserves the bioactivity of the incorporated components. Furthermore, exosomes and cell membranes demonstrate exceptional biocompatibility, enabling efficient fusion with target cell membranes, promoting endocytosis, and improving intracellular delivery, which collectively enhances drug targeting and therapeutic outcomes. To optimize adjuvant delivery, various strategies have been developed to enhance the targeting specificity and sustained-release capacity of active components in TCMs, including surface modification with specific molecules (such as antibodies, ligands, or peptides), modulation of membrane composition (*e.g.*, altering cholesterol content), and the construction of hybrid membrane structures.

5.1.5. Microneedles

The skin serves as a critical habitat for various APCs, which are pivotal in triggering the initial immune defenses[222]. Despite the limitations posed by poor patient compliance and the high risk of infection, the stratum corneum barrier necessitates subcutaneous injection as the primary method of vaccination. Microneedles, an emerging penetration enhancement method, contain numerous micrometer-sized needles anchored to a base to effectively breach the stratum corneum, enabling non-invasive and convenient drug delivery[223]. Moreover, some microneedles with adjuvant properties are made of swellable materials, allowing for the slow release of antigens to enhance immunological activation[224]. Wang et al.[225] utilized dissolvable microneedles to load *Panax notoginseng* polysaccharide (PNPS), significantly enhancing its loading capacity. These "natural" microneedles exhibited strong mechanical strength and rapidly dissolved within 30 min of penetrating the dermis. Upon dissolution, PNPS were achieved a penetration depth of up to 450 μm, significantly surpassing the initial insertion depth of 200 μm. Compared to PNPS solution or nanoparticles, the microneedle-facilitated delivery system more effectively promoted subcutaneous DC maturation and enhanced the transdermal immune response in SD rats.

The application of microneedles in delivering TCM-derived active components has become increasingly prevalent[226−228]. Microneedles typically exhibit a high drug-loading capacity and can rapidly release drugs at elevated concentrations directly at the target site, making them particularly suitable for herbal components with low bioavailability. The microneedle system can also be designed as a composite drug delivery platform, facilitating the simultaneous administration of multiple drugs. This composite approach provides significant advantages in enhancing immune responses, enabling combination therapies, and improving overall therapeutic efficacy. Microneedles can directly penetrate the skin barrier, delivering adjuvants to the epidermal and dermal layers, thereby circumventing the first-pass effect commonly associated with oral administration and improving drug bioavailability. Additionally, the microneedle system is capable of delivering large-molecule adjuvants that are unsuitable for oral administraion or injection, protecting them from degradation and ensuring drug stability and therapeutic effectiveness.

5.2. Inorganic nanocarriers

In recent years, a growing number of inorganic materials with excellent biocompatibility and high stability have emerged as promising candidates for drug delivery carriers. While their long-term safety remains to be investigated, the benefits of inorganic carriers such as ease of preparation and strong drug-loading capacity cannot be ignored. Moreover, inorganic nanoparticles exhibit enhanced uptake by macrophages and DCs, underscoring their significant potential in vaccine applications[229,230]. In the following sections, we will explore the application and value of inorganic carriers in delivering natural adjuvants, focusing on gold nanoparticles, mesoporous silica nanoparticles, and calcium-based particles.

5.2.1. Gold nanoparticles

The appeal of gold nanoparticles (AuNPs) lies in their potential for diverse surface modifications and the ability to regulate interactions with cells by customizing their shapes. It also represents versatile inorganic nanocarriers due to their dual capacity for

therapeutic agent delivery through internal encapsulation and surface attachment. The high specific surface area and abundant active sites of AuNPs make surface attachment an increasingly preferred drug-loading approach. Polysaccharide adjuvants possess inherent reducing capabilities attributed to their repeated hemiacetal structural units. These molecules serve as effective reducing agents in AuNP synthesis, facilitating the conversion of Au^{3+} to Au^{0}. Following nanoparticle formation, polysaccharide molecules adsorb onto the AuNP surface, imparting enhanced colloidal stability. However, this approach relies predominantly on non-covalent interactions, such as hydrogen bonding, resulting in relatively weak molecular associations. An alternative strategy utilizing Au-S covalent bonds addresses the limitations of non-covalent binding. In this approach, thiol-containing drug molecules form stable Au-S bonds with the AuNP surface, ensuring robust molecular anchorage. Moreover, these Au-S linkages provide a structural foundation for targeted delivery, enabling site-specific drug release in environments with elevated glutathione (GSH) concentrations[231].

Recent studies have demonstrated that AuNPs serve as an effective delivery platform for various natural adjuvants, significantly enhancing their therapeutic efficacy[232]. For instance, the combination of *Pleurotus ferulae* polysaccharide (PFPS) with AuNPs (PFPS Au NPs) has been shown to strongly stimulate DC immune function through the TLR4 and NLRP3 pathways[233]. Notably, PFPS Au NPs have improved the utilization and anti-human papillomavirus (HPV) efficiency of PFPS, achieving immune effects comparable with the PFPS solution with only one-tenth of the required adjuvant dosage. Additionally, research by Pang et al.[234] has demonstrated that APS-modified AuNPs effectively act on DCs to initiate cellular immunity, particularly by increasing the proportion of Tems. Furthermore, GLP-loaded AuNPs (GLP-Au) not only upregulated the expression of CD80, CD40, and MHC-II on DCs, but also increased the percentage of T_M in 4T1-bearing mice (Fig. 7A)[235].

AuNPs offer the advantage of precise size and shape adjustments, which are crucial for targeted delivery to specific sites. In comparison to other metallic nanoparticles, AuNPs exhibit superior biocompatibility and reduced immunogenicity. Traditional herbal active ingredients often face challenges such as poor solubility and susceptibility to degradation; AuNPs can shield these compounds from external degradation, significantly enhancing

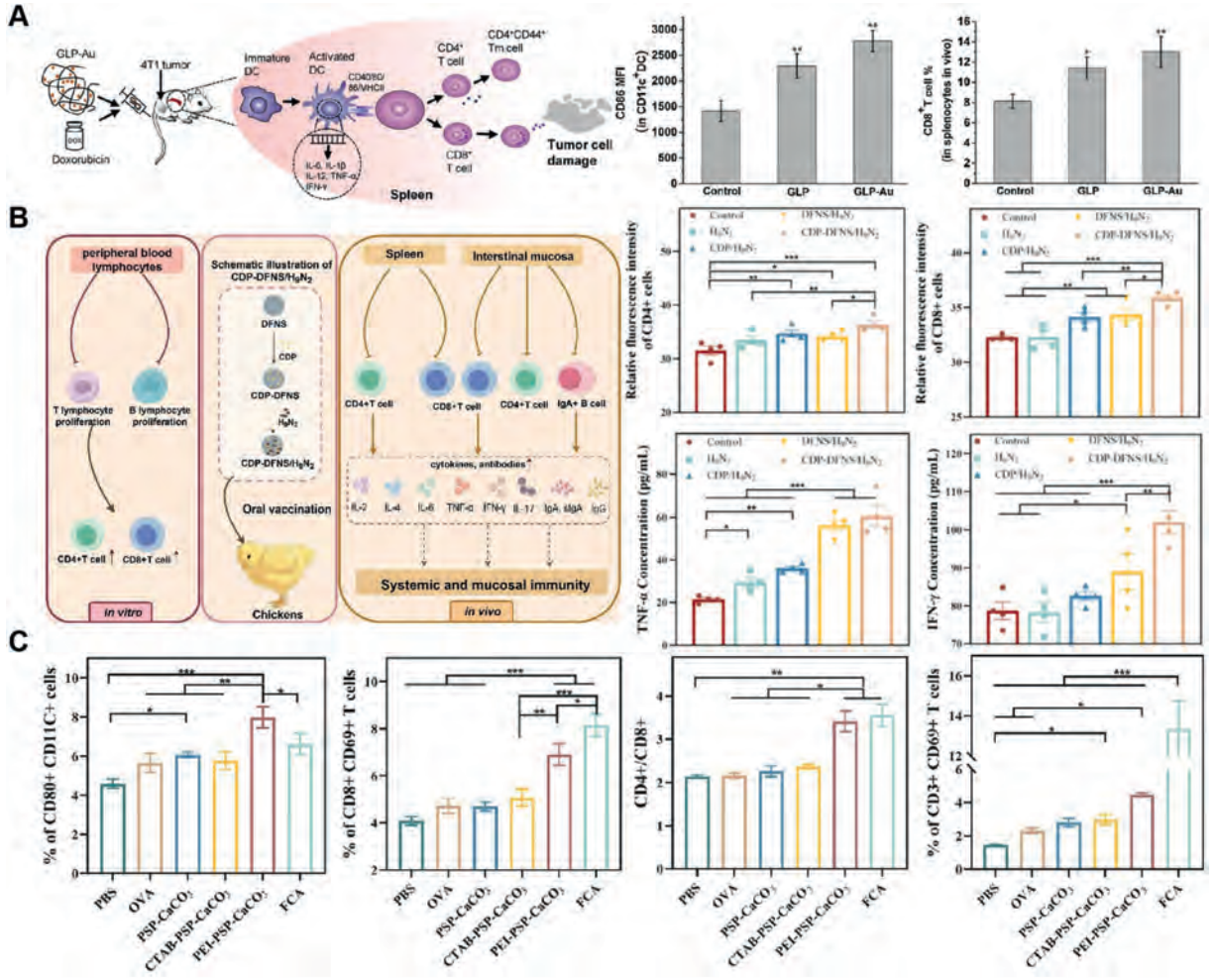

Figure 7 Inorganic material-based vaccine design for delivery of natural adjuvants. (A) AuNPs containing GLP enhanced DC activation and subsequent T cell response. Reprinted with permission from Ref. 235. Copyright 2019, Elsevier. (B) CDP-modified MSNs resisted mucosal viral infections through T cell-mediated immune activation. Reprinted with permission from Ref. 239. Copyright 2024, Elsevier. (C) PEI-modified CaP nanoparticles loaded with PSP induced robust DC activation and elevated levels of both cellular and humoral immune responses *in vivo*. Reprinted with permission from Ref. 243. Copyright 2024, American Chemical Society.

Table 3 Formulation approaches for enhancing the delivery efficiency and *in vivo* fate of natural adjuvants.

Adjuvant	Carrier	Disease model/ Antigen	Strategy	Administration	Ways to enhance immunity	Ref.
Ganoderma lucidum polysaccharide	Liposome	OVA	Loaded in internal aqueous phase	s.c.	Regulate Th1/Th2 Boost T cell function	191
	Liposome	PCV2	Loaded in internal aqueous phase	s.c.	Regulate Th1/Th2 Boost T cell function	192
	Gold nanoparticle	4T1 breast cancer	React with chloroauric acid after thiolation	i.v.	Promote DC maturation Boost T cell proliferation	235
Lycium barbarum polysaccharide	Liposome	OVA	Loaded in internal aqueous phase	Only *in vitro* studies	Promote DC maturation	53
Astragalus polysaccharide	Liposome	OVA	Loaded in internal aqueous phase	s.c.	Promote DC maturation Enhance macrophages phagocytosis	193
	Polymer nanoparticle	LLC lung cancer	Internally loaded in maleimide-modified nanoparticle	Intratumoral injection	Promote DC maturation	24
	Gold nanoparticle	4T1 breast cancer	React with chloroauric acid	i.v.	Promote DC maturation Promote Tem proportion	234
	Calcium phosphate nanoparticle	B16 melanoma	CaP-encapsulated adjuvant nanoparticle	s.c.	Promote DC maturation Boost T cell function	99
Rehmannia glutinosa polysaccharide	Liposome	Not mentioned	Loaded in internal aqueous phase	Only *in vitro* studies	Active M1 macrophages	194
Astragalus saponin	Liposome	CT26 colorectal cancer	Loaded in internal aqueous phase	s.c.	Boost $CD8^+$ T cell function Inhibit tumor angiogenesis	195
Berberine	Liposome	CT26 colorectal cancer	Loaded in internal aqueous phase	i.v.	Reduce PD-L1 and IDO1 expression Combined with ICD	196
Angelica sinensis polysaccharide	Polymer nanoparticle	PCV2	Internal loading	s.c.	Active macrophages Regulate Th1/Th2	200
Ginsenoside Rg1	Nano emulsion	Hepatitis B	Loaded in the aqueous phase of W/O emulsion	i.m.	Regulate Th1/Th2	206
Poria cocos polysaccharide	Emulsion	OVA	Adsorbed at the oil-water interface	s.c.	Promote DC maturation Regulate Th1/Th2	207
Ginseng-derived EV	Biomimetic nanocarrier	B16 melanoma CT26 colorectal cancer 4T1 breast cancer MC38 colorectal cancer	Self-adjuvant carrier	i.p.	Polarization and active macrophages	219,220
Ginsenoside Rg3	Biomimetic nanocarrier	Glioblastoma	Rg3-surface inserted and therapeutic agent-loaded tumor exosome	i.v.	Promote DC maturation Regulate macrophage polarization	217
Garlic-derived nanoparticle	Biomimetic nanocarrier	B16 melanoma	Self-adjuvant carrier	*p.o.*	Boost $\gamma\delta$ T cell function	221
Dendrobium devonianum Polygonatum	Biomimetic nanocarrier	OVA	PEI-modified macrophage membrane-coated nanoparticle	i.p.	Enhance macrophages phagocytosis active macrophages	214

(*continued on next page*)

Table 3 (*continued*)

Adjuvant	Carrier	Disease model/ Antigen	Strategy	Administration	Ways to enhance immunity	Ref.
Panax notoginseng polysaccharide	Microneedle	Not mentioned	Self-adjuvant carrier	Transdermal Administration	Enhance skin penetration Promote DC maturation	225
Pleurotus ferulae polysaccharide	Gold nanoparticle	HPV-induced TC-1 lung cancer	React with chloroauric acid	i.p.	Promote DC maturation	233
Cistanche deserticola polysaccharide	Mesoporous silica nanoparticle	Avian influenza	Amide-conjugated to nanoparticle	*p.o.*	Boost T cell and B cell function Promote antigen secretion	239
Polygonatum sibiricum polysaccharide	Calcium carbonate microparticle	OVA	Internal loading	s.c.	Promote DC maturation Boost T cell function Promote antigen secretion	242

their stability and bioavailability, as well as improving absorption and therapeutic efficacy. Furthermore, due to their strong antioxidant properties and chemical stability, AuNPs create an environment conducive to controlled drug release. By modifying the structure and surface properties of the nanoparticles, the drug release rate can be precisely regulated. This slow-release mechanism prolongs the effective duration of the drug in the body, continuously boosting the immune response.

5.2.2. *Mesoporous silica nanoparticles*

Mesoporous silica nanoparticles (MSNs), with their superior biocompatibility and precisely arranged pore structure, are widely recognized as exemplary inorganic nano-platforms. Additionally, MSNs possess exceptional drug delivery capabilities due to their high specific surface area and distinctive pore architecture, which facilitate efficient molecular adsorption, loading, and delivery. Drug encapsulation within MSNs typically occurs *via* two principal mechanisms. The first mechanism involves drug−carrier interactions, including hydrogen bonding, van der Waals forces, and electrostatic interactions. The second mechanism relies on the nanoconfinement effect, where the precisely tuned pore dimensions of MSNs enhance drug adsorption efficiency and loading capacity through spatial restriction of drug molecules in their amorphous state[236]. These characteristics make MSNs not only effective as common drug carriers but also highly suitable for vaccine design. Additionally, they can efficiently load various antigen proteins and offer substantial protection to these antigens, making them promising candidates for oral vaccine delivery systems[237,238]. For example, He et al.[239] developed a *Cistanche deserticola* polysaccharide (CDP)-modified nanosilica (CDP-DFNS/H9N2) as an adjuvant for an avian influenza virus (H9N2) vaccine targeting mucosal viral infections. This silica carrier, with its dendritic pore structure, increased the loading capacity for antigens and adjuvants while enabling the slow release of antigens. Compared to the unmodified CDP/H9N2, the CDP-DFNS/H9N2 formulation significantly elevated antigen-specific immune responses in both systemic and mucosal tissues, and promoted the secretion of corresponding antibodies (Fig. 7B).

Mesoporous silica features tunable pore sizes and structures, typically ranging from 2 to 50 nm, which allows for the effective loading of a wide variety of drug adjuvants. Its large specific surface area (exceeding 500 m^2/g) provides ample space for achieving high drug loading. This makes mesoporous silica particularly suitable for active components of TCMs that require high therapeutic concentrations. Additionally, mesoporous silica facilitates controlled drug release through mechanisms such as pore closure, or responsive release systems, including pH-responsive, temperature-responsive, or enzyme-responsive systems.

5.2.3. *Calcium-based nanoparticles*

Calcium-based inorganic materials, primarily calcium carbonate ($CaCO_3$) and calcium phosphate (CaP), are widely utilized in drug delivery systems. Like other inorganic drug carriers, their excellent biodegradability and high drug loading capacity are key factors contributing to their effectiveness. Additionally, their pH-responsive properties provide a distinct benefit in achieving spatial specificity and organelle-targeted drug release. When used to carry immunostimulants, the calcium shell effectively prevents cargo inactivation and significantly enhances the immunogenicity of the delivered agents[229]. Calcium-based nanoparticles exhibit

Table 4 Commercially veterinary vaccines with natural adjuvants.

Vaccine	Adjuvant	Platform type	Antigens	Types of vaccine	Diseases	Company
Equilis® Te	ISCOM	Suspension	Tetanus toxoid	Inactivated vaccines	Tetanus	MSD animal health
Equilis® Prequenza	ISCOM	Suspension	Equine influenza virus strains	Inactivated vaccines	Influenza	MSD animal health
Equip® F	ISCOM	Suspension	Equine influenza virus inactivated strains	Inactivated vaccines	Influenza	Zoetis
Strangvac®	ISCOM	Suspension	Recombinant protein from *Streptococcus equi*	Recombinant vaccines	Equine strangles	Intervacc
Equilis® Prequenza Te	ISCOM	Suspension	Equine influenza virus strains and Tetanus toxoid	Inactivated vaccines	Tetanus influenza	MSD animal health
Equip® FT	ISCOM	Suspension	Equine influenza virus strains and Tetanus toxoid	Inactivated vaccines	Tetanus Influenza	Zoetis
Suivac® APP	Oil emulsion and Saponin	Emulsion	Toxoids, outer membrane proteins, and LPS of inactivated bacterium	Inactivated vaccines Recombinant vaccines	Porcine pleuropneumonia	Dyntec
Suivac® DNT	Oil emulsion and Saponin	Emulsion	Pasteurella multocida type D and inactivated bacterial suspension Bordetella bronchiseptica	Inactivated vaccines Recombinant vaccines	Atrophic rhinitis of pigs	Dyntec
Suivac® EDT	Oil emulsion and Saponin	Emulsion	Inactivated verotoxin VT2e	Inactivated vaccines Recombinant vaccines	Edematous disease of pigs	Dyntec
Suivac® Ery-IN	Oil emulsion and Saponin	Emulsion	Serovars S1a, S1b, S2a and S2b of inactivated bacterium Erysipelothrix rhusiopathiae	Inactivated vaccines	Swine erysipelas	Dyntec
Suivac® Parvo Ery-IN	Oil emulsion and Saponin	Emulsion	Inactivated virus of parvovirosis of pigs and serovars	Inactivated vaccines	Swine erysipelas	Dyntec
Suivac® PRRS-IN Suivac® PRRS-INe	Oil emulsion and Saponin	Emulsion	Inactivated virus of PRRS	Inactivated vaccines	Porcine reprodiuction and respiratory syndrome	Dyntec

ISCOM: immuno stimulating complex (containing Quillaja saponins); LPS: lipopolysaccharide.

inherent structural advantages through their porous architecture and high specific surface area, which facilitate the non-covalent adsorption of therapeutic agents and biomolecules *via* physical interactions. These characteristics have established calcium-based materials as some of the most extensively studied inorganic vaccine carriers.

Therefore, Li et al.[99] proposed a nanovaccine strategy involving a CaP-shielded adjuvant-antigen self-assembly (APS-NVs). In this approach, the natural adjuvant APS and the OVA antigen peptide SIINFEKL formed an assembled complex, which was then encapsulated in a CaP shell to enhance stability and resistance to degradation *in vivo*. Following subcutaneous injection, APS-NVs exhibited efficient accumulation in lymph nodes and significantly influenced DC maturation. Moreover, APS-NVs increased the proportion of splenic T cells, which was essential for the activation of adaptive immunity. Additionally, a chitosan-CaP composite formulation co-loaded with RGb1 and IL-4 demonstrated a high IgG2a/IgG1 ratio post-inoculation and promoted the secretion of various inflammatory factors[241]. He et al.[242] further explored immune regulation by comparing PEI- and CTAB-engineered $CaCO_3$ particles using *Polygonatum sibiricum* polysaccharide (PSP) as a model adjuvant. Their findings suggested that PEI modification enhanced the adjuvant activity of PSP, as evidenced by increased activation of lymph node DCs and improved lymphocyte immune function (Fig. 7C).

Calcium, an abundant mineral, offers excellent biocompatibility and biodegradability, making calcium nanoparticle a promising carrier in drug delivery systems. In the body, calcium nanoparticles naturally degrade into calcium ions, which neither accumulate nor pose risks of toxicity and side effects. This makes calcium nanoparticles highly suitable for delivering TCM-based adjuvants, especially when long-term treatment or high doses are required. Calcium nanoparticles not only possess excellent drug loading and release capabilities but also interact with immune cells via calcium ions on their surface, thereby promoting the activation of immune responses. As a result, calcium nanoparticles serve not only as drug delivery carriers but also as active modulators of immune responses, further enhancing the immune activation potential of these adjuvants (Table 3).

6. Conclusions and perspectives

Exploring new and applicable adjuvants represents a persistent technological challenge intimately linked to the future of vaccine innovation. Many compounds inspired by TCMs can actively interact with multiple immune processes through multiple mechanisms and levels, ultimately assisting the body in initiating adaptive immunity. These compounds are considered promising candidates for the development of novel adjuvants. However, immune stimulatory performance alone is not sufficient to assess adjuvant quality; their safety must also be carefully evaluated[243]. Notably, TCM-derived adjuvants typically exhibit high biocompatibility and good tolerance, meeting the safety standards required for vaccine development[244]. Given the significant potential of TCMs as a natural reservoir for novel adjuvants, here we emphasize the structural diversity of TCM-derived adjuvants in modulating the immune system. Furthermore, advances in understanding the properties of natural adjuvants have led to numerous optimization strategies for their chemical structures and delivery methods, enhancing their applicability in modern vaccine formulations. These developments are crucial for advancing the clinical application of natural adjuvants and promoting innovation in vaccine systems.

TCMs have been used for thousands of years in China, with their medicinal properties extensively validated. Compared to newly developed adjuvants, TCM-derived adjuvants are readily recognized and accepted, and this unique advantage further underscores their potential in vaccine development. While exploring potential adjuvant candidates from TCMs indicates a promising direction for the development of novel vaccine systems, several significant challenges warrant careful consideration. Firstly, the efficacy of natural products is heavily influenced by their purity, which is determined by factors such as the quality of the source plants, purification methods, and reagents used during purification. Although techniques like nuclear magnetic resonance (NMR) analysis and liquid chromatography can mitigate the interference of impurities in extracts, purifying large-molecule adjuvants, such as polysaccharides, remains challenging[31]. Furthermore, crude extracts from natural products, such as decoctions or tinctures, often exhibit significant therapeutic benefits due to their multi-component synergistic effects[245]. However, these benefits come with inherent risks. Ensuring consistent quality standards and improving production reproducibility, particularly in controlling the content of active ingredients, remains a significant issue that must be addressed. Secondly, the costs and batch differences associated with plant growth, cultivation, and harvesting cannot be overlooked, posing challenges for the clinical application and large-scale production of natural adjuvants[246−248]. Finally, current mainstream research predominantly focuses on the preliminary evaluation of the effects of natural adjuvants. However, in-depth studies on their mechanisms of action and structure−activity relationships are crucial for the discovery and development of new adjuvants. Understanding these aspects will significantly advance the field and contribute to the successful integration of natural adjuvants into vaccine systems.

In addition to the aforementioned challenges, the selection and extraction of active components from TCMs face issues such as low extraction efficiency, high costs, and potential environmental and safety concerns. Future studies should pay attention to the development of more efficient extraction and purification techniques, such as enhancing the applicability of high-throughput screening methods to accelerate the discovery of natural adjuvants. Utilizing computational chemistry and related techniques can provide a comprehensive understanding of the relationship between compound structure and immune activity. This insight can help mitigate the inherent drawbacks of natural adjuvants through chemical modifications or biological transformations, thereby promoting the development of improved adjuvants with enhanced effects[168]. Moreover, to achieve large-scale production, reduce costs, and minimize environmental impact, exploring the route of total synthesis for TCM-derived adjuvants should be a promising direction for further research[249]. To meet the high market demand for sanguinarine, Guo et al.[250] utilized the high efficiency of biosynthetic pathways and the multifunctionality of pathway enzymes in engineered yeast strains to achieve the complete biosynthesis of sanguinarine and its halogenated derivatives using halogenated tyrosine as the raw material. This approach represents a significant improvement over the traditional, insufficient method of extracting sanguinarine from plant roots, offering a more sustainable solution for production.

TCM-derived adjuvants encounter several obstacles, including poor stability, low bioavailability, and limited targeting capability[251]. Therefore, utilizing appropriate delivery carriers is

crucial for improving the efficacy of vaccine immunotherapy. Commonly used adjuvant delivery systems currently include emulsions, polymer nanoparticles, and liposomes[252−254]. To enhance the applicability and delivery efficiency of TCM-derived adjuvants, more effective delivery platforms need to be developed. For instance, novel delivery systems based on cell membrane structures and microneedles have gained attention in the drug delivery field due to their unique advantages[255,256]. TCMs consist of diverse components, and by selecting compounds with appropriate physicochemical properties, they can be effectively loaded into these formulations to reach target immune cells. Additionally, delivery platforms need to be optimized to better match various application scenarios and the different properties of adjuvants. Achieving high efficiency and long-lasting effects in vaccines requires excellent targeting ability and favorable drug release characteristics from any delivery system. It is also noteworthy that many nanoparticles derived from TCMs have played significant roles in immune modulation. These nanoparticles naturally carry active ingredients and possess exosome-like delivery advantages, which deserve further attention[257,258].

Currently, most TCM-derived adjuvants in human and veterinary vaccines remain at the research and experimental stages. Propolis adjuvants (*e.g.*, avian cholera propolis vaccine) and the saponin Quil A are among the few plant-derived adjuvants that have been approved for use as adjuvants in commercial veterinary vaccines (Table 4). Other TCM-derived components are primarily used as additives or auxiliary agents, often in combination with aluminum-based or squalene-based adjuvants. Among the adjuvants that have been licensed for human vaccines, only QS-7, QS-17, and QS-21 are currently in use[259]. The major challenges hindering the clinical translation of TCM-based adjuvants include standardization, quality control issues, and unclear mechanisms of action. These barriers continue to limit their widespread adoption. Therefore, we advocate for intensified research efforts utilizing advanced technologies to comprehensively elucidate the mechanisms of these natural adjuvants and explore scalable production and quality control strategies, which is also the central focus of this review.

In summary, although the widespread applications of natural adjuvants in vaccine formulations face significant challenges, these limitations do not diminish their potential in the revolution of vaccines. Advances in terms of chemical synthesis, bioengineering, and nanotechnology will aid in addressing these issues and enhancing the prospects for the clinical translation of natural adjuvants. With ongoing interdisciplinary collaboration and deeper research, the full potential of natural adjuvants is expected to be realized, leading to their successful integration into modern vaccines for effective disease prevention and treatment.

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Author contributions

Xiaoyuan Fan: Conceptualization, collecting references and writing-original draft; Fengxiang Liu: Collecting references and writing-original draft; Fei Sun: Writing-review & editing; Yiyang Wang: Writing-review & editing; Wenwen Shen: Writing-review & editing; Shujun Wang: Writing-review & editing; Jin Sun: Conceptualization, supervision, funding acquisition and writing-review & editing; Kaiyuan Wang: Supervision and writing-review & editing.

Conflicts of interest

The authors declare no conflicts of interest.

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