They found that the direct contact area between DCs and T cells was approximately four-fold higher in the group treated with L-GO nanosheets compared to untreated DCs, and approximately two-fold higher compared to small nanosheets[58]. The L-GO nanosheets acted like a "nanozipper", adhering to the surface of DCs and bringing together large clusters of DCs and T cells, creating a stable microenvironment for effective cell interactions and T cell activation[58]. The scientists described this phenomenon as the first evidence that L-GO nanosheets showed selective adherence to different cell membranes[58]. The high binding affinity with DCs membranes facilitated DCs-T cell clustering, while the low binding affinity with T cells prevented interference with DCs-T cell interactions[58].

These clusters of DCs and T cells induced a more than twenty-fold higher antigen-specific T cell response compared to conventional cytokine-cocktail adjuvants, and resulted in >99.7% clearance of viral RNA from lung tissues in mice inoculated with SARS-CoV-2[58]. The researchers concluded that the robust immune responses induced by DC vaccines using GO nanosheets could serve as a promising reference for developing personalized antiviral therapy against the global COVID-19 pandemic[58]. The summary of how L-GO increased DCs-T cells clustering to activate Rho-ROCK-MLC pathway and stimulate the binding of ICAM-1 and LFA-1 to fully activated $CD8^+$ T cells to diminish COVID-19 was illustrated in **Figure 7**. This approach can be implemented to increase the efficacy of DC vaccine for cancer treatment using L-GO.

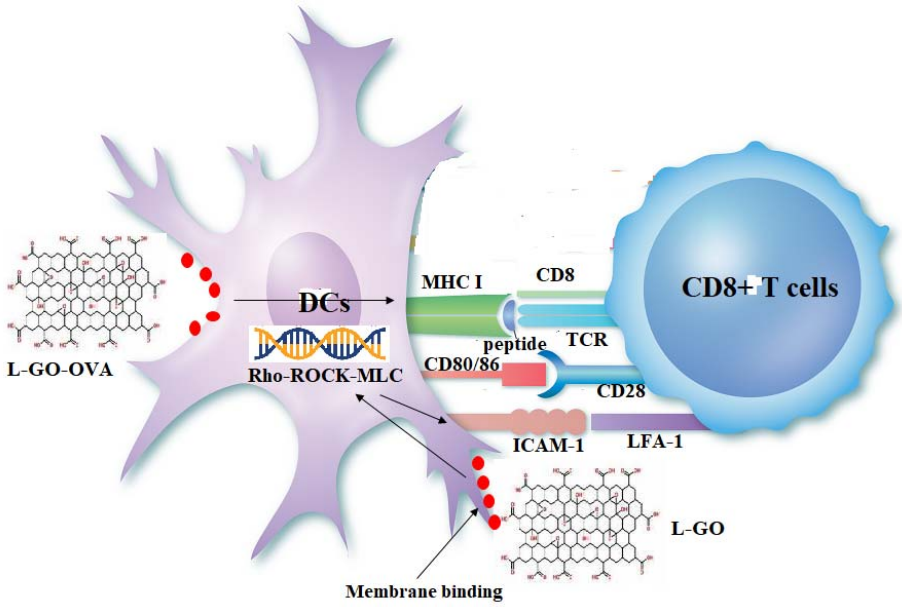

Figure 7. The intense binding capacity of L-GO to DCs membrane increase DCs-T cells receptors interaction which facilitate the full activation of $CD8^+$ T cells.

2.2 Other perspectives on how GO can enhance DC vaccine performance

Immunotherapeutic strategies for various diseases now include vaccination, with the addition of adjuvants to enhance vaccine performance and reduce the need for high-dose vaccines. In recent years, research has focused on developing adjuvants for vaccine preparations, such as alum, liposomes, and GO nanosheets. GO nanosheets have shown potential as universal adjuvants for DC vaccines, particularly in cancer treatment, as they can be tailored to different antigens. GO has been used as a delivery vehicle for vaccine antigens, effectively internalizing antigens into DCs and promoting cross-presentation to $CD8^+$ T cells, leading to anti-tumor responses[55]. However, GO's low solubility and poor stability in the human body, as well as its cytotoxicity and potential DNA damage, have

limited its application[59,60]. To overcome these limitations, functionalized GOs (FGOs) have been extensively studied as carriers and adjuvants. The surface chemistry of nanomaterials is crucial for biocompatibility, and covalent modification with oxygen-based functional groups has been a common method to fabricate FGOs with improved biocompatibility[61]. Some examples of FGOs that have been successfully synthesized through this method include GO-PEI, GO-PEG, GO-PEG-PEI, GO-PAMAM, and others. For instance, GO-PEG-PEI has a positively charged surface that can adsorb negatively charged antigens with ultra-high loading efficiency, promoting DC aggregation and antigen uptake[61,62]. GO-PEG-PEI has also been shown to induce DC maturation, upregulate co-stimulatory molecules, and enhance T cell proliferation and cytokine secretion, suggesting its potential as an adjuvant for improved immunogenicity and cellular immunity[63]. Additionally, GO can be covalently linked with alum for higher antigen loading efficiency, and OVA-loaded GO-AlO(OH) has been shown to increase antigen uptake by DCs and facilitate cytosolic delivery, indicating its potential as an effective antigen delivery system[64]. GO has also been proposed to protect proteins from proteolysis, and functionalization with chitosan has been shown to improve biocompatibility and stimulate cytokine production for enhanced cellular immune response[64]. RNA-based antigen approaches have shown promise in clinical studies, and GO-bound total RNA of tumors may elicit more potent antitumor responses in DC vaccines, as GO can enhance RNA stability and hinder RNase degradation[65].

2.3 Clinical translational challenges of using GO for DC vaccine-based immunotherapy

GO has shown tremendous results in enhanced DC vaccine-based immunotherapy, but there are several clinical translational challenges that need to be addressed. These challenges emerge from the complex interactions between GO and the immune system, safety concerns, and the need for optimized delivery strategies. GO has been displayed to have immunomodulatory effects that can modulate the immune response[52]. While this can be beneficial for eliciting the efficacy of DC vaccines, the exact mechanisms and long-term effects of GO on the immune system are not fully understood. Further research is required to enlighten the immunomodulatory effects of GO and decide the optimal dosage and treatment regimen for safe and effective immunotherapy. The biocompatibility and potential toxicity of GO are critical concerns for clinical translation[51]. While GO has demonstrated low toxicity in many in vitro and animal studies, its long-term effects and potential accumulation in vivo need to be carefully evaluated[51]. Comprehensive preclinical studies that involve toxicity assessments, biodistribution studies, and long-term safety evaluations, are necessary to ensure the safe use of GO in humans[51]. Understanding the pharmacokinetics and biodistribution of GO is essential for its clinical translation. The size, shape, surface charge, and functionalization of GO can influence its distribution, accumulation, and clearance from the body[66]. Detailed studies investigating the fate of GO after administration, including its systemic distribution and elimination pathways, are required to ensure proper dosing and minimize potential side effects[66]. Efficient delivery of GO to target cells, such as DCs, is crucial for the success of immunotherapy. Strategies for GO delivery need to be optimized to ensure effective uptake by DCs and minimal off-target effects. Various delivery systems, such as nanoparticles, liposomes, or biomaterial-based carriers, can be explored to enhance the specificity and efficiency of GO delivery to DCs[67]. Establishing standardized protocols for GO synthesis, functionalization, characterization, and quality control is essential for clinical translation[68]. Consistent and reproducible production of GO with well-defined physicochemical properties is necessary to ensure reliable and comparable results across different studies and to meet regulatory requirements.

3. Conclusion

GO has shown great promise in its ability to play a pivotal role in activating DCs and influencing various aspects of T cell effector function. This phenomenon appears to be dependent on factors

such as size, shape, and functionalization of GO. Notably, recent studies have revealed the crucial role of L-GO in promoting the assembly of the DC-T cell immune synapse, offering new insights for engineering DC vaccines based on enhancing DC-T cell communication. The potential of GO and its functionalized derivatives as adjuvants in vaccine formulations is significant, as they can enhance vaccine stability, antigen delivery, and immune response. However, interdisciplinary collaboration between researchers, clinicians, and regulatory authorities are required in addressing several aforementioned clinical translational challenges. Rigorous preclinical evaluation, including comprehensive toxicity studies and optimization of delivery strategies, is crucial to establish the safety and efficacy of GO in DC vaccine-based immunotherapy.

Author contributions

RM, MMR, MYA and MSM conceived of the scope of the review, drafted and revised the manuscript.

AHR and NHO helped in revise the manuscript and provided many valuable comments about the manuscript. All authors read and approved the final manuscript.

Funding

This research was funded by strategic research fund (ENRICH) (311/CIPPT/411988) and Research University Fund for BCTRP (1001/CIPPT/8070033) from Universiti Sains Malaysia.

Conflict of interest

The authors declare no potential conflict of interest.

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