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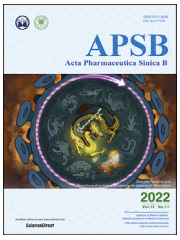

REVIEW

The anti-inflammatory feature of glucagon-like peptide-1 and its based diabetes drugs—Therapeutic potential exploration in lung injury

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Abstract Since 2005, GLP-1 receptor (GLP-1R) agonists (GLP-1RAs) have been developed as therapeutic agents for type 2 diabetes (T2D). GLP-1R is not only expressed in pancreatic islets but also other organs, especially the lung. However, controversy on extra-pancreatic GLP-1R expression still needs to be further resolved, utilizing different tools including the use of more reliable GLP-1R antibodies in immune-staining and co-immune-staining. Extra-pancreatic expression of GLP-1R has triggered extensive investigations on extra-pancreatic functions of GLP-1RAs, aiming to repurpose them into therapeutic agents for other disorders. Extensive studies have demonstrated promising anti-inflammatory features of GLP-1RAs. Whether those features are directly mediated by GLP-1R expressed in immune cells also remains controversial. Following a brief review on GLP-1 as an incretin hormone and the development of GLP-1RAs as therapeutic agents for T2D, we have summarized our current understanding of the anti-inflammatory features of GLP-1RAs and commented on the controversy on extra-pancreatic GLP-1R expression. The main part of this review is a literature discussion on GLP-1RA utilization in animal models with chronic airway diseases and acute lung injuries, including studies on the combined use of mesenchymal stem cell (MSC) based therapy. This is followed by a brief summary.

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1. Introduction

Glucagon-like peptide-1 (GLP-1) is an incretin hormone produced in gut endocrine L cells[1−4]. Postprandial GLP-1 secretion leads to reduced plasma glucose levels by mechanisms including the stimulation of insulin secretion, the inhibition of glucagon release, as well as the delay of gastric emptying[4]. Furthermore, plasma GLP-1 elevation or GLP-1-based drug administration may directly reduce food intake, involving their function in the brain, mediated by GLP-1 receptor (GLP-1R), which is known to be expressed in the brain hypothalamus and elsewhere[5−7]. Various GLP-1-based drugs (also known as GLP-1R agonists, GLP-1RAs) have been developed and approved by US Food and Drug Administration (FDA), European Medicines Agency, or other authorities for diabetes treatment since 2005[8]. They are now widely utilized in treating type 2 diabetes (T2D) without side effects of weight gain and hypoglycemia, compared with various sources of commercial insulin[9]. GLP-1-based drugs, such as liraglutide (commercially known as Victoza®) and semaglutide (Ozempic®) were also approved by FDA for chronic weight management in patients with obesity, overweight, or weight related comorbid conditions.

Studies on native GLP-1 and GLP-1-based drugs in animal models or in treating patients with T2D have also uncovered their profound anti-inflammatory function[10,11]. Since inflammatory responses also play important roles in the development and progression of diseases other than T2D, repurposing GLP-1-based drugs has been attracting researchers' attention in various fields.

In this review, we will briefly summarize the discovery of GLP-1 as an incretin hormone and the development of GLP-1-based diabetes drugs. We will then discuss literature that leads to the recognition of the anti-inflammatory and immune-regulatory functions of GLP-1 and its based drugs. This will be followed by a brief discussion of controversies on GLP-1R expression in extra-pancreatic organs. The main content of this review, however, is a literature discussion on the discovery and functional assessment of potential therapeutic effects of GLP-1-based drugs in chronic airway diseases and acute lung injuries. For more detailed discussions on utilization or potential utilization of GLP-1-based drugs, as well as dipeptidyl peptidase 4 (DPP-4) inhibitors in the treatment of diabetes, cardiovascular diseases, non-alcoholic fatty liver diseases (NAFLD), kidney disorders, and neurodegenerative brain disorders, please see excellent review articles elsewhere[1,12−18].

2. The incretin GLP-1 and its plasma elevation during inflammation

GLP-1 was recognized as the 2nd incretin hormone back in 1983[19,20]. Ebert and colleagues observed that in a rat model, incretin activity was still preserved after gastric inhibitory polypeptide (GIP, also known as glucose-dependent insulinotropic polypeptide) was removed from gut extracts by immune-adsorption[20]. Following the isolation of the proglucagon gene (*GCG*/*Gcg*) cDNA from fish, hamsters, rats, mice, and humans, it was evident that in addition to encoding glucagon, a counter-regulatory hormone of insulin, *GCG/Gcg* cDNAs also encode two additional polypeptides defined as glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2)[19,21−27].

Gcg (*GCG* in humans) is abundantly expressed in pancreatic α-cells, intestinal endocrine L cells, and certain neuronal cells in the brain[3,22,28]. Post-translational processing of the pro-hormone proglucagon occurs in tissue-specific manners *via* prohormone convertases (PC) known as PC1/3 and PC2. As shown in Fig. 1A, in pancreatic α-cells, which mainly express PC2, proglucagon is processed to produce the active hormone glucagon and other products including major proglucagon fragments. In the brain and the intestinal endocrine L cells, the expression of PC3 (also known as PC1) leads to the catalysis of proglucagon into GLP-1 and GLP-2, as well as glicentin and oxyntomodulin[23,25,26,29−33].

Full-length GLP-1 consists of 37 or 36 amino acid residues, and it becomes biologically active after it is truncated at the N-terminus to form GLP-1_{7-37} or GLP-$1_{7-36amide}$ (Fig. 1B)[34,35]. As mentioned above, GLP-1 is the 2nd incretin hormone recognized to date[36−39] while GIP is the first one[20,40,41]. Incretins are defined as gut-produced hormones that can stimulate insulin secretion in a glucose concentration-dependent manner. The inhibitory effect of GLP-1 on glucagon secretion, however, was not shared by GIP[42]. Instead, a study showed that GIP might stimulate glucagon secretion from pancreatic islet α-cells[42]. Native GLP-1 (both GLP-1_{7-37} and GLP-$1_{7-36amide}$) can be cleaved by the ubiquitously expressed enzyme dipeptidyl peptidase 4 (DPP-4) to produce GLP-1_{9-37} or GLP-$1_{9-36amide}$, while further cleavage by neutral endopeptidase 24.11 leads to the production of GLP-$1_{28-36amide}$ and GLP-$1_{32-36amide}$[43−48]. Although certain biological functions of GLP-$1_{9-36amide}$, GLP-$1_{28-36amide}$ and GLP-$1_{32-36amide}$ have been described in pre-clinical investigations by our team and others[46−49], those are generally considered as inactive "degradation" products of GLP-1. The half-life of GLP-1 is relatively short, around 1.5 min in human plasma. For mechanisms underlying GLP-1 secretion, please see review articles by our team and by others elsewhere[50−53].

In humans, circulating GLP-1 level starts to ramp up only a few minutes after nutrient intake. It reaches the peak around 1 h[54]. Among the nutrient components, glucose was shown to be the strongest stimulus of GLP-1 secretion, followed by sucrose, starch, triglycerides (TG), and certain amino acids[50,51]. Studies in animal models have suggested that systemic inflammation induced by endotoxin (lipopolysaccharide, LPS) can also stimulate GLP-1 secretion in mice[55−57]. Kahles and colleagues observed that among the inflammatory stimuli including endotoxin, interleukin 6 (IL-6), and IL-1β, it appears that IL-6 was sufficient and necessary to directly stimulate GLP-1 production and release; as in IL-6 knockout (KO) mice, endotoxin-induced GLP-1 secretion was found to be blunted[57]. It is worth mentioning that in rodent species especially in mice, plasma GLP-1 measurement is still a technical challenge and data obtained may not always be reliable. Nevertheless, Kahles and colleagues[57] have also reported that in a cohort in intensive care unit (ICU), GLP-1 plasma levels correlated with inflammation markers and the disease severity. Consequently, they suggested that GLP-1 serves as a link between

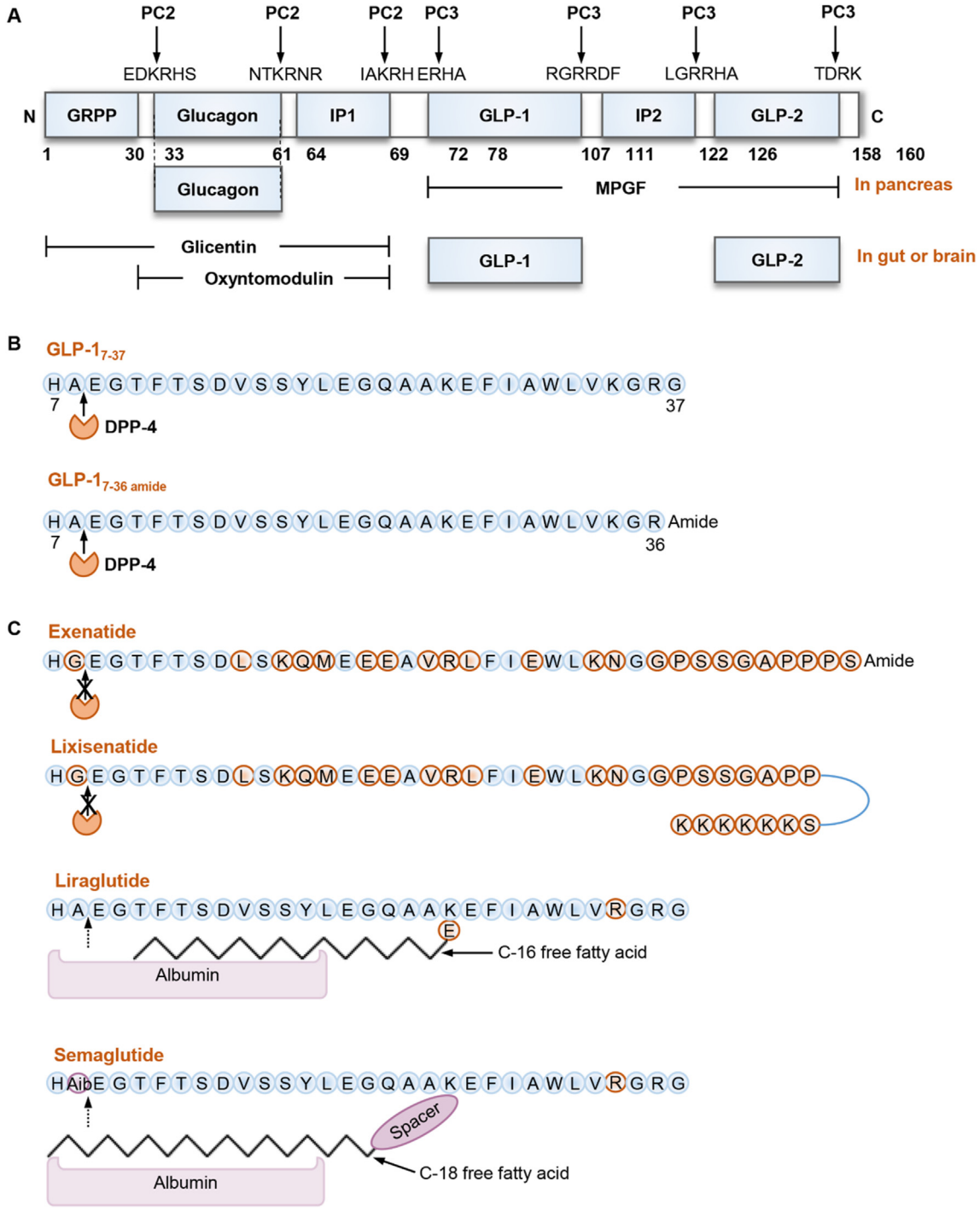

Figure 1 GLP-1 and GLP-1-based drugs. (A) The structure of proglucagon and proglucagon-derived polypeptides (PGDPs). GRPP, glicentin-related pancreatic polypeptide. IP-1 and IP-2, intervening peptides 1 and 2; MPGF, major proglucagon fragment. GLP-1 and GLP-2, glucagon-like peptide 1 and 2. (B) The primary amino acid sequences of human GLP-1_{7-37} and GLP-$1_{7-36amide}$. The ubiquitously expressed dipeptidyl peptidase 4 (DPP-4) will cleave X-alanine dipeptides from the N-terminus of GLP-1_{7-37} or GLP-$1_{7-36amide}$ to form GLP-1_{9-37} or GLP-$1_{9-36amide}$, respectively. (C) Chemical structures of four GLP-1R agonists (GLP-1RAs). Due to amino acid substitution or non-covalent binding to albumin or immunoglobulin, these GLP-1RAs are protected from DPP-4 mediated degradation, thus having a much longer half-life.

the immune system and the gut[57]. Indeed, metabolic illness and inflammatory diseases share certain common therapeutic targets[58]. Individuals who underwent cardiac surgery or autologous stem cell transplantation had up to 2-fold higher levels of circulating GLP-1, as reported by Lebherz and colleagues, as well as by Ebbesen and colleagues[59,60]. Patients with severe burn injury produced 3-fold more plasma GLP-1, while patients who died from severe burn injury had 5-fold higher GLP-1 levels than those who survived[61]. In addition, patients that suffered from sepsis combined with T2D displayed an enhanced activation of endogenous GLP-1 system compared to non-diabetic patients[62]. Thus, in both rodent models and in human subjects, systematic

inflammation can cause plasma GLP-1 elevation. Further investigations are required to determine whether plasma GLP-1 level can be developed as a biomarker for the diagnosis and prognosis of inflammatory responses and inflammatory diseases. Patho-physiologically, elevated GLP-1 level during systematic inflammation may serve as a self-defense mechanism.

3. GLP-1R agonists as diabetes drugs

Although GIP was discovered more than a decade earlier than GLP-1, for various reasons, it has not yet been developed as a therapeutic agent. In 2005, the first GLP-1-based drug, exenatide (with the commercial name Byetta®), was approved by FDA for T2D treatment. Since then, ten additional GLP-1R agonists (GLP-1RAs) have been approved for T2D treatment. Table 1 lists those GLP-1RAs, as well as four DPP-4 inhibitors (DPP-4i) and DPP-4i-based compound drugs.

Fig. 1C shows the structures of four GLP-1RAs, including exenatide, lixisenatide, liraglutide and semaglutide. Exenatide was developed based on studies in a peptide isolated from the saliva of the Gila monster, known as exendin-4. Exendin-4 contains 39 amino acid residues with a half-life of around 30 min, sharing 53% amino acid sequence homology with human GLP-1[63,64]. As a synthetic version of exendin-4, exenatide is resistant to DPP-4-induced degradation which contributes to a longer half-life of about 2.4 h after subcutaneous injection[65,66]. Lixisenatide, another derivative of exendin-4, was approved by FDA in 2016[67,68]. Liraglutide (Victoza®) was approved by FDA in 2010, which is a modified human GLP-1, sharing 97% sequence identity with native GLP-1. The non-covalent binding with albumin prevents its renal elimination. It is the first long-acting compound of GLP-1RAs, with a much longer half-life of 13 h[69–71]. Semaglutide (Ozempic) is the most recently approved long-acting GLP-1RAs for T2D in 2017, with a half-life of 7 days[72,73]. An equipotent once-daily oral administration form of semaglutide was approved in 2019[74]. Table 1 also lists a few other GLP-1RAs. Among them, albiglutide consists of a dimer of human GLP-1 molecules fused to a recombinant human albumin, while dulaglutide consists of a dimer of human GLP-1 molecules fused to a modified human immunoglobulin G4 heavy chain[9].

As shown in Fig. 1B, native GLP-1 can be cleaved by DPP-4, which is a ubiquitously expressed peptidase. DPP-4 can also inactivate GIP. Thus, DPP-4 inhibition can prevent the degradation of both native GLP-1 and GIP. DPP-4i can specifically inhibit the enzymatic degradation activity of DPP4 by over 80%, leading to a doubling of active GLP-1 level[75]. Sitagliptin (Januvia), developed by Merck & Co, was the first DPP-4i approved by the FDA as a T2D drug in 2006, followed by saxagliptin, linagliptin and alogliptin[76–78]. DPP-4i can be administered orally and formulated either as a single-ingredient product or in combination with other diabetes medicines, including metformin (Table 1). Although investigations have also been conducted in assessing the effects of DPP-4i in lung injury models, we will not cover those studies in current manuscript. Information on such studies can be found elsewhere[79–81].

As a relatively novel category of T2D drugs, adverse drug reactions (ADRs) of GLP-1RAs have been intensively studied globally. As reviewed very recently by Shetty and colleagues, GLP-1RAs are most commonly associated with ADRs in the

Table 1 FDA approved GLP-1 receptor agonists (GLP-1RA) and DPP-4 inhibitors (DPP-4i).

Brand name	Active ingredient	FDA-approved year
GLP-1RA		
Byetta	Exenatide	2005
Bydureon	Exenatide (extended release)	2012
Victoza	Liraglutide	2010
Saxenda	Liraglutide	2014
Xultophy 100/3.6	Liraglutide and insulin degludec	2016
Tanzeum	Albiglutide	2014
Trulicity	Dulaglutide	2014
Adlyxin	Lixisenatide	2016
Soliqua 100/33	Lixisenatide and insulin glargine	2016
Ozempic	Semaglutide	2017
Rybelsus	Semaglutide (oral)	2019
DPP-4i		
Januvia	Sitagliptin	2006
Janumet	Sitagliptin and metformin	2007
Janumet XR	Sitagliptin and metformin (extended release)	2012
Steglujan	Sitagliptin and ertugliflozin	2017
Onglyza	Saxagliptin	2009
Kombiglyze XR	Saxagliptin and metformin (extended release)	2010
Qtern	Saxagliptin and dapagliflozin	2017
Qternmet XR	Saxagliptin, dapagliflozin and metformin (extended release)	2019
Tradjenta	Linagliptin	2011
Jentadueto	Linagliptin and metformin	2012
Jentadueto XR	Linagliptin and metformin (extended release)	2016
Glyxambi	Linagliptin and empagliflozin	2015
Tradjenta XR	Linagliptin, empagliflozin and metformin	2020
Nesina	Alogliptin	2013
Kazano	Alogliptin and metformin	2013
Oseni	Alogliptin and pioglitazone	2013

gastrointestinal tract, particularly pancreatitis[82]. Cardiovascular, renal, hematologic, dermatologic, neurologic, autoimmune, hepatic and metabolic associated ADRs were also identified for GLP-1RAs[82]. For more than a decade, the development of pancreatitis or even pancreatic cancer has been the major concern in utilizing GLP-1RAs. It has been summarized by Ryder in 2013, that for animal studies, the worrying pancreatic histological changes are not reproducible and are variable among the use of different GLP-1RAs; and that increased reports of pancreatitis and pancreatic cancer by FDA are likely due to 'notoriety bias'[83]. He then concluded that although we should remain vigilant, the balance of evidence at current stage is in supporting GLP-1-based therapy strongly, with beneficial effects far outweighing those potential risks[83]. For further information on common and rare ADRs of GLP-1RAs, please see review articles elsewhere[82,84–86].

4. The anti-inflammation features of GLP-1 and its based diabetes drugs

Systemic inflammation is usually characterized by elevated pro-inflammatory cytokines and imbalanced immune cells in the circulation. As the first FDA-approved GLP-1-based diabetes drug, the anti-inflammatory features of exenatide have been extensively investigated in patients with T2D. As early as 2007, Viswanathan et al.[10] demonstrated that in subjects with T2D, exenatide had two "non-metabolic actions": the effect on attenuating plasma C-reactive protein (CRP) levels and the effect on lowering systolic blood pressure. A few years later, Kim et al.[87] showed in mice that cardiomyocyte GLP-1R activation promoted the translocation of the rap guanine nucleotide exchange factor Epac2 to the membrane, leading to atrial natriuretic peptide (ANP) elevation, which lowers blood pressure. Interestingly, they have also located GLP-1R expression in mouse cardiac atria[87]. In 2011, Wu et al.[88] showed that in patients with T2D, 16-week exenatide treatment had not only reduced body mass index and improved hemoglobin A1c and glucose profiles; but also decreased circulating levels of inflammatory markers including high-sensitivity CRP and monocyte chemoattractant protein-1. Furthermore, the level of oxidative stress marker 8-*iso*-prostaglandin F2a, was also reduced following exenatide treatment[88]. The protein and mRNA levels of a battery of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), IL-1β and IL-6 in peripheral blood mononuclear cells (PBMC or MNC) were also shown to be suppressed by *in vivo* exenatide treatment in subjects with T2D[89,90]. Moreover, both investigations have revealed the anti-inflammatory effect of exenatide in the absence of body weight loss in patients with T2D with 12-week exenatide treatment[89,90]. Thus, the anti-inflammatory effect of exenatide may not always be secondary to its body weight-lowering effect[89–91]. The anti-inflammatory effect of liraglutide was also demonstrated recently by Zobel and colleagues in subjects with T2D[92]. In this clinical trial, subjects with T2D were on 26-week liraglutide treatment. Zobel and colleagues observed the discrete modulatory effect of liraglutide on the expression of inflammatory genes in PBMCs. Importantly, such modulatory effect was not observed in the *in vitro* settings with direct liraglutide treatment in the human monocytic cell line THP-1[92]. Furthermore, Zobel and colleagues[92] reported that GLP-1R expression could not be detected in the THP-1 cell line or PBMCs.

The anti-inflammatory effects of GLP-1RAs were also observed in various animal models. Although GLP-1RAs showed no improvement in patients with type 1 diabetes (T1D), Sherry et al.[93] demonstrated that exenatide could facilitate the reversal of T1D in NOD mice treated with the "therapeutic" anti-CD3 monoclonal antibody. Mechanistically, the facilitation is likely involving the increase of anti-inflammatory subsets of T lymphocytes, such as T helper 2 and regulatory T cells in mice[93–95]. More recent studies have further demonstrated the T lymphocyte regulatory function of liraglutide and dulaglutide, as well as the DPP-4i sitagliptin[96–98]. The DPP-4i linagliptin was also shown to attenuate insulin resistance and inflammation by modulating M1/M2 macrophage polarization, as reported by Zhuge and colleagues[99]. In a Wistar rat model with intraperitoneal LPS challenge, exenatide treatment was shown to attenuate neutropenia, associated with decreased levels of a battery of pro-inflammatory cytokines, including IL-1α, IL-1β, IL-6, TNFα, and IFNγ[100]. Utilizing the pro-adipocytic 3T3-L1 and RAW264.7 macrophage cellular models, several studies have shown that the DPP-4i anagliptin or GLP-1RA liraglutide can inhibit nuclear factor-kappa B pathway and secretion of a series of pro-inflammatory cytokines[101–103]. Although a few studies have indicated the expression of GLP-1R in rodent immune cells[95,98], as mentioned above, a more recent human study by Zobel and colleagues[92] showed that the repressive effect of liraglutide on the expression of inflammatory genes in PBMCs was not observed in the *in vitro* settings with direct liraglutide treatment[92]. In addition, Zobel and colleagues[92] could not detect GLP-1R in THP-1 or primary PBMCs. Fig. 2 summarizes our current understanding of the anti-inflammatory features of GLP-1RAs. As shown, intra-pancreatic functions of GLP-1 or GLP-1RAs are known to be mediated by GLP-1R. It remains to be determined whether *in vivo* immunoregulatory functions of GLP-1RAs on immune cells are mediated by GLP-1R that are expressed in those cells or by yet to be further explored mechanism.

5. Controversy on GLP-1R expression in extra-pancreatic organs

During the past two and a half decades, there are substantial controversies in the literature regarding GLP-1R expression in extra-pancreatic organs, including the liver, heart, and adipose tissues[8,104–106], in addition to that in immune cells we have mentioned above[92,95,98]. Nevertheless, *in vivo* effects of GLP-1 and GLP-1RAs on the liver and other extra-pancreatic organs are clear and substantial[104–109].

cDNA that encodes rat GLP-1R was initially isolated by Thorens et al.[110] in 1992, while the first GLP-1R KO mouse line was created by Scrocchi et al.[111] in 1996. In 1994, Campus et al.[112] investigated the expression of mouse *Glp1r* using a combination of Northern blotting and RT-PCR. They reported the detection of *Glp1r* in small and large intestines, pancreas, liver, lung, and kidney. Wei and Mojsov[113] then reported in 1995 that for human GLP-1R, the brain, heart and pancreatic forms have the same deduced amino acid sequence. In 1996, utilizing more specific approaches including RNase protection assay and *in situ* hybridization, along with RT-PCR, Bullock et al.[114] reported the detection of *Glp1r* in the gastric pit of the stomach, large-nucleated cells in the lung, crypts of the duodenum, and pancreatic islets. They, however, cannot detect *Glp1r* signal in the

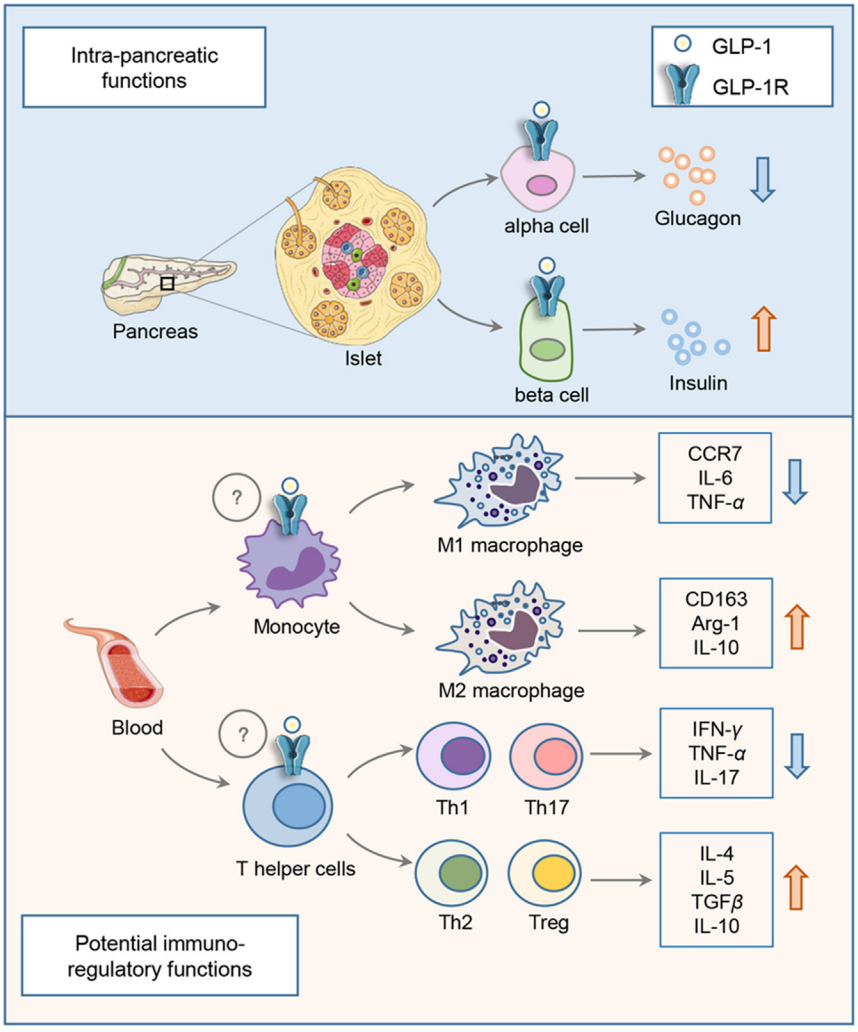

Figure 2 Illustration of intra-pancreatic and potential immune-regulatory functions of GLP-1RAs. In pancreatic islets, GLP-1 or GLP-1RAs stimulates insulin secretion and represses glucagon secretion by pancreatic β-cells and α-cells, respectively, events that depend on GLP-1R. GLP-1RA *in vivo* administration exerts its regulatory function in both macrophages and T lymphocytes (T helper cells). It is unclear whether this is mediated by GLP-1R that is expressed in these two cell lineages (indicated with a question mark). *In vivo* GLP-1RA administration inhibits differentiation of M1 macrophage and the production of pro-inflammatory cytokines and chemokines including CCR7, IL-6 and TNF-α. Conversely, M2 macrophage differentiation and the production of CD163, Arg-1 and IL-10 can be stimulated by *in vivo* GLP-1RA treatment. Meanwhile, GLP-1RA treatment may inhibit the differentiation of pro-inflammatory T helper cells, including Th1 and Th17, leading to reduced production of pro-inflammatory cytokines including interferon γ, TNF-α and IL-17. The differentiation of the anti-inflammatory T helper 2 and regulatory T cells, as well as the production of IL-4, IL-5, TGFβ and IL-10, however, could be promoted by *in vivo* GLP-1RA treatment.

kidney, skeletal muscle, heart, liver, or adipocytes[114]. They have suggested that the GLP-1R expressed in the kidney and heart might be structural variants of the known receptor[114]. The 2nd GLP-1 receptor theory, however, has not been proved or disproved during the past two and a half decades. A more recent study by Sato et al.[115] showed the *Glp1r* expression in the lung alveoli utilizing the *in situ* hybridization approach.

Due to the profound hepatic function of GLP-1 and GLP-1RAs, efforts have been made in determining GLP-1R expression in the liver and hepatocytes. Several studies have shown the detection of *Glp1r* mRNA and GLP-1R protein in mouse or human hepatic cell lines and the mouse liver[107,116,117], in contrast to the early report by Bullock et al.[114] Investigations by Panjwani et al.[105] and by Baggio et al.[106] showed that the controversy could be partially due to the lack of reliable anti-GLP-1R antibodies, raising the issue of the development of more ones. With the none-bias RNA-seq and other approaches, we and others have shown that mouse or human liver does not express mRNA that encodes mouse or human GLP-1R[104,108,109].

More reliable GLP-1R antibodies (3F52 for humans and 7F38 for mice) have been generated by Knudsen's team, which could be utilized in detecting GLP-1R expression by immunohistochemistry (IHC) method[118,119]. When the human 3F52 antibody was utilized in monkey and human tissues, Pyke et al.[118] reported the detection of GLP-1R signal in smooth muscle cells in the walls of arteries and arterioles. This observation correlates with a few functional studies, showing that exenatide treatment attenuated NR4A orphan nuclear receptor NOR1 in vascular smooth muscle cells[120], and that GLP-1R over-expression in airway smooth muscle cells attenuated cell proliferation and migration, as well as

secretion of pro-inflammatory cytokines[121]. It appears that both 3F52 and 7F38 could not be utilized for detecting GLP-1R in tissue samples by Western blotting. Utilizing 7F38, we have shown the detection of GLP-1R in the lung of wild-type mice but not in GLP-1R KO mice[122]. Co-immune staining approaches need to be adopted, in combination with the utilization of GLP-1R KO mouse tissue samples, for determining which cell lineages in the lung that express GLP-1R. As discussed above, whether PBMCs and other immune cells express GLP-1R also remains controversial[92,98]. The immune-staining approaches should also be utilized for clarifying whether certain immune cells express GLP-1R, and whether their GLP-1R expression can be regulated in physiological and patho-physiological conditions.

As GLP-1R is known to be expressed in the brain, we have suggested that *in vivo* extra-pancreatic functions of GLP-1 and GLP-1RAs are either mediated by certain brain-peripheral tissue axis or by a small portion of GLP-1R-positive cells that are scattered within each of those organs. Very recently, McLean et al.[123] conducted their investigation on potential murine *Glp1r* expression within endothelial and hematopoietic cells. They have created a mouse line with targeted inactivation of *Glp1r* in $Tie2^{+}$ cells. Those mice exhibited reduced levels of *Glp1r* mRNA transcripts in aorta, liver, spleen, blood, and gut. Importantly, they have located liver *Glp1r* expression to $\gamma\delta$ T lymphocytes while semaglutide mediated hepatic metabolic beneficial effects were observed in high fat diet challenged $Glp1r^{Tie2+/+}$ mice but not in $Glp1r^{Tie2-/-}$ mice[123]. Hence, they have suggested that observed *in vivo* functions of GLP-1-based drugs in certain extra-pancreatic organs could be attributed to endothelial and hematopoietic-cell expressed GLP-1R[123].

6. GLP-1-based drugs in airway diseases and lung injury studies

We have learned for more than 25 years that lung is an extra-pancreatic organ, which exhibits the highest level of *Glp1r* mRNA[112,114]. Hence, great efforts have been made in clinical trials and various lung injury animal models, seeking the possibility to repurpose GLP-1-based drugs in chronic airway diseases and acute lung injury treatment. Here we will present our literature review on clinical investigations as well as studies with chronic airway diseases and acute lung injury animal models. We will then summarize a few very recent studies on "therapeutic effects" of the combined use of human mesenchymal stem cells and GLP-1-based drugs in mouse acute lung injury models.

6.1. Studies in chronic airway diseases

Chronic airway diseases mainly include asthma, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, bronchiectasis, and bronchitis. As pre-clinical studies on GLP-1RAs have been conducted mainly on asthma and COPD, below we focus on presenting our literature review on these two categories of diseases.

6.1.1. Asthma

In a recent retrospective cohort study, Foer et al.[124] have compared rates of asthma exacerbations and symptoms between patients with T2D and asthma prescribed GLP-1RAs and those prescribed sodium-glucose cotransporter-2 inhibitors, or DPP-4i, or sulfonylureas, or basal insulin. They observed that patients prescribed GLP-1RAs had lower counts of asthma exacerbation and encountered asthma symptoms after 6 months of the treatment, when compared with patients who received each of the other four categories of drugs[124]. In a human study, Mitchell et al.[125] have measured the expression of GLP-1R, using flow cytometry staining and analysis, on eosinophils and neutrophils in normal and asthmatic subjects and then evaluated *in vitro* effects of a GLP-1RA on functions of eosinophils[125]. They reported that GLP-1R is expressed in human eosinophils and neutrophils. In eosinophils but not in neutrophils, GLP-1R expression is significantly higher in normal subjects when compared to subjects with allergic asthmatics. GLP-1R expression did not change on either eosinophils or neutrophils following the allergen challenge. Their *in vitro* study showed that GLP-1RA significantly decreased expression of eosinophil-surface activation markers following LPS stimulation and decreased eosinophil production of IL-4, IL-8 and IL-13, but not the IL-5, a key pro-inflammatory cytokine relevant to chronic airway disorders[125].

IL-33, a member of the IL-1 family, is constitutively produced in fibroblasts, endothelial cells, and epithelial cells of the skin, lung, and gastrointestinal tract[126]. It is among crucial mediators of both innate and adaptive immune responses induced by aeroallergens. Genome-wide association studies have revealed the implication of the *IL33* locus in the development of asthma[127,128]. To date, there is no known therapeutic agent that can inhibit the release of IL-33 from airway cells[129]. When *Alternaria* extract, an aeroallergen with protease activity, is intranasally administrated in mice, asthma attack can be induced. In this mouse model, Toki and colleagues assessed both "preventative" and "therapeutic" effects of liraglutide. Either administrated before or after *Alternaria* extract challenge, liraglutide suppressed IL-33 secretion, associated with decreased numbers of group 2 innate lymphoid cells, and reduced mucus production[129]. However, further mechanistic explorations are needed for clarifying the involvement of GLP-1R and the downstream signaling events. In another asthma mouse model challenged with ovalbumin for 81 days, intraperitoneal injection of liraglutide at 2 mg/kg twice daily in the last 66 days inhibited airway inflammation and mucus hyper-secretion through a protein kinase A (PKA)-dependent signaling pathway[130].

6.1.2. COPD

In a meta-analysis study, Wei and colleagues[131] have reported that the utilization of GLP-1-based drugs showed reduced trends in the risks of nine categories of respiratory diseases, including pneumonia, bronchitis, pulmonary fibrosis, asthma, and COPD. However, GLP-1-based drug utilizations were shown to increase trends in interstitial lung disease.

COPD is among the top leading cause of death worldwide. Up to date, no approved therapy can reverse lung injury caused by COPD. Huang and colleagues reported that the expression of GLP-1R in PBMC isolated from COPD patients is lower than that in non-COPD subjects[132]. *In vitro* liraglutide treatment, however, upregulated GLP-1R expression and restored antigen-stimulated interferon γ production in T lymphocytes[132]. Considering the literature controversy on GLP-1R expression in extra-pancreatic organs, further investigations are needed for clarifying GLP-1R expression in immune cells with newly developed GLP-1R antibodies and other tools such as RNA-seq[104,108,109,119,133]. There is

an on-going clinical trial operated by Hospital South-West Jutland, University of Southern Denmark on assessing the effects of liraglutide treatment in patients with COPD. This prospective, randomized, placebo-controlled, double-blinded, parallel-group two-center clinical trial, headed by Dr. Claus B. Juhl, will determine various pharmacological effects and functional outcomes of 4-, 20-, 40- and 44-week liraglutide treatment in 40 patients with COPD.

Pulmonary surfactant is a surface-active complex of proteins and phospholipids formed by type II alveolar cells, which plays important role in regulating the alveolar size and lung innate immunity, as well as in preventing fluid accumulation and maintaining dryness of the airway. In human type II pneumocytes isolated from cadaveric organ donors, Vara et al.[134] found that native GLP-1 or exenatide could stimulate cAMP formation and phosphatidylcholine secretion; and such effects were shown to be reversed by the GLP-1R antagonist exendin (9−39). Early investigations have generated ovalbumin induced-asthma model and long-term LPS-induced rodent COPD model[135,136]. Combining these two models, Viby and colleagues[137] have assessed the effect of liraglutide on improving lung functions in a female COPD mouse model. They found that mice treated with liraglutide or exenatide showed a much better clinical appearance and increased survival rate. They also observed reduced expression of surfactant proteins in their COPD female mouse model, associated with increased expression of pro-inflammatory cytokines. However, levels of surfactants and pro-inflammatory cytokines in the lung were largely unaffected with liraglutide treatment in the female COPD mouse model[137]. One may speculate that long-term (>10 days) liraglutide administration may exert more profound "metabolic" beneficial effects in addition to its anti-inflammatory effect observed in the acute injury model. Nevertheless, the stimulatory effect on surfactant secretion was not observed in this *in vivo* model, in contrast with the *in vitro* assay with human type II pneumocytes isolated from cadaveric organ donors[134]. Thus, mechanisms underlying the improvement effect of liraglutide treatment in COPD are complicated, involving not only surfactants and pro-inflammatory cytokines, but also other yet to be identified components.

As mentioned above, Kim and colleagues[87] have located mouse GLP-1R expression in mouse cardiac atria and shown that GLP-1R activation increased cardiac atria ANP secretion, leading to the reduction of blood pressure. As an atrial natriuretic peptide hormone, ANP is also recognized as a potent pulmonary vasodilator[138]. Although ANP is mainly produced in the heart, pulmonary ANP expression was reported, at least in rodent species at its mRNA level[139]. Utilizing the mouse COPD model, Balk-Moller et al.[139] have assessed the lung function of GLP-1-based drugs. Although mouse lung functions did not differ between mice receiving PBS and exendin (9−39) (a GLP-1R antagonist) treatment, or between GLP-1R KO mice and their wild-type littermates, COPD mice receiving GLP-1-based drugs (liraglutide or exenatide) showed improved pulmonary functions, with less inflammation and 10-fold more ANP at the mRNA level. In isolated mouse bronchial sections, direct ANP treatment showed a moderate broncho-dilatory effect, while such effect was also observed, although less effective, with direct liraglutide treatment. Based on these findings, the authors suggested the existence of a link between GLP-1 and ANP in COPD. Balk-Moller and colleagues, however, did not assess pulmonary ANP production at peptide hormone level. Hence, it remains to be determined whether observed beneficial effects of liraglutide treatment is generated by ANP produced in cardiac atria only, or with the contribution of pulmonary produced ANP[139]. It is worth recalling that in 1993, a study by Richter et al.[140] have identified GLP-1 binding site on rat mucous glands in the trachea and on vascular smooth muscle of the pulmonary artery. In isolated rings of rat arteries, GLP-1 was shown to induce relaxation of pre-constricted arteries, involving the secretion of macromolecules. Whether such macromolecules include ANP is worth to be investigated.

6.2. *Nosocomial infection in the lung*

Nosocomial infection especially that in the lung is a critical complication world widely. Lung chronic infections can be generated by respiratory pathogens including the most notorious pathogen *Pseudomonas aeruginosa*, the virulence factor of which is known as pyocyanin, was shown to attenuate the expression of forkhead box A2 (FOXA2), a key transcription factor of a battery of genes that are involved in mucus homeostasis[141]. Choi et al.[142] have shown that FOXA2 expression was severely depleted in surface airway epithelial cells in patients with COPD, while exenatide treatment can restore FOXA2 expression in *P. aeruginosa* challenged mouse model.

6.3. *Studies in acute lung injury*

Acute lung injury (ALI) may lead to the development of acute respiratory distress syndrome (ARDS) which is the major cause of respiratory failure in ICU. ARDS occurs when fluid builds up in alveoli of the lung. The fluid prevents the lungs from filling with enough air, leading to reduced oxygen in the bloodstream. There is no cure for ARDS yet, while the treatment focuses on supporting the patient while the lung heals. In serious conditions, extracorporeal membrane oxygenation (ECMO) is needed. To our knowledge, GLP-1-based drugs have not been utilized in clinical trials for ALI. Nevertheless, as mentioned above, a very recent retrospective study has shown that the utilization of GLP-1-based drugs reduced trends in the risks of pneumonia, in addition to asthma and COPD[131]. Extensive investigations have, however, been conducted in ALI animal models, mainly with intratracheally LPS administration in mice[143].

In 2011, Lim and colleagues[144] have developed a "nanomedicine" designated as GLP1-SSM, in which human GLP-1 (7−36) is self-associated with PEGylated phospholipid micelles (SSM). They then demonstrated that in the LPS-induced ALI mouse model, subcutaneous GLP1-SSM administration decreased lung neutrophil influx, myeloperoxidase activity, and IL-6 levels in a dose-dependent manner[144]. In 2017, GLP-1-SSM was shown by this team to alleviate gut inflammation in a dextran sodium sulfate-induced mouse colitis model[145].

Several recent studies have explored mechanisms underlying the attenuating effect of GLP-1RA in ALI animal models. Reduction of pulmonary surfactant is tightly associated with decreased pulmonary compliance and edema in ALI. Thyroid transcription factor-1 (TTF-1) is known to play an important role in regulating levels of surfactant protein-A, the most abundant protein component of pulmonary surfactant. Romaní-Pérez et al.[146] have reported that in rats, administration of exenatide or liraglutide to the mother from gestational day 14 to the birth increased SP-A and SP-B mRNA levels and amounts of SPs in the amniotic fluid at the end of pregnancy. Furthermore, they have reported that lung *Glp1r* mRNA level increased 4-fold on the 1st day of life in both male and female rats, while the level of

expression was subsequently maintained into the adulthood[146]. In 2018, Zhu et al.[147] found that in the ALI mouse model, LPS administration reduced lung SP-A and TTF-1 levels, while the reduction was reversed by simultaneous administration of liraglutide with LPS challenge. In 2019, in a similar mouse model, Xu and colleagues[148] found that LPS challenge-induced polymorphonuclear neutrophil extravasation, lung injury, along with alveolar-capillary barrier dysfunction. Concomitant liraglutide administration prevented polymorphonuclear neutrophil-endothelial adhesion by inhibiting the expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1. Other documented functions of GLP-1-based drugs in ALI models include the stimulation of the eNOS/sGC/PKG signaling cascade, the induction of vasorelaxant expression, and the inactivation of the nuclear factor-kappa B inflammatory signaling pathway[149–151]. However, none of these investigations have directly assessed the involvement of pulmonary GLP-1R.

In 2020, our team has directly assessed the involvement of GLP-1R in mediating effects of liraglutide treatment in the LPS-induced ALI mouse model. In this study, conducted by Zhou and colleagues[122], liraglutide was not administrated simultaneously with LPS challenge but as a "preventative agent" which was subcutaneously administrated 2 h before intratracheal LPS delivery. In such experimental settings, we observed that liraglutide pre-treatment significantly reduced LPS-induced acute lung injury, including the reduction in lung injury score, wet/dry lung weight ratio, immune cell counts, protein concentration in bronchoalveolar lavage fluid, and cell apoptosis in the lung. Those effects were highly associated with reduced pulmonary mRNA expression of genes that encode inflammatory chemokines and cytokines. Importantly, none of those "preventative" effects were observed in GLP-1R KO mice, highlighting the essential role of lung GLP-1R in mediating the effect of liraglutide in preventing lung injury[122]. Based on such "preventative" effect observed, we suggested that retrospective studies should be conducted in T2D subjects treated with or without GLP-1-based drugs, asking whether T2D patients are less vulnerable to ALI as well as chronic lung inflammatory injury after receiving GLP-1-based drug treatment[122,152,153].

The study conducted by Zhou et al.[122] has also revealed that liraglutide treatment attenuated LPS-induced pulmonary thioredoxin-interacting protein (TxNIP) over-expression, and such attenuation is also GLP-1R dependent. TxNIP is a member of the NLR family pyrin domain containing 3 (NLRP3) inflammasome component[154–156], a mediator of glucotoxicity[157,158], and a therapeutic target of T2D and other disorders[155,158–161]. In addition to the high glucose challenge, TxNIP level in pancreatic β-cells was also shown to be stimulated by dexamethasone and streptozotocin, an antibiotic utilized in generating the T1D rodent model. Importantly, the LPS challenge caused approximately 2.5-fold elevation in lung TxNIP levels in wild-type littermates, while in GLP-1R KO mice, lung TxNIP increased about 7-fold after the challenge with the same amount of LPS. Thus, lung GLP-1R itself may represent a native defense system. In contrast to the observation made by Balk-Moller and colleagues[139] in their COPD model, we did not see a stimulatory effect of liraglutide treatment on pulmonary *nppa* (which encodes ANP) expression. However, we observed that the LPS challenge led to a 3-fold activation on pulmonary *nppa* level. Whether such activation represents a protective or defensive response remains to be explored[122]. Fig. 3 summarizes our current understanding of pulmonary GLP-1R mediated protection in the ALI mouse model, in response to GLP-1RA treatment, involving the attenuation of the inflammasome component TxNIP. Further investigations are needed to determine the exact involvement of GLP-1R expressed in lung alveoli smooth muscle cells, epithelial cells, or both. GLP-1RAs may also exert their immune-regulatory functions on immune cells in the lung and the circulation.

6.4. *Combined effect of MSC and GLP-1-based drugs*

Mesenchymal stem cells (MSCs) are pluripotent adult stem cells[162]. They possess both self-renewal capacity and differentiation potential into several mesenchymal lineages including bones, cartilages, adipose tissues and tendons. MSCs can repair tissue injuries and prevent immune cell activation and proliferation, involving the secretion of growth factors and other macromolecules. MSC-based therapy may apply to lung injuries including ALI and radiation-induced lung injury, as well as other disorders[163–168].

More than 18 years ago, Ortiz and colleagues[162] demonstrated that when male mouse bone marrow-derived MSCs were intravenously administrated, they were able to home to the recipient female mouse lung in response to bleomycin-induced injury. Those MSCs were shown to adopt an epithelium-like phenotype, reducing both inflammation and collagen deposition[162]. Mechanistic exploration studies have then demonstrated that those MSCs can produce paracrine factors, such as IL-1 receptor antagonist (IL-1RA), IL-10, keratinocyte growth factor, and prostaglandin E2[167,169]. In LPS challenge induced ALI mouse model, Mei and colleagues demonstrated that bone-marrow derived MSCs with overexpressed angiopoietin 1 (Agn-1) further reduced the severity of lung injury[170]. Gupta and colleagues[171] then demonstrated that in the LPS-induced ALI mouse model, intrapulmonary delivery of bone marrow-derived MSCs 4 h after LPS-challenge was still able to improve survival rate and attenuate lung injury. During the last decade, functions of MSCs from various sources including bone marrows, adipose tissues, lung tissues, as well as human chorionic villi were also assessed in multiple disease models. For studies on additional paracrine factors released by MSCs and mechanistic exploration of MSC therapy in lung injuries, please see review articles elsewhere[172–175]. Below we will discuss a few recent studies that involve GLP-1 and GLP-1R.

In 2010, Sanz and colleagues[176] reported the detection of GLP-1R in hMSC, derived from bone marrow. They found that in hMSC, GLP-1 treatment stimulated cell proliferation and reduced cell apoptosis. Furthermore, GLP-1 treatment prevented cell differentiation into adipocytes, associated with the repression of peroxisome proliferator-activated receptor-γ, C/EBPβ, and lipoprotein lipase. A few follow-up studies then tested the effect of the combined use of MSC and GLP-1 in myocardial infarction[177–179]. MSCs with GLP-1 conditioned media were shown to possess anti-apoptotic effects on ischaemic human cardiomyocytes[179]. MSCs that were engineered to secrete a GLP-1 fusion protein were shown to possess therapeutic effects in myocardial infarction in a pig model[177,179].

More recently, attempts have also been made in testing the combined use of hMSC and liraglutide in ALI mouse model[180,181]. Last year, Yang and colleagues reported that LPS treatment could attenuate the proliferation of human chorionic villus-derived MSCs (hCMSCs), human bone marrow-derived MSCs (hBMSCs), and human adipose-derived MSCs (hAMSCs). In the LPS-induced ALI mouse model, liraglutide combined with MSCs showed a more significant therapeutic

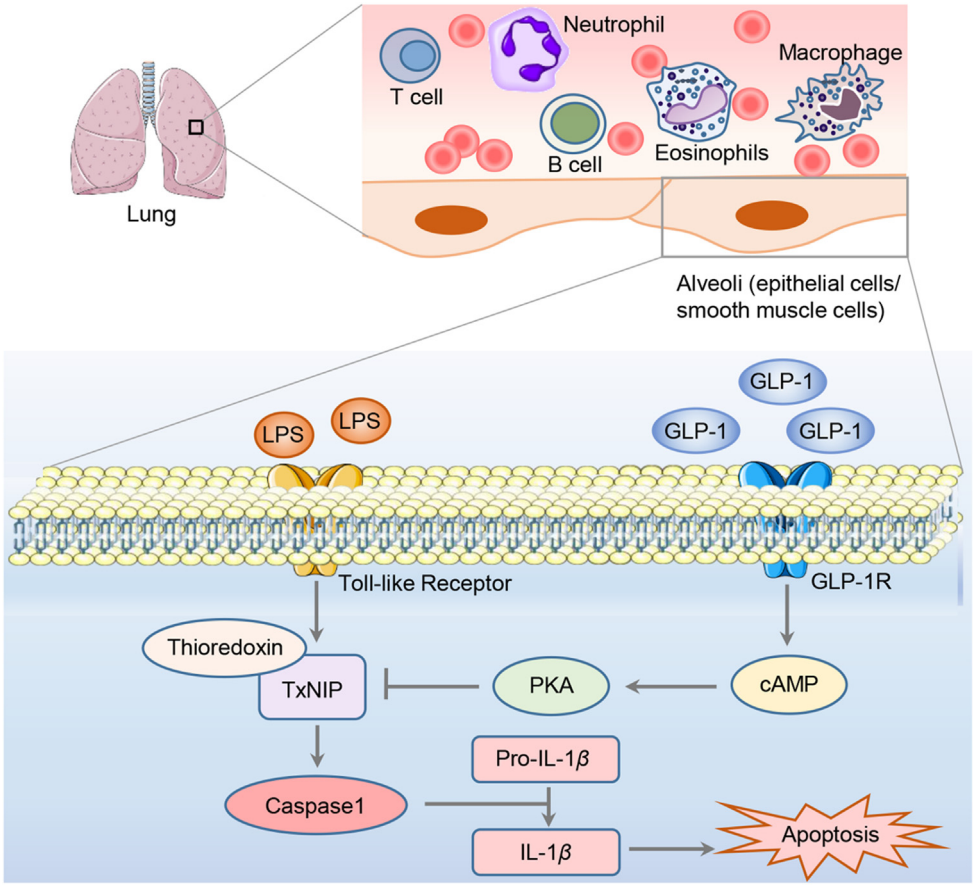

Figure 3 The effect of GLP-1RAs on LPS-induced ALI involving TxNIP reduction. GLP-1R is highly expressed in the lung, and likely includes alveoli epithelial cells and smooth muscle cells in the walls of arteries and arterioles. In addition, GLP-1RAs possess potent immunoregulatory functions in the lung, by reducing pro-inflammatory cytokines and increasing anti-inflammatory cytokines produced by immune cells in the lung as well as in the circulation. *Via* the Toll-like receptor, the LPS challenge induces overexpression of TxNIP, a member of the NLRP3 inflammasome. NLRP3 inflammasome activation leads to the activation of caspase 1 and over-production of active IL-1β, which initiates the apoptosis of alveolar epithelial cells and adhesion of immune cells (including monocyte-macrophages and neutrophils) to the capillary. Interaction between GLP-1RA and GLP-1R may lead to elevated intracellular cAMP level and the activation of PKA, which inhibits the expression of TxNIP.

effect[180]. Dose-dependent reduction effects of LPS on hCMSC proliferation and expression of GLP-1R, Ang-1 and FGF-10 were then demonstrated in another study conducted by the same group by Fang and colleagues[181]. Furthermore, the study by Fang and colleagues[181] demonstrated that liraglutide treatment dampened the above reductions, involving the cAMP/PKAc/β-catenin−TCF4 signaling pathway. The same study also reported that combined use of liraglutide and hCMSCs exhibited enhanced therapeutic efficacy than liraglutide alone in reducing lung injury in their mouse ALI model.

7. Summary

In this review, we have discussed both clinical and pre-clinical investigations on the anti-inflammatory and immune cell modulatory features of GLP-1 and GLP-1RAs. We commented that *in vivo* repressive effect of liraglutide on the expression of pro-inflammatory genes in PBMCs was not recaptured in the *in vitro* setting with direct liraglutide treatment[92]. Thus, it remains to be determined whether GLP-1RAs exert their anti-inflammatory and immune-modulatory functions *via* indirect mechanisms. This could involve a brain−peripheral tissue axis, or *via* interaction with a small population of immune cells, such as intestinal intraepithelial lymphocytes (IEL) or certain T lymphocytes ($\gamma\delta$ cells)[123,182]. Recent observations made by McLean et al.[123] also indicated that functions of GLP-1RAs in extra-pancreatic organs could be attributed to GLP-1R expressed in endothelial and hematopoietic cell lineages, in agreement with the detection of GLP-1R in smooth muscle cells in the walls of arteries and arterioles of monkey and human lungs[118].

GLP-1R is most abundantly expressed in mouse lung, demonstrated 25 years ago by Bullock et al.[114] with methods including RNase protection and *in situ* hybridization. As lung GLP-1R level elevated 4 times on the 1st day of birth, and elevated plasma GLP-1 level was observed in patients with systematic inflammation, it is likely that GLP-1 and lung GLP-1R represent a yet to be further explored defense system of our body. Observations made in a few clinical trials and retrospective studies have supported the beneficial effect of GLP-1RAs in asthma and lung injury. Detailed understanding of this defense system and properly utilizing the tools in regulating this system may lead to better treatment of chronic airway diseases and ALI.

The key inflammasome component TxNIP, a known therapeutic target of diabetes, is also among the major targets of GLP-1/GLP-1R signaling pathway activation in the lung. Lung TxNIP elevation can be stimulated by plasma glucose level elevation or the release of the stress hormone glucocorticoid[122], which is a recognized double-edged sword in ARDS treatment. Whether a moderate stimulation on lung TxNIP elevation in response to glucose and glucocorticoid elevation also represents a defensive response remains to be investigated. It is also worth determining whether TxNIP depletion brings beneficial or deleterious outcomes in mice with LPS or other inflammatory challenges.

Nanomedicine and hMSC-based cell therapy are the cutting-edge skills in translational medicine. GLP-1-SSM, a putative nanomedicine tool has already been tested in the ALI model, while combined hMSC and GLP-1-based drugs have been studied in a pig myocardial infarction model; and more recently, in the mouse ALI model. We anticipate seeing further applications of these two "therapies" in preclinical studies and clinical trials in near future.

The whole world has been undergoing the astonishing COVID-19 pandemic. There is literature debating whether GLP-1-based drugs may serve as a cure or adjuvant for COVID-19 treatment[152,153,183,184]. A recent meta-analysis conducted by Hariyanto and colleagues[185] covered nine studies with 19,660 patients of T2D who were infected by SARS-CoV-2. The study suggested that pre-administration of GLP-1-based drugs was associated with a reduced mortality rate[185]. Further retrospective studies and pre-clinical studies should be conducted to determine the therapeutic and preventative potential of GLP-1RAs on COVID-19 animal models, as our battle with such pandemic is likely a long journey.

To repurpose GLP-1RAs for future treatment of lung injury including asthma, COPD and others, attention should be made to their known and yet to be identified ADRs. As mentioned above, the most common ADR of GLP-1RAs is pancreatitis, demonstrated in certain animal model studies and clinical observations[82,83]. In conducting a recent clinical comparative study on asthma patients with GLP-1RAs versus other T2D drugs, Foer and colleagues[124] did not report the development of pancreatitis or other ADRs. This could be due to the relatively small sample size (n = 448 for patients treated with GLP-1RAs)[124]. In the most recent meta-analysis study conducted by Wei and colleagues[131], GLP-1RA utilizations were shown to increase trends in interstitial lung disease. None of the previous animal studies, including the one conducted by our team[122], have paid attention to the development of ADRs in the lung. Hence, future animal studies should be designed to verify whether the use of certain GLP-1RAs in the dosages for treating lung injury can cause different profiles of ADRs, or cause ADRs specifically in the lung.

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Author contributions

Juan Pang: conceptualization, investigation, and writing-original draft. Jia Nuo Feng: conceptualization and investigation. Wenhua Ling: writing-review and editing. Tianru Jin, writing-review and editing, supervision and funding acquisition.

Conflicts of interest

The authors declare no conflicts of interest.

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