FcRn for Biologics' Nasal Delivery

Subjects: Immunology

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It was registered on the international prospective register of systematic reviews PROSPERO, which helped in identifying articles that met the inclusion criteria. Clinical and preclinical studies involving FcRn and the nasal delivery of biologics were screened, and the risk of bias was assessed across studies using the Oral Health Assessment Tool (OHAT). Among the 12 studies finally included in this systematic review (out of the 758 studies screened), 11 demonstrated efficient transcytosis of biologics through the nasal epithelium.

Keywords: neonatal Fc receptor ; monoclonal antibody ; immunoglobulin G ; Fc-fusion protein ; transcytosis ; nasal route ; biologics

1. Introduction

FcRn is expressed in endothelial cells and haematopoietic cells, as well as in airway epithelial cells, including human nasal epithelial cells (HNECs) [1][2], alveolar epithelial cells [3][4][5][6] and porcine olfactory mucosa Moreover, FcRn impacts the biodistribution of IgG-like monoclonal antibodies (mAbs); it transports antibodies across the cellular barrier [7][8][9][3][4][10][11][12][13][14][15][16] and delivers IgGs across mucosal surfaces to confer protective immunity [17][18][19][20][21][22][23]. The role of FcRn in the transport of molecules in epithelial cells has been extensively analyzed [3][4][12][13][14][15][24][25].

Balin et al. demonstrated that horseradish peroxidase was detectable in the olfactory bulb of rodents and monkeys within 45 to 90 min after intranasal delivery [26], thus suggesting that axons of olfactory neurons may represent a pathway to the brain for protein drugs such as Abs [27]. In addition, the generation of micron-sized aerosol droplets can have tremendous effects on proteins. The shear stress occurring during aerosol formation can induce the formation of unfolded proteins or protein aggregation Therefore, it is of great importance to further study the behaviour of biologics after their intranasal delivery in various preclinical models.

The current review will be the first to specifically discuss the use of FcRn in the transcytosis of biologics after intranasal delivery. This review is a prerequisite to further developments of the nasal route for the administration of biologics, which can considerably improve patients' quality of life, especially in those who undergo multiple lifelong intravenous and/or subcutaneous injections for the treatment of chronic diseases.

To address this question, we hereby provide a systematic review of the implications of FcRn in the transport of biologics after intranasal delivery. Systematic reviews tend to be more comprehensive and less biased than other types of literature reviews. Therefore, systematic reviews have become an increasingly central pillar of basic science and represent a substantive contribution to knowledge [28], especially as the number of publications increases overtime. To increase the transparency of our review, it was registered in the international prospective register of systematic reviews PROSPERO (CRD42021236019,https://www.crd.york.ac.uk/prospero/#recordDetails, accessed on 8 March 2021), following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [29][30].

In this review, the recorded outcomes were the effects of biologics after nasal administration following FcRn-mediated transcytosis across the epithelium. In vivo studies were more specifically investigated for pharmacokinetic (absorption, distribution, metabolism, elimination, transcytosis efficiency, time and plateau effect), pharmacodynamic (dose-effect response and drug activity) and toxicity (cellular viability, epithelial permeability, cell differentiation, ciliary analysis) outcomes. In vitro studies were investigated for pharmacokinetic (transcytosis efficiency, time effect, plateau effect and repeated administration effect), pharmacodynamic (dose-effect response and drug activity) and toxicity (cellular viability, epithelial permeability, cell differentiation and ciliary analysis) outcomes. Studies reporting any other types of outcome were excluded.

2. Development and Findings

Among these, an in vitro HNEC model was used in one study [1], an in vivo murine model was used in six studies [16][17][18][19][31][32], an ex vivo porcine model was used in 3 studies [33][34][35] and an ex vivo human model was selected in one study [2].**Table 1** describes the general characteristics of the included studies. Additional details about the in vivo and in vitro studies are described in theSupplementary Materials in Supplmentary Files S3 and S4, respectively.

Table 1. Characteristics of the included studies.

First Author Date	Design	Species	Intervention	Application	Biotherapy Tested	Dose Administered	Concentration	Formulation	Primary Outcome	Blocking FcRn
Rawool [27] * 2008, India	In vivo	Mice FcRn WT	Assess FcRn-mediated mucosal vaccine delivery with an Ft model	Vaccine delivery against Ft	mAb-iFT (fusion of iFT and IgG)	2 × 10^7 iFT/mAB-iFT/F(ab')2-iFT organisms	2 × 10^7 CFU/20 µL	PBS	FcRn-targeted immunogen enhanced immunogen-specific IgA production and protection against subsequent infection. It is a highly effective vaccination strategy against Ft.	Yes
Lu [28] * 2011, USA	In vivo	Mice FcRn WT and KO	Assess ability of FcRn to deliver Gag-Fc fusion protein in a HIV model	Vaccine delivery against VIH	HIV Gag-Fc fusion protein	20 µg	1 mg/mL	PBS	FcRn-targeted mucosal immunization was effective at inducing Gag specific Ab responses in serum or mucosal secretions, and high levels of stable immune memory were obtained.	Yes
Ye [29] * 2011, USA	In vivo	Mice FcRn WT and KO	Assess FcRn-mediated mucosal vaccine delivery with an HSV-2 model	Vaccine delivery against HSV	gD-Fc/wt (HSV-2 gD fused with an IgG Fc fragment)	20 µg	1 mg/mL	PBS	Intranasal immunization with an engineered fused protein resulted in complete protection of wild-type, but not FcRn KO, mice that were intravaginally challenged with virulent HSV-2.	Yes
Bitsatksis [36] * 2015, USA	In vivo	Mice FcRn WT	Assess FcRn-mediated mucosal vaccine delivery with an Ft model	Vaccine delivery against Ft	mAb-iFT IC (fusion of iFT and IgG)	2 × 10^7 CFU mAb-iFT IC organisms	NA	PNS	FcRn targeting increases the frequency and activation status of DCs in the lungs of immunized mice and mediates the generation of Ft-specific effector memory $CD4^+$ T cells.	No

First Author Date	Design	Species	Intervention	Application	Biotherapy Tested	Dose Administered	Concentration	Formulation	Primary Outcome	Blocking FcRn
Kumar [32] 2018, USA	In vivo	Rats FcRn WT	Assess CNS IgG distribution after intranasal administration	Intracerebral mAb delivery	Radiolabeled Ab: [125I]-IgG	50 µg to 2.5 mg for [125I]-IgG	1-20-50 g/mL	PBS	[125I]-IgG concentrations in the CNS was higher following intranasal delivery compared to intra-arterial delivery for doses presenting similar endpoint blood concentrations.	No
Bern [25] 2020, Biorxiv	In vivo	Mice FcRn WT and hFcRn	Assess ability of FcRn for delivery of albumin based biologics	Haemophilia	Fluorescently labeled Ab: AF488-IgG	0.7 mg for AF488-IgG	20 mg/mL	PBS	Nasal FcRn enabled efficient transcytosis of albumin fusion proteins.	Yes
Röhm [33] 2017, Germany	In vitro	RPMI cells	Assess IgG quality after transcytosis	Enhancement of IgG permeation rate	Biotinylated albumin (WT, KAHQ or QMP) IgG1 and scFv-Ab	10 to 30 µg for a 10 g mouse	20 µL à la dose de 1 mg/kg to 3.2 mg/kg	PBS	Aggregation of native IgG was reduced and transepithelial permeation, D-rate was enhanced up to 2.8-fold with the used of specific formulation (F1) for intranasal aerosol-cell delivery.	No
Bequignon [8] 2019, France	In vitro	HNEC	Assess mAb transcytosis via FcRn	Anti-cancer immunotherapy	mAb HIRMab 83-14	4 mg	1.4 mg/mL for Fab; 4 mg/mL for IgG	L-arginine, HBC, PS20, sorbitol, trehalose	Transepithelial passage of therapeutic mAb was dose-dependent.	No
Ladel [16] * 2019, Germany	In vitro	OEPC and RPMI cells	Assess permeation rates, hIgGs through the nasal mucosae	Anti-cancer immunotherapy	WT pIgG, WT hIgG and DG hIgG (biosimilar of Bevacizumab)	50 µg	1.5 mg/mL	PBS	hIgG permeation was faster than pIgGs over the models in OEPC ALI cultures, but it converges from 8 h to 48 h. DG hIgG showed a higher permeation rate than WT hIgG in the RPMI ALI model. hIgG permeation was 14 times higher after 5 h than the one of pIgG. The permeation rate of DG hIgG over WT hIgG did not show differences.	No
Ex vivo	Porcine olfactory mucosa									

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First Author [38] * Date	Design	Species	Intervention	Application	Biotherapy Tested	Dose Administered	Concentration	Formulation	Primary Outcome	Blocking FcRn
Samson 2012, France	Ex vivo	Porcine nasal mucosa	Assess the transport of bevacizumab through porcine nasal mucosa	Rendu-Osler Disease	Bevacizumab	500 µg	25 mg/mL	trehalose, sodium phosphate, RSA20, water	Total recovery of intranasally-delivered bevacizumab was 83% of the initial dose, which 3% localized at the mucosal surface and 11% that had gone through the mucosa	No
Heidl 2015 Austria	Ex vivo	HNEC	Assess FcRn expression and localization in HNEC	Intranasal administration of mAb	None	NA	NA	NA	FcRn was detected in ciliated and basal cells of the basal epithelium as well as in vascular endothelial cells and in gland tissue.	No
Ladel 2018 Germany	Ex vivo	Porcine olfactory mucosa	Assess ability of FcRn to transport IgGs through the nasal lamina propria	Anti-cancer immunotherapy	Elec-trofused hIgG (Biosimilar of Bevacizumab)	5 µg	8 mg/mL	PBS	FcRn is expressed in the olfactory mucosa and enabled the apical uptake of allogeni, and xenogenic IgG in a species-specific manner.	No

The risk of bias was assessed using the OHAT, even though we analyzed in vivo and in vitro studies (**Table 2**). The majority of studies using animal models [33][34][16][17][18][19][35][31][32] investigated the ability of FcRn to enhance transcytosis (n= 8, [33][34][16][17][18][19][31][32]), while only two investigated the epithelial toxicity of this procedure (n= 2, [16][35]). In studies performed on human cells [1][2][34][25], three considered transcytosis efficiency [1][34][25] and one considered transcytosis toxicity [1].

Table 2. Risk of bias summary using OHAT.

Study	Selection Bias	Performance Bias	Attrition Bias	Detection Bias	Selective Reporting	Other Bias
Rawool [27], 2008	--	--	+	+	++	+
Lu [28], 2011	--	--	+	+	++	+
Ye [29], 2011	--	--	++	++	++	+
Bitsaktsis [32], 2015	--	--	+	+	++	+
Kumar [37] 2018	--	--	+	+	+	-
Bern [26] 2020	--	--	++	+	++	+
Röhm [35] 2017	--	--	++	+	++	+
Bequignon [31] 2016	--	--	++	+	++	+
Samson [38] 2012	--	--	+	+	++	+
Heidl [10] 2015	--	--	+	-	+	+
Ladel [15] 2018	--	--	+	+	++	+

Study	Selection Bias	Performance Bias	Attrition Bias	Detection Bias	Selective Reporting	Other Bias
Ladel [16] 2019	+	+	+	+	++	+

(--) Definitely high risk; (-) probably high risk; (+) probably low risk; (++) definitely low risk.

Among the 12 studies included in this systematic review, six first confirmed the expression of FcRn in their different models chosen to assess biologic-transcytosis across airway epithelial cells [1][2][33][34][18][19]. The expression pattern of FcRn in larger animal models and humans was studied using FcRn mRNA or FcRn protein measurements in cell cultures derived from in vivo tissue extraction [1][2][33][34]. In humans, Heidl et al. was the first to confirm the localization of the FcRn α-chain in ciliated cells of the epithelium in blood vessels and subepithelial glands using an affinity-purified antibody against the cytoplasmic tail of the FcRn α-chain in nasal tissue sections [2]. They showed that the steady-state distribution of FcRn was predominantly observed at the basolateral side of ciliated epithelial cells and gland cells.

Bequignon et al. also demonstrated the expression of FcRn in HNECs. High expression of FcRn was found in the cytosol of ciliated, mucus and basal cells [1]. Interestingly, FcRn expression varies depending on the degree of cell differentiation [1].

Some of them used cell lines and mouse models that were already known to express FcRn [16][17][32], or the aim of the study was different [25]. In contrast, Samson et al. suggested that FcRn expression might explain bevacizumab permeability through the nasal epithelium, as it can bind IgG. However, FcRn expression in the nasal mucosa was not studied in this paper, and it had not been reported previously at that time [35]. More recently, Kumar et al. showed an AF488-IgG signal in the underlying lamina propria on high magnification confocal imaging of sections from the olfactory epithelium, but did not look at FcRn expression [31].

Taken together, the combined expression of FcRn in the endothelium, glands and ciliated nasal epithelial and basal cells, as well as the localization of IgG in these tissues, suggests that FcRn could play a role in IgG transport in the nasal mucosa [2]. Among the 12 studies included in this systematic review, 11 demonstrated efficient transcytosis of biologics through the nasal epithelium [1][33][34][16][17][18][19][25][35][31][32]. The biologics that were studied were highly heterogeneous across the studies, as were their doses (**Table 1**). Most studies focused on immunization following intranasal delivery of biologics [17][18][19][32], whereas others aimed to characterize FcRn-mediated transport of biologics and their subsequent biodistribution [33][34][16][25][35][31]

Globally, all studies about immunization reported that intranasal delivery of biologics was efficient for inducing an appropriate immune response directed against the pathogen of interest. Rawool et al. demonstrated that (i) the FcR-targeted immunogen enhances immunogen-specific IgA production and protection against subsequent Ft infection in an IgA-dependent manner; (ii) (IFN-γ)-secreting, effector memory CD4+T cells during infection, thus further elucidating the immunological mechanisms involved in enhanced immune protection utilizing this novel mucosal vaccine platform. Furthermore, 100% of the C57BL/6 mice that were immunized with mAb-iFT ICs survived the Ft LVS challenge, while immunization with iFT alone provided only 50% protection.

Gag-Fc fusion protein (fusion of p24 protein from HIV Gag with IgG heavy chain) was used for the HIV vaccine challenge model [18], and gD-Fc/wt (HSV-2 glycoprotein D fused with an IgG Fc fragment) was used to test the effect of glycosylation in the HSV vaccine challenge model [19]. Moreover, intranasal immunization induced an immune response sufficiently potent to protect mice from infection with HIV after the intravaginal challenge. Finally, FcRn-targeted immunization induced strong antibody and cellular immune responses to HIV Gag at mucosal and systemic sites [18]. The authors identified that FcRn-targeted mucosal immunization differs notably between WT and FcRn KO mice or between gD-Fc/wt and gD-Fc/mut immunized mice in terms of mucosal and systemic immune responses, cytokine expression profiles, the maintenance of T and B cell memory and long-lived bone marrow plasma cells, and the resistance to infection.

FcRn-mediated transcytosis after intranasal delivery was also studied in applications other than immunization. Significantly higher [125I]-IgG concentrations were found in the CNS after intranasal delivery than after intra-arterial delivery for doses producing similar endpoint blood concentrations. Importantly, the concentration of [125I]-IgG in the CNS significantly increased in a dose-dependent manner following intranasal administration of increasing doses, from the picomolar range after administration of small doses (50 µg) up to the nanomolar range after administration of higher doses (1 mg and 2.5 mg). The authors showed that it might be feasible to achieve therapeutic levels of IgG in the CNS, especially when delivering high doses in the nose, and they provided insights about the nose-to-brain pathways the Abs took after intranasal delivery.

Bequignon et al. used an in vitro model of HNEC primary culture to study infliximab transcytosis. Infliximab is an IgG1 therapeutic mAb mostly used for the treatment of autoimmune disorders. The apical-to-basal experiment demonstrated effective and dose-dependent infliximab transfer across the HNECs on both day 7 and day 21 of cell differentiation.

Samson et al. demonstrated the transmucosal transport and bioavailability of bevacizumab [35]. Bevacizumab is an IgG1 therapeutic mAb directed against the vascular endothelial growth factor, and is mostly used for the treatment of some cancers. After intranasal delivery of bevacizumab in porcine olfactory mucosa, the total recovery of bevacizumab throughout the 2.5 h experiment was 83%. Histopathological analyses revealed that bevacizumab was distributed at the mucosal surface (53%), intracellularly (19%) and throughout the nasal mucosa (11%).

in 2018 demonstrated the potential FcRn-mediated transport of epithelial and basal cells towards the lamina propria, facilitating the apical uptake of allogenic and, in lesser amounts, xenogenic IgG [33]. A year later, they also demonstrated that only traces of porcine IgGs (pIgGs) could be recovered at the basolateral compartment in ex vivo olfactory tissue, while human IgGs (hIgGs) reached far higher levels [34]. They also demonstrated comparable permeation rates for human and porcine IgG in primary cells from porcine olfactory epithelium (OEPC), which displayed the highest expression of FcRn. Nevertheless, at early time points in OEPC ALI cultures, the permeation of the hIgGs was significantly faster than that of the pIgGs [34].

Röhm et al. used a nebulization platform to evaluate different formulations of therapeutics containing biologics, and five different excipients (i.e., arginine, cyclodextrin, polysorbate, sorbitol, and trehalose) with three concentration levels were tested for each. Three different formulations (F1, F2, and F3) containing different concentrations of trehalose, sorbitol, arginine, polysorbate and cyclodextrin were tested to minimize aerosolization-induced protein aggregation and maximize permeation through an in vitro epithelial cell barrier [25]. After a 90 min or 240 min incubation time, protein concentrations were determined in the abluminal media by either ELISA or fluorescence spectroscopy. F1 reduced the aggregation of native Fab and IgG relative to vehicle by up to 50% and enhanced the transepithelial permeation rate up to 2.8-fold in comparison to the vehicle.

Interestingly, the permeation rate and flux through OEPC cells were significantly higher than the permeation rate through RPMI cells for both DG hIgGs and hIgGs, thus suggesting that transcytosis efficiency also depends on cell type. Finally, species-dependent binding to IgG receptors or species-dependent IgG trafficking and/or degradation pathways influenced the permeation of IgGs through the olfactory mucosa, and it was shown that hIgGs reached higher levels than pIgGs. The authors concluded that the permeation behaviour of DG and WT hIgG displayed similar patterns in in vitro and ex vivo models [34].

Globally, all of these studies found that the intranasal delivery of biologics was generally safe and did not induce specific side effects. Bequignon et al. assessed the toxicity of infliximab intranasal delivery using two methods. In addition, Samson et al. performed histological analyses after intranasal delivery of bevacizumab. There was no evidence of histological effects, confirming that nasal delivery of bevacizumab was harmless [35].

Drawing strong conclusions about intranasal delivery of biologics may be premature owing to the small number of studies included and the heterogeneity in the models used, as well as biologics tested and the reported results.

The doses of biologics tested varied a lot among the studies, even for similar study designs: (i) for in vivo studies from 1 µg [32] to 2.5 mg [31], considering 10 to 20 g per mouse; (ii) for in vitro studies from 12.5 ng [1] to 4 mg [25]; and (iii) for ex vivo studies from 8 µg [33] to 500 µg [35]. None of the studies tried to identify a saturation kinetic.

The time for collecting data varied widely across studies. In vivo assessment of the efficiency and/or toxicity of biologics generally used a 1 month follow-up in the majority of the studies, but some had a follow-up of over 6 months after intranasal delivery [19]. The time of incubation used in the in vitro and ex vivo studies ranged from 30 min [31] to 6 days [33] [34][16][35]. During these periods, samplings were performed regularly.

The nasal epithelial cells used for transcytosis assays of biologics were either primary cultures of HNECs [1][2], human-derived RPMI cells [34][25] or porcine-derived OEPCs [34]. In vivo studies were performed on mice in five studies [16][17][18] [19][32] and on rats in one study [31]. also specifically studied a human FcRn transgenic mouse model that lacked expression of both mouse FcRn and mouse albumin [B6.Cg-Albem12 Some studies used both in vitro and in vivo models to study transcytosis and interactions with FcRn, but the cells used in vitro were not airway epithelial nasal cells; thus, their results are not described in this review [16][18][19].

3. Conclusions and Future Perspectives

This systematic review confirmed the expression of FcRn in nasal airway and olfactory epithelium, as well as its potential role in IgG transcytosis across airway-polarized cell layers. Choosing intranasal delivery over oral delivery for biologics may be a great non-invasive method to induce systemic effects, as it would avoid biologics' degradation by the extremely low pH and the presence of digestive enzymes in the gastrointestinal tract [39]. In addition, intranasal delivery may represent a comfortable and non- to minimally invasive way of self-administration [40].

However, further developments are required for better understanding the pharmacokinetic and pharmacodynamics properties of FcRn-mediated IgG-transcytosis through mucosal tissue. Further, variations in these characteristics across cell types, such as olfactory cells vs. ciliated cells, are unexplored. Altogether, the characterization of intranasal delivery of biologics and the role of FcRn in this setting are expected to increase in the near future. This would be helpful for the clinical development of the intranasal delivery of various biologics, including mAbs or modified Fc-fusion proteins, for the treatment of a wide range of diseases.