Journal of Autoimmunity 122 (2021) 102674

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Intestinal permeability in type 1 diabetes: An updated comprehensive overview

Mia Øgaard Mønsted *, Nora Dakini Falck, Kristina Pedersen, Karsten Buschard, Laurits Juulskov Holm, Martin Haupt-Jorgensen

The Bartholin Institute, Department of Pathology, Rigshospitalet, Copenhagen N, Denmark

ARTICLE INFO

Keywords:
Type 1 diabetes
Intestinal permeability
Barrier integrity
Leaky gut
Paracellular permeability
Transcellular permeability

ABSTRACT

The etiopathogenesis of the autoimmune disease type 1 diabetes (T1D) is still largely unknown, however, both genetic and environmental factors contribute to the development of the disease. A major contact surface for environmental factors is the gastrointestinal (GI) tract, where barrier defects in T1D likely cause diabetogenic antigens to enter the body tissues, contributing to beta-cell autoimmunity. Human and animal research imply that increased intestinal permeability is an important disease determinant, although the underlying methodologies, interpretations and conclusions are diverse. In this review, an updated comprehensive overview on intestinal permeability in patients with T1D and animal models of T1D is provided in the categories: *in vivo* permeability, *ex vivo* permeability, zonulin, molecular permeability and blood markers. Across categories, there is consistency pointing towards increased intestinal permeability in T1D. In animal models of T1D, the intestinal permeability varies with age and strains implying a need for careful selection of method and experimental setup. Furthermore, dietary interventions that affect diabetes incidence in animal models does also impact the intestinal permeability, suggesting an association between increased intestinal permeability and T1D development.

1. Introduction

Type 1 diabetes (T1D) is an autoimmune T cell-mediated disease, in which the pancreatic beta-cell mass is partially or completely destroyed, leading to hypoinsulinemia and hyperglycemia. Genetic predisposition is an important part of the pathogenesis and the majority of T1D patients have the haplotypes HLA-DR3-DQ2 and/or HLA-DR4-DQ8 [1]*. Genetic drift alone cannot explain the global increase in the disease incidences over the past few decades [2–4]*. Additionally, the concordance rates for T1D among monozygotic twins are not identical [5–7]*, which underlines that environmental factors are key components of the T1D pathogenesis.

T1D is associated with microbiota changes [8,9*], enterovirus infections [10–13]*, increased intestinal permeability [14–17] and is moreover strongly correlated with celiac disease [18,19]*. Furthermore, a proinflammatory environment in the small intestinal mucosa has been observed in T1D patients, specifically with increased abundance of IFN-γ-, IL-1α- and IL-4-producing cells [20]* and reduced abundance of FoxP3+ T regulatory cells (Tregs) [21]*. This suggests that the tolerogenic environment, which is maintained by the mucosal immune system and acts against food and bacterial antigens, is skewed in T1D. Also, lymphocytes targeting beta-cell specific antigens from T1D patients were found to express the gut homing receptor α4β7 [22]*. This receptor was found on the majority of lymphocytes in the pancreatic islet-infiltrate of pre-diabetic Non-Obese Diabetic (NOD) mice besides an aberrant homing behavior, where most α4β7 positive lymphocytes were located to non-mucosal tissues, including pancreatic lymph nodes (PLN) [23]*. This implies that lymphocytes infiltrating the pancreatic islets have been primed by the milieu in the gut before migrating to the pancreas. Thus, increased intestinal permeability could lead to an excess passage of diabetogenic antigens over the epithelial barrier, skewing the mucosal immune response towards inflammation and activating auto-reactive T cells. Taken together, the GI is likely central in the pathogenesis of T1D.

This review aims to provide an interpretable schematic overview of results from different methods used to assess intestinal permeability in T1D patients. Included is also evidence from animal models with autoimmune diabetes; namely the widely used NOD mouse and the Biobreeding Diabetes-Prone (BBDP) rat (Fig. 1 depicts relevant characteristics of the two animal models) [24]*.

* Corresponding author. The Bartholin Institute, Biocenter Copenhagen, Ole Maaløes Vej 5, 2200, Copenhagen N, Denmark.
E-mail address: mia.oegaard.moensted@regionh.dk (M.Ø. Mønsted).

https://doi.org/10.1016/j.jaut.2021.102674
Received 5 March 2021; Received in revised form 29 May 2021; Accepted 29 May 2021
Available online 25 June 2021

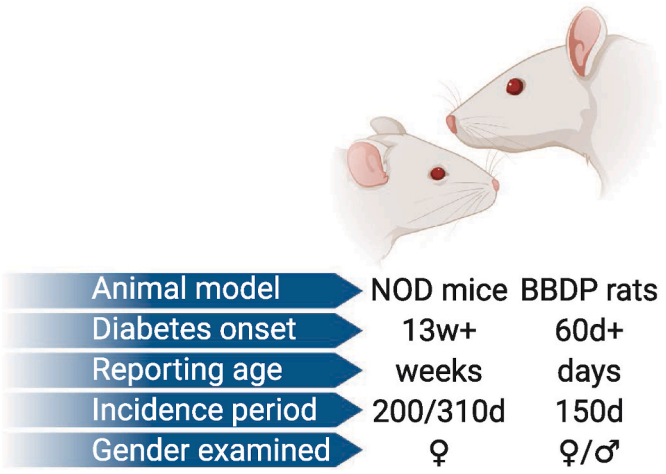

Fig. 1. NOD mice and BBDP rats in type 1 diabetes research, differences in characteristics. The development of autoimmune diabetes in NOD mice and BBDP rats share many characteristics with human T1D development including development of insulitis, islet autoantibody and susceptibility to environmental factors [24]*. Hyperglycemia appears in NOD mice around 13 weeks of age and in BBDP rats around 60 days. In the literature, the age of NOD mice is typically indicated in weeks, whereas the age of BBDP rats is given in days. In NOD mice, the standard period for measuring diabetes incidence is 200 or 310 days compared to 150 days in BBDP rats. Female NOD mice are preferred for experiments over male NOD mice, as a higher diabetes incidence is typically reached in female NOD mice. In BBDP rats, no gender difference is present in relation to diabetes incidence. BBDP=Biobreeding Diabetes-Prone, d = days, NOD=Non-Obese Diabetic, T1D = Type 1 diabetes, w = weeks.

To find relevant articles, PubMed was searched with the keywords (final search date: May 19th, 2021):

(intestinal permeability OR intestinal barrier dysfunction OR gut permeability OR leaky gut OR gastrointestinal permeability OR intestinal integrity OR gut barrier dysfunction)
AND
("Diabetes Mellitus, Type 1" [Mesh] OR T1D OR insulin dependent diabetes OR juvenile diabetes OR juvenile-onset diabetes OR type 1 diabetes)

Supplementary articles that are cited in this review but not identified during the search are marked with an asterix (*) throughout the review. Articles identified in the search were examined for methods in the following categories: *in vivo* permeability, *ex vivo* permeability, zonulin, molecular permeability and blood markers. In each category two tables were constructed, one summarizing methods and results from human and animal model experiments while the other one summarizes methods and results from animal models either genetically modified or from intervention studies. Methods within each category are separated in rows to clarify which methods that are relevant to use in T1D research, while also highlighting the importance of age and potential association with diabetes incidence. Furthermore, the tables are divided into rows of either increased (↑), unchanged (↔) or decreased (↓) permeability or signs of intestinal damage. This provides an accessible way to interpret the relation between the observed experimental change and intestinal permeability. We believe that this provides a comprehensive and intelligible overview of current knowledge, which will be helpful in planning experiments for the assessment of intestinal permeability in T1D and for data interpretation.

2. *In vivo* permeability

A common method for assessment of small intestinal permeability in humans is measurement of the concentration of non-metabolizable sugars, radioisotopes or polyethylene glycols (PEG) in the urine following ingestion. The outcome is often noted as the ratio of a paracellular and a transcellular marker. Trans- and paracellular permeability refers to transport through and between enterocytes, respectively. Paracellular markers that are discussed in this review include lactulose (LA), cellobiose, ^{15}Cr-EDTA and PEG-4000, whereas transcellular markers include mannitol (MA) and rhamnose (Fig. 2). This method is also applicable in rodents, but a more common method for assessing paracellular permeability of the small intestine, especially in mice, is performed by oral administration of body weight-adjusted fluorescein isothiocyanate (FITC)-dextran 4000 (FD4) and subsequent measurement of the level in blood.

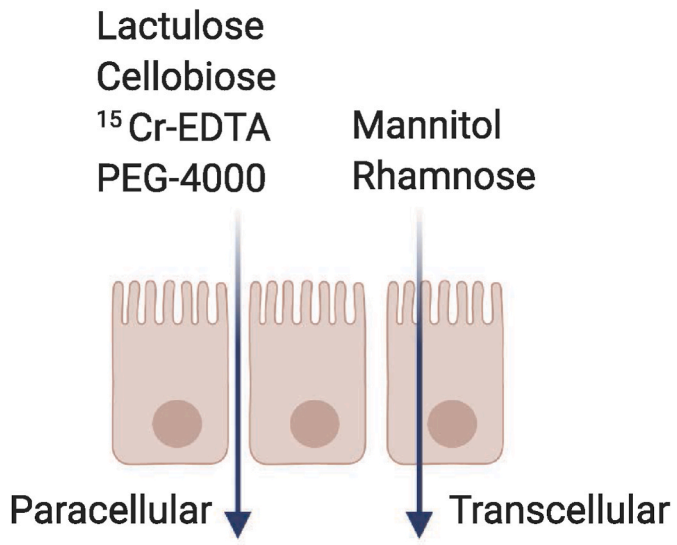

Fig. 2. Transcellular and paracellular markers for in vivo assessment of intestinal permeability. The transcellular permeability markers mannitol and rhamnose are absorbed through the enterocytes. Paracellular permeability markers, lactulose, cellobiose, ^{15}Cr-EDTA and PEG-4000, passes the epithelial barrier between the enterocytes and are thus subjected to the semipermeable tight junction barrier. PEG-4000 = polyethylene glycol-4000.

2.1. Humans and animal models

The majority of articles included in this review demonstrate an increased paracellular permeability in the small intestine of T1D patients compared to healthy controls [14–17,25] (Table 1). Interestingly, pre-onset individuals also show increased paracellular permeability [14,26]. Only a few studies do not find an increase in the small intestinal paracellular permeability in T1D patients vs controls [27,28]. The discrepancies between findings are likely caused by differences in size of study groups, analytical methodology or country that the study was performed in, since exposure to environmental agents differ between countries [29]* just as the incidence of T1D [3]*. This could also merely reflect the multifactorial nature of T1D development. Still, an increased small intestinal paracellular permeability assessed *in vivo* seems quite established in human T1D. Results on the transcellular permeability in T1D patients are more variable. Conflicting articles have reported increased [27,28], decreased [16,28] or unchanged transcellular permeability [14,15,17] in T1D patients compared to controls (Table 1). These results are largely overlooked in the T1D literature, where T1D is cited as a disease with increased paracellular permeability. However, it appears that alterations in the transcellular permeability in T1D patients are also common.

The paracellular/transcellular permeability ratio is often reported as increased in T1D patients but also in pre-onset individuals [14–17,26,30]. Especially the fact that T1D progressors with 2 ≥ islet autoantibodies (IA) have increased intestinal permeability compared to non-progressors with 2 ≥ IA [30] (Table 1) suggests an involvement of

Table 1
In vivo permeability in humans and animal models. The table depicts whether paracellular- (para), transcellular (trans) permeability (perm) or the ratio between them was changed (↑ increased, ↔ unchanged or ↓ decreased), which method was used to identify the change (↑ significantly increased, ↔ unchanged, ↓ significantly decreased), what organism was used and which groups that were compared and at what age. "Tissue" refers to the intestinal segment that the *in vivo* method examines, which is measured in either urine* or blood**. B6=C57BL/6, BBDP=Biobreeding Diabetes-Prone, BBDR=Biobreeding Diabetes-Resistant, FD4 = fluorescein isothiocyanate (FITC)-dextran 4000, IA = islet autoantibodies, LA = lactulose, MA = mannitol, NOD=Non-Obese Diabetic, NOR=Non-Obese Diabetes-Resistant, T1D = Type 1 Diabetes.

		Method	Organism	Group comparison (age)	Tissue	Ref.
Para perm	↑	↑ LA	Human	Pre-clinical, new-onset and long-term T1D vs controls	Small intestine*	[14]
				T1D vs relatives and controls	Small intestine*	[15]
				T1D vs controls	Small intestine*	[16]
				T1D (DQB1*0201 pos) vs T1D (DQB1*0201 neg)	Small intestine*	[28]
				T1D vs controls	Small intestine*	[17]
				IA-positive vs controls	Small intestine*	[26]
			Rat	BBDR and BBDP vs Wistar (42, 49, 53 days)	Small intestine*	[32]
		↑ ^{15}Cr EDTA	Human	T1D vs controls	Small intestine*	[25]
		↑ FD4	Mouse	NOD vs NOR (4–6 weeks)	Small intestine**	[35]
				NOD vs NOR and BALB/c (10–12 weeks)	Small intestine**	[35]
				NOD vs BALB/c and B6 (16–18 weeks)	Small intestine**	[35]
				NOD at diabetes onset vs NOD (10 weeks)	Small intestine**	[36]
				NOD at diabetes onset vs NOD (10 weeks)	Small intestine**	[37]
				NOD (4 weeks) vs NOD (10 weeks)	Small intestine**	[37]
				NOD vs B6 and NOR (12 weeks)	Small intestine**	[34]
		↑ Sucrose	Rat	BBDP vs BBDR (50–110 days)	Stomach, duodenum*	[33]
	↔	↔ LA	Human	T1D vs controls	Small intestine*	[28]
		↔ Cellobiose	Human	T1D vs children controls and adult controls	Small intestine*	[27]
		↔ FD4	Mouse	NOD vs B6 and BALB/c (4–6 weeks)	Small intestine**	[35]
				NOD vs B6 (10–12 weeks)	Small intestine**	[35]
				NOD at diabetes onset vs NOD (6, 12 weeks)	Small intestine**	[37]
				NOD (12 weeks) vs NOD (6, 10 weeks)	Small intestine**	[37]
		↔ Sucrose	Rat	BBDP vs BBDR (25–~43 days)	Stomach/duodenum*	[33]
		↔ Sucralose	Rat	BBDP vs BBDR (25–110 days)	Colon*	[33]
Trans perm	↑	↑ MA	Human	T1D (DQB1*0201 pos) vs T1D (DQB1*0201 neg)	Small intestine*	[28]
				T1D vs children controls and adult controls	Small intestine*	[27]
	↔	↔ MA	Human	Pre-clinical, new-onset and long-term T1D vs controls	Small intestine*	[14]
				T1D vs relatives and controls	Small intestine*	[15]
				T1D vs controls	Small intestine*	[17]
				IA-positive vs controls	Small intestine*	[26]
			Rat	BBDR and BBDP vs Wistar (42, 49, 53 days)	Small intestine*	[32]
	↓	↓ MA	Human	T1D vs controls	Small intestine*	[16]
				T1D vs controls	Small intestine*	[28]
Para/trans ratio	↑	↑ LA/MA	Human	Pre-clinical, new-onset and long-term T1D vs controls	Small intestine*	[14]
				T1D vs relatives and controls	Small intestine*	[15]
				T1D vs controls	Small intestine*	[16]
				T1D vs controls	Small intestine*	[17]
				IA-positive vs controls	Small intestine*	[26]
			Rat	BBDP vs BBDR (50–75 days)	Small intestine*	[33]
				BBDR and BBDP vs Wistar (42, 49, 53 days)	Small intestine*	[32]
		↑ LA/Rhamnose	Human	T1D vs T1D sibling and controls	Small intestine*	[30]
				2 ≥ IA progressors vs non-progressors, T1D siblings and controls	Small intestine*	[30]
	↔	↔ LA/MA	Human	T1D vs controls	Small intestine*	[28]
				T1D (DQB1*0201 pos) vs T1D (DQB1*0201 neg)	Small intestine*	[28]
			Rat	BBDP vs BBDR (25–~43 days)	Small intestine*	[33]
		↔ LA/Rhamnose	Human	2 ≥ IA and 1 ≥ IA vs controls	Small intestine*	[30]
		↔ Cellobiose/MA	Human	T1D vs children controls and adult controls	Small intestine*	[27]

increased small intestinal permeability in the pathogenesis of T1D as the number of serum IAs markedly increase the risk of progressing to overt T1D [31]*. Nonetheless, some studies report an unchanged ratio in both autoantibody-positive individuals and T1D patients compared to controls [27,28,30]. An increased ratio is often interpreted as an increase in paracellular permeability, however, the transcellular permeability is, as already mentioned, not necessarily constant [16,27,28] (Table 1), which causes difficulties interpreting solely the ratio. Accordingly, it is important to report the results from both permeability measurements to make the correct conclusion. This is emphasized in the study by Kuitunen et al., as they observed an unchanged LA/MA ratio, even though the T1D patients with the HLA-DQB1*0201 allele, which genetically predispose to celiac disease, had both an increased para- and transcellular permeability compared to T1D patients without the allele [28] (Table 1). Nevertheless, the use of these *in vivo* markers in human T1D seems relevant and generates consistent results regarding paracellular permeability. Furthermore, the varying results regarding transcellular permeability should be investigated in future T1D permeability experiments, since alterations in the transcellular permeability could be caused by structural changes in the morphology of the small intestine allowing a breech in the intestinal barrier.

Similar results regarding para- and transcellular permeability are observed in animal models; however, the studies are few, possibly because of the difficulties and animal welfare problems of keeping rodents in metabolic cages for a longer period of time to collect urine. Neu et al. observed an increased paracellular permeability, no change in transcellular permeability and hence an increased LA/MA ratio in the small intestine of BBDP and Biobreeding Diabetes-Resistant (BBDR) rats

Table 2
In vivo permeability in animal models - genetic modifications and interventions. The table depicts whether paracellular (para) permeability (perm) or the paracellular/transcellular (trans) perm ratio was changed (↑ increased, ↔ unchanged, ↓ decreased), which method was used to identify the change (↑ significantly increased, ↔ unchanged, ↓ significantly decreased), what organism was used and which groups that were compared and at what age. The "intervention" column denotes which treatment the animals were subjected to. "Incidence effect" is the observed effect of the intervention on diabetes incidence either in numbers, if available or as delayed diabetes onset, accelerated diabetes onset or the effect on insulitis score ("-" marks if none of these diabetes endpoints were evaluated). "Tissue" refers to the intestinal segment that the *in vivo* method examines, which is measured in either urine* or blood**. BBDP=Biobreeding Diabetes-Prone, BBDR=Biobreeding Diabetes-Resistant, FD4 = fluorescein isothiocyanate (FITC)-dextran 4000, FZI/0 = blocker of the zonulin receptor, HC = hydrolyzed casein, HFD = high fat diet, IRT5 = Immune Regulation and Tolerance 5, LA = lactulose, MA = mannitol, NOD=Non-Obese Diabetic.

		Method	Organism	Group comparison (age)	Intervention	Incidence effect	Tissue	Ref.
Para perm	↑	↑ Sucrose	Rat	BBDP vs BBDR (50–110 days)	HC diet	50%–20%	Stomach, duodenum*	[33]
		↑ FD4	Mouse	$NOD^{MR1-/-}$ vs $NOD^{MR1+/-}$ (15 weeks)	–	Accelerate	Small intestine**	[36]
				NOD (4 weeks)	C. *rodentium*	Increased insulitis score	Colon (enema administered)**	[34]
				HFD *in utero* - vs control NOD (16 weeks)	HFD (*in utero*)	–	Small intestine**	[43]
				$NOD^{Trac-/-}$ (8–10 weeks)	BDC2.5 T cells	0%–~70%	Small intestine**	[37]
	↔	↔ Sucrose	Rat	BBDP vs BBDR (25–~43 days)	HC diet	50%–20%	Stomach/duodenum*	[33]
		↔ Sucralose	Rat	BBDP vs BBDR (50–110 days)	HC diet	50%–20%	Colon*	[33]
		↔ FD4	Mouse	NOD (12 weeks)	HFD	–	Small intestine**	[43]
	↓	↓ FD4	Mouse	NOD (7 weeks)	XOS diet	Delayed	Small intestine**	[45]
				NOD (16 weeks)	IRT5	~80%–~45%	Small intestine**	[47]
				NOD (13 weeks)	Fingolimod	~58%–25%	Small intestine**	[46]
Para/trans ratio	↑	↑ LA/MA	Rat	BBDP vs BBDR (50–110 days)	HC diet	50%–20%	Small intestine*	[33]
	↔	↔ LA/MA	Rat	BBDP (65 days)	Amino acid mix	Delayed	Small intestine*	[40]
				BBDP vs BBDR (25–~43 days)	HC diet	50%–20%	Small intestine*	[33]
	↓	↓ LA/MA	Rat	BBDP (44–72 days)	FZI/0	80%–27%	Small intestine*	[42]
				BBDP (65 days)	HC diet	~95%–~55%	Small intestine*	[41]
				BBDP (65 days)	HC diet (Pancase S)	~85%–~60%	Small intestine*	[40]
				BBDP (65 days)	HC diet (Nutramigen)	~85%–~45%	Small intestine*	[40]

compared to control Wistar rats [32]. Meddings et al. found an age-dependent increase in the LA/MA ratio in BBDP vs BBDR rats [33]. Measuring permeability in other segments of the GI tract is less common. An age-dependent increase in paracellular permeability was found in the stomach and duodenum of BBDP vs BBDR rats using sucrose as a marker, but on the other hand sucralose paracellular permeability in the colon was unaltered [33] (Table 1).

Using FD4 as a marker, NOD mice were found to have an increased small intestinal paracellular permeability compared to Non-Obese Diabetes-Resistant (NOR) mice [34,35] (Table 1). An age dependent increase in paracellular permeability was also found between NOD mice and the control strains C57BL/6 (B6) and BALB/c mice [35]. Furthermore, NOD mice had a higher paracellular permeability at diabetes-onset than at the age of 10 weeks [36,37] (Table 1).

Increased intestinal permeability in BBDR rats and NOD mice compared to control strains seems age-related, which may reflect alterations in the intestinal permeability in the ageing animal or as the disease progresses with age. Other possible explanations to the varying results observed between NOD mice at different ages include differences in the exact execution of the FD4 analysis (length of fasting time and/or water deprivation, dose of FD4 administered per body weight etc.). Another reason could be differences in the diabetes incidence between animal facilities [38]*, which very well could be caused by variation in microbiota composition across the facilities and also vendors [39]*. Since NOD mice at the onset of diabetes show a higher paracellular permeability than younger NOD mice [36,37], failure to find this difference in the younger NOD mice is likely because not all of these mice progress to diabetes. Due to the age-related permeability differences, care must be taken when choosing groups and assessment age for permeability experiments in rodents. It would be relevant with a reliable non-fatal *in vivo* permeability method for evaluating the progression of paracellular permeability in T1D animal models and how it relates to T1D development.

2.2. Animal models - genetic modifications and interventions

Different dietary components have been found to modulate autoimmune diabetes incidence in T1D animal models. Casein is a major protein component of cow's milk. Hydrolyzed casein (HC) has protective effects on diabetes in BBDP rats, possibly due to absence of cow's milk proteins [40]. BBDP rats were fed diets, where HC was the amino acid source, which decreased LA/MA ratios and reduced the diabetes incidence [40,41] (Table 2). Furthermore, a control diet, where HC was replaced with an amino acid mixture, postponed the diabetes development, but did not alter the LA/MA ratio [40]. This suggests a protective effect from specific peptides in the HC diets that the amino acid mixture does not have. Despite a decrease in the LA/MA ratio in BBDP rats on HC diet, the rats still had a higher permeability in stomach/duodenum and the small intestine than BBDR rats [33]. No difference was observed in the permeability of the colon [33].

In another study, treatment of BBDP rats with FZI/0, a zonulin antagonist that hinders zonulin-mediated disruption of tight junctions (see Section 4), also decreased the LA/MA ratio and reduced the diabetes incidence [42] (Table 2). This suggests that zonulin is involved in the pathogenesis of T1D, possibly through the regulation of paracellular permeability in the small intestine.

To investigate a potential link between maternal obesity and gut epithelial barrier function, a high fat diet (HFD) was provided to NOD mice [43]. FD4 intestinal permeability was examined and found to be unchanged in the HFD-fed mothers but increased in their offspring, suggesting that the intestinal permeability is alterable already *in utero* (Table 2). It is unclear if the increased permeability led to a higher T1D incidence, as it was not measured in the study.

Citrobacter rodentium, a bacterium with the capability of disrupting the epithelial barrier, was administered orally to NOD mice, which increased the FD4 intestinal permeability compared to control NOD mice [34]. In this study, FD4 was enema administered, thus the observed

Table 3
Ex vivo permeability in animal models. The table depicts whether paracellular (para) permeability (perm) was changed (↑ increased, ↔ unchanged, ↓ decreased), which method was used to identify the change (↑ significantly increased, ↔ unchanged, ↓ significantly decreased), what organism was used and which groups that were compared and at what age. "Tissue" refers to where the permeability outcome was examined. BBDP=Biobreeding Diabetes-Prone, BBDR=Biobreeding Diabetes-Resistant, FD4 = fluorescein isothiocyanate (FITC)-dextran 4000, MA = mannitol, NOD=Non-Obese Diabetic, NOR=Non-Obese Diabetes-Resistant, TEER = trans-epithelial electrical resistance.

		Permeability method	Organism	Group comparison (age)	Tissue	Ref.
Para perm	↑	↑ Tissue conductance	Mouse (*ex vivo*)	NOD vs NOR (7–10 weeks)	Jejunum	[49]
		↓ TEER	Rat (*ex vivo*)	BBDP vs BBDR (50 days)	Jejunum, ileum	[42]
				BBDP vs BBDR (75 days)	Ileum	[42]
				BBDP vs BBDR (65 days)	Ileum	[41]
	↔	↔ FD4	Mouse (*ex vivo*)	NOD vs NOR (7–10 weeks)	Jejunum	[49]
				Diabetic NOD vs age-matched control NOD	Jejunum	[49]
		↔ Tissue conductance	Mouse (*ex vivo*)	Diabetic NOD vs age-matched control NOD	Jejunum	[49]
		↔ TEER	Rat (*ex vivo*)	BBDP vs BBDR (20, 50, 75 days)	Colon	[42]
				BBDP vs BBDR (20 days)	Jejunum, Ileum	[42]
				BBDP vs BBDR (75 days)	Jejunum	[42]
	↓	↓ MA	Rat (*ex vivo*)	BBDP vs Wistar rats (80 days)	Ileum	[32]

increase in permeability was colon derived. Concomitantly, the infected NOD mice showed an increased insulitis score at 12 weeks of age (Table 2).

Mucosal-associated invariant T cells (MAIT cells) recognizes metabolites derived from the microbiota when presented by the MHC class I-related gene protein, MR1 [44]*. MAIT cells play a role in maintenance of the gut integrity and they have been found in lower numbers in recent-onset T1D patients compared to controls and in NOD mice compared to B6 mice [36]. $NOD^{MR1-/-}$ mice are incapable of producing MAIT cells and have increased FD4 intestinal permeability in addition to accelerated diabetes onset [36]. $NOD^{Trac-/-}$ lack the T cell receptor alpha chain constant and when injected with diabetogenic T cells (BDC2.5 T cells), autoimmune diabetes is induced. In this model, an increased FD4 permeability was observed together with the rise in T1D incidence [37] (Table 2). Higher proportions of the BDC2.5 T cells were found in ileum than in mesenteric lymph nodes (MLN) and PLN. The cells also produced local inflammatory cytokines strengthening a causality between the mucosal immune response and T1D development.

In yet another study, NOD mice were administered a prebiotic xylooligosaccharide (XOS) diet aiming at propagating a beneficial microbiota composition [45] (see Section 5.2 for immunomodulatory effects). Here, a decrease in FD4 intestinal permeability was observed and the diabetes onset was moreover delayed [45]. Treatment with fingolimod, a sphingosine-1-phosphate receptor agonist with anti-inflammatory properties among others, decreased FD4 permeability and reduced diabetes incidence in NOD mice (Table 2) [46]. Similarly, when administering NOD mice a probiotic combination termed Immune Regulation and Tolerance 5 (IRT5) that contains 5 bacteria with autoimmune therapeutic effects, a decrease in FD4 permeability and reduced diabetes incidence was observed [47] (Table 2).

Interestingly, it seems that interventions or genetic modification aimed to accelerate T1D development also increased intestinal permeability and vice versa. Thus, by altering the intestinal permeability it may be possible to control the development of T1D at least in rodents. Likely, the degree of intestinal permeability determines the extent of which environmental antigens can pass the intestine, which again drive the mucosal immune system in either a pro- or anti-inflammatory direction, facilitating or preventing diabetes development in rodent models of T1D.

3. *Ex vivo* permeability

The *ex vivo* paracellular permeability can be measured by mounting small pieces of intestine in Ussing chambers, where the intact intestinal tissue maintains its polarization. With this method, passive permeability to ions, also entitled tissue conductance, can be measured besides the reciprocal transepithelial electrical resistance (TEER) that estimates tissue integrity [48]*. Furthermore, permeability markers (see Section 2) (Fig. 2) can be added to the apical side of the mounted intestinal piece to trace the degree of permeation. This method has the advantage of examining conditions in specific intestinal segments of interest separately.

3.1. Animal models

No difference was observed in *ex vivo* FD4 permeability in the jejunum of NOD vs NOR mice or in diabetic NOD vs age-matched non-diabetic NOD mice [49] (Table 3). *Ex vivo* MA is a measure of paracellular permeability not to be confused with its *in vivo* transcellular properties. Decreased MA permeability was observed in ileum of BBDP vs Wistar rats [32].

Watts et al. examined jejunum, ileum and colon TEER differences in Biobreeding rats [42]. A decrease in ileum, an age-related decrease in jejunum but no differences in colon was observed in BBDP compared to BBDR rats (Table 3). Compromised ileal integrity (decreased TEER) in BBDP compared to BBDR rats was further supported by a study from Visser et al. [41]. The ion permeability (tissue conductance) was increased in jejunum of NOD mice vs NOR mice but not in diabetic NOD mice vs age-matched controls [49] (Table 3).

The *ex vivo* assessment of permeability and integrity in T1D appears to be more varying than the aforementioned physiological measurements (see Section 2). One reason for this is most likely the usage of different methods. Thus, the permeability to sugars is not directly comparable to ion conductance because of the obvious size differences of sugars and ions. Furthermore, different intestinal segments were used in the few studies. As with the physiological permeability measurements, it seems that *ex vivo* permeability depends on the age of the rodent and also the investigated intestinal segment. The *ex vivo* permeability results from animal models of T1D are presently too variable to clearly state that the intestinal permeability is increased or that the intestinal integrity is disrupted, but several studies point in that direction.

3.2. Animal models - genetic modifications and interventions

Short-chain fatty acids (SCFAs) are produced by bacterial fermentation of non-digestible fibers in the colon. The majority of SCFAs are constituted by acetate, butyrate, formate and propionate, which have a number of physiological properties, exemplified by butyrate that has the ability to regulate tight junction proteins [50]* (see Section 5.2 for immunomodulatory effects of SCFA-promoting diets). Supplementation

Table 4
Ex vivo permeability in animal models - genetic modifications and interventions. The table depicts whether paracellular (para) permeability (perm) was changed (↑ increased, ↔ unchanged, ↓ decreased), which method was used to identify the change (↑ significantly increased, ↔ unchanged, ↓ significantly decreased), what organism was used and which groups that were compared and at what age. The "intervention" column denotes which treatment the animals were subjected to. "Incidence effect" is the observed effect of the intervention on diabetes incidence either in numbers, if available or the effect on insulitis score. "Tissue" refers to where the permeability outcome was examined. BBDP=Biobreeding Diabetes-Prone, GLP-2 = glucagon-like peptide 2, HC = hydrolyzed casein, MA = mannitol, NOD=Non-Obese Diabetic, PEG-4000 = polyethylene glycol 4000, TEER = transepithelial electrical resistance.

		Method	Organism	Group comparison (age)	Intervention	Incidence effect	Tissue	Ref.
Para perm	↑	↑ Tissue conductance	Mouse (*ex vivo*)	NOD-DQ8 (8–10 weeks)	Gliadin sensitization	No change in insulitis score	Jejunum	[52]
	↔	↔ MA	Rat (*ex vivo*)	BBDP (66–80 days)	Butyrate	No change	Ileum	[51]
		↔ PEG-4000	Rat (*ex vivo*)	BBDP (66–80 days)	Butyrate	No change	Ileum	[51]
		↔ Tissue conductance	Rat (*ex vivo*)	BBDP (66–80 days)	Butyrate	No change	Ileum	[51]
	↓	↓ Tissue conductance	Rat (*ex vivo*)	BBDP (66–80 days)	Butyrate	No change	Colon	[51]
			Mouse (*ex vivo*)	NOD (8 weeks and 14 weeks)	GLP-2 analog	No change	Jejunum	[49]
		↑ TEER	Rat (*ex vivo*)	BBDP (diabetes onset or 80–85 days)	FZI/0	80%–27%	Ileum	[42]
				BBDP (65 days)	HC diet	~95%–~55%	Ileum	[41]

Table 5
Zonulin in humans and animal models. The table depicts whether paracellular (para) permeability (perm) was changed (↑ increased, ↔ unchanged), which method was used to identify the change (↑ significantly increased, ↔ unchanged), what organism was used and which groups that were compared and at what age. "Tissue" refers to where the permeability outcome was examined. BBDP=Biobreeding Diabetes-Prone, BBDR=Biobreeding Diabetes-Resistant, IA = islet autoantibodies, NOD=Non-Obese Diabetic, NOR=Non-Obese Diabetes-Resistant, T1D = Type 1 Diabetes.

		Method	Organism	Group comparison (age)	Tissue	Ref.
Para perm	↑	↑ Zonulin	Human	T1D vs relatives and controls	Serum	[15]
				T1D vs controls	Serum	[8]
			Rat	BBDP vs BBDR (50, 75, 100 days)	Serum, intraluminal (small intestine)	[42]
				BBDP (diabetes onset) vs BBDP (80–85 days)	Serum, intraluminal (small intestine)	[42]
			Mouse	NOD (early/late diabetes onset) vs NOR	Duodenum, jejunum, ileum, colon	[62]
				NOD (early/late diabetes onset) vs NOD (no diabetes onset)	Duodenum, jejunum, ileum	[62]
	↔	↔ Zonulin	Human	IA-positive vs controls	Serum	[61]

with butyrate did not affect neither diabetes incidence nor *ex vivo* paracellular permeability of MA and PEG-4000 in ileal tissue from BBDP rats [51] (Table 4).

TEER was increased in the ileum of BBDP rats treated with FZI/0 [42] and on HC diet [41], suggesting an improved ileal integrity. Both interventions also decreased the diabetes incidence.

The transgenic NOD-DQ8 mouse, which has the human HLA-DQ8 background instead of its MHC-II background, does not spontaneously develop autoimmune diabetes as the normal NOD mouse does. When sensitizing the NOD-DQ8 mice with gluten-derived gliadin, the tissue conductance increased in jejunum, suggesting an increased ion permeability, even though the insulitis score did not change as expected [52] (Table 4). In the butyrate-fed BBDP rats, ileal tissue conductance was not changed but colonic tissue conductance was decreased [51], which would be expected since butyrate mainly exerts its effect in the colon. Glucagon-like peptide (GLP)-2 affects both paracellular and transcellular intestinal permeability, enhancing the barrier function [53]*. A decrease in jejunal tissue conductance was seen in NOD mice treated with GLP-2, although diabetes incidence was not altered [49] (Table 4).

The few articles that investigate the *ex vivo* permeability in genetically modified animals or in intervention studies show that it is possible to alter the permeability to ions. Still, tissue conductance was altered in models where interventions did not have any effect on diabetes incidence, suggesting that changing the permeability of ions is not sufficient to alter T1D development.

4. Zonulin

Zonulin is a widely used marker for small intestinal paracellular permeability. The protein disrupts tight junctions by activating a multistep phosphorylation pathway that leads to polymerization and rearrangement of actin filaments and displacement of the scaffolding protein zonula occludens (ZO)-1 (see Section 5) (Fig. 3) [54,55]*. Some environmental factors, such as bacteria and gluten, can stimulate the intestinal zonulin release [56,57]*. In this context, serum zonulin levels was found to correlate with enterovirus density in the small intestinal mucosa of patients with concomitant celiac disease and T1D [58]*. This implies that zonulin could also be involved in the pathogenesis of T1D as a mediator of increased paracellular permeability. Recent studies have questioned the use of commercially available zonulin ELISA kits due to uncertainties of whether these kits actually detect zonulin [59,60]* and care must therefore be taken to choose the right methodology for the measurement of zonulin levels.

4.1. Humans and animal models

Serum zonulin levels were increased in T1D patients [8,15] but not in IA-positive individuals vs controls [61] (Table 5). Also, BBDP rats had higher zonulin levels in both serum and lumen of the small intestine compared to BBDR rats [42]. Furthermore, BBDP rats at diabetes-onset showed higher levels of zonulin in serum and lumen of the small intestine than non-diabetic BBDP rats of 80–85 days of age. Joesten et al. examined the spatial variation of zonulin levels in the intestine of NOD mice [62]. Interestingly, the study demonstrated that NOD mice with early or late-onset autoimmune diabetes had higher levels of zonulin compared to NOR mice in all investigated intestinal segments [62]. Also, early and late-onset autoimmune diabetes NOD mice had higher zonulin levels in the small intestinal segments than their diabetes-free littermates [62] (Table 5).

The level of zonulin seems to be increased in both serum of T1D patients and in serum and/or intestinal segments of animal models. Furthermore, results from NOD mice and BBDP rats suggests that zonulin levels in the small intestine is higher in diabetic than in non-diabetic animals [42,62], which corresponds well with the FD4 observations in Table 1.

Table 6
Zonulin in animal models - genetic modifications and interventions. The table depicts whether paracellular (para) permeability (perm) was changed (↔ unchanged, ↓ decreased), which method was used to identify the change (↔ unchanged, ↓ significantly decreased), what organism was used and which groups that were compared and at what age. The "intervention" column denotes which treatment the animals were subjected to. "Incidence effect" is the observed effect of the intervention on diabetes incidence either in numbers, if available or as delayed diabetes onset. "Tissue" refers to where the permeability outcome was examined. BBDP=Biobreeding Diabetes-Prone, HC = hydrolyzed casein, NOD=Non-Obese Diabetic, SNase = Staphylococcal nuclease, XOS = xylooligosaccharide.

		Method	Organism	Group comparison (age)	Intervention	Incidence effect	Tissue	Ref.
Para perm	↔	↔ Zonulin	Rat	BBDP (diabetes onset or 80–85 days)	FZI/0	80%–27%	Intraluminal (small intestine)	[42]
			Mouse	NOD (13 weeks)	XOS diet	Delayed	Serum	[45]
				NOD (5, 7, 9, 15, 17, 19 weeks)	SNase	Delayed	Serum	[66]
	↓	↓ Zonulin	Rat	BBDP rats (50–70 days)	HC diet	~95%–~55%	Serum	[41]
			Mouse	NOD (11, 13 weeks)	SNase	Delayed	Serum	[66]

4.2. Animal models - genetic modifications and interventions

Intraluminal levels of zonulin in the small intestine of BBDP rats was unchanged after FZI/0 treatment, despite the lower LA/MA ratio that was observed [42] (Table 6). This suggests that the FZI/0 treatment is efficient in hindering the zonulin-mediated disruption of the tight junctions via the zonulin receptor making it a candidate for treating increased intestinal permeability in pre-T1D individuals. Currently, Larazotide, the drug name of the zonulin receptor antagonist, is being tested in a phase III trial for alleviating persistent symptoms in celiac disease patients by hindering zonulin-derived increased paracellular permeability [63]*.

Neutrophil extracellular traps (NETs), the neutrophilic response to pathogens, consists of DNA, histones and enzymes from within the cell. The levels of NETs and the circulation of neutrophil serine proteases are increased in T1D patients [64]*. Staphylococcal nuclease (SNase) has the ability to degrade DNA and with that also NETs [65]*. In this regard, intestinal permeability changes in SNase-treated NOD mice was examined [66]. The mice had an age-dependent decrease in serum zonulin levels, while diabetes onset was delayed [66]. Decreased serum zonulin levels were also seen in BBDP rats on HC diet [41] whereas serum zonulin levels were unchanged in NOD mice fed an XOS diet [45] (Table 6).

Even though the studies are few, it seems, as with the *in vivo* estimation of FD4 small intestinal permeability, that differences in the level of zonulin were age dependent. Furthermore, zonulin was not persistently altered in intervention studies that were able to alter diabetes incidence or delay diabetes development.

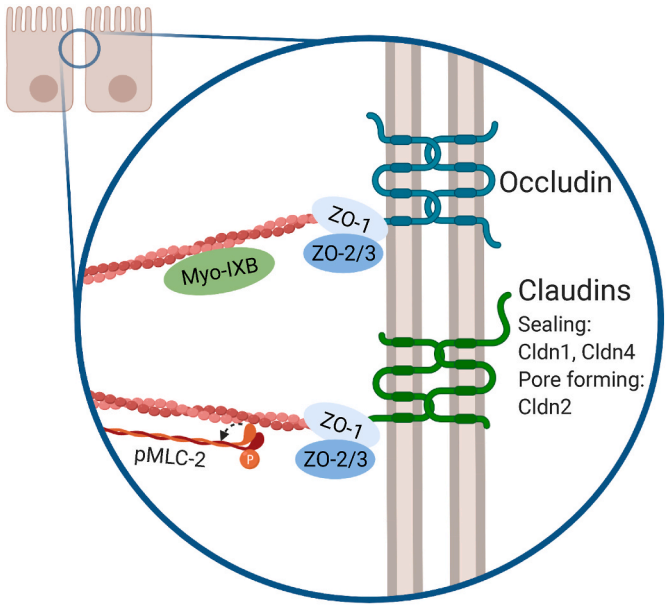

Fig. 3. Simplified illustration of tight junction (or related) protein markers used for assessment of molecular permeability. Tight junctions between enterocytes are situated at the apical junctional complex in conjunction with adherens junctions and are tightly linked to the perijunctional actomyosin ring. Tight junctions form a semipermeable barrier restricting and regulating the paracellular permeability besides being a crucial structure for maintaining cell polarity. Tight junctions are a network of transmembrane proteins (occludin and claudins) mediating cell-cell adhesion that interacts with scaffolding proteins (zonula occludens (ZO)), which in turn connects the junction with the perijunctional actomyosin ring. Cldn = Claudin, Myo-IXB = myosin-IXB, pMLC-2 = Phosphorylated myosin light chain-2, ZO = zonula occludens.

Table 7
Molecular permeability in humans and animal models. The table depicts whether paracellular (para) permeability (perm) was changed (↑ increased, ↔ unchanged), which method was used to identify the change (↔ unchanged, ↓ significantly decreased), what organism was used and which groups that were compared and at what age. "Tissue" refers to where the permeability outcome was examined at mRNA or protein level. B6=C57BL/6, BBDP=Biobreeding Diabetes-Prone, BBDR=Biobreeding Diabetes-Resistant, Cldn = claudin, Myo-IXB = myosin-IXB, NOD=Non-Obese Diabetic, Ocln = occludin, T1D = Type 1 Diabetes, ZO = zonula occludens.

		Method	Organism	Group comparison (age)	Tissue	Ref.
Para perm	↑	↓ ZO-1	Mouse	NOD female vs NOD male (5 weeks)	Colon (mRNA)	[67]
				NOD at diabetes onset vs NOD (8–10 weeks)	Ileal epithelium (mRNA)	[37]
		↓ Cldn1	Rat	BBDP vs BBDR and Wistar (34, 41 days)	Small intestine (protein)	[32]
				BBDP vs BBDR (51–70 days)	Ileum (mRNA)	[41]
		↓ Cldn4	Mouse	NOD at diabetes onset vs NOD (8–10 weeks)	Ileal epithelium (mRNA)	[37]
		↓ Ocln	Mouse	NOD female vs NOD male (5 weeks)	Colon (mRNA)	[67]
				NOD at diabetes onset vs NOD (8–10 weeks)	Ileal epithelium (mRNA)	[37]
	↔	↔ ZO-1	Human	T1D vs controls	Small intestine (mRNA)	[15]
			Mouse	NOD vs BALB/c (4 weeks)	Colon (mRNA)	[35]
				NOD vs BALB/c and B6 (5 weeks)	Colon (mRNA)	[67]
		↔ Cldn1	Human	T1D vs controls	Small intestine (mRNA)	[15]
			Rat	BBDP vs BBDR (21–50 days)	Ileum (mRNA)	[41]
			Mouse	NOD vs BALB/c (4 weeks)	Colon (mRNA)	[35]
				NOD female vs NOD male (5 weeks)	Colon (mRNA)	[67]
		↔ Cldn2	Human	T1D vs controls	Small intestine (mRNA)	[15]
		↔ Ocln	Human	T1D vs controls	Small intestine (mRNA)	[15]
			Rat	BBDP vs BBDR and Wistar (34, 41 days)	Small intestine (protein)	[32]
			Mouse	NOD vs BALB/c and B6 (5 weeks)	Colon (mRNA)	[67]
		↔ Myo-IXB	Human	T1D vs controls	Small intestine (mRNA)	[15]

5. Molecular permeability

Tight junctions are the gate keepers of paracellular permeability, determining the rate and size of macromolecules passing the intestinal barrier. Determining the mRNA and/or protein levels of different tight junction (or related) proteins is commonly used to evaluate the paracellular permeability at the molecular level. Fig. 3 depicts the proteins used as molecular markers of permeability in the articles included in this review and what association they have to the tight junction complex.

5.1. Humans and animal models

ZO proteins (ZO-1, ZO-2 and ZO-3) are scaffolding proteins ensuring linkage between the tight junctions and the cytoskeleton (Fig. 3). ZO-1 mRNA was decreased in the colon of female vs male NOD mice [67] (Table 7), which is interesting since the diabetes incidence in female NOD mice is higher than in male NOD mice (Fig. 1). The same was seen in ileal epithelium at diabetes-onset in NOD mice compared to younger NOD mice [37]. No difference in ZO-1 mRNA was observed in the small intestine of T1D patients compared to controls [15] nor in the colon of NOD mice compared to BALB/c and B6 mice [35,67].

Claudins (Cldn) are a large group of transmembrane proteins that either seal or form leaky pores in the paracellular gap between e.g. enterocytes (Fig. 3). The mRNA levels of the sealing Cldn1 were unchanged in the small intestine of T1D patients [15]. The Cldn1 protein levels were decreased in BBDP rats compared to BBDR and Wistar rats [32] and mRNA levels were decreased in BBDP rats compared to BBDR rats [41]. No change in Cldn1 mRNA levels were evident in the colon of NOD mice compared to BALB/c mice or between gender [35,67]. Moreover, no difference was found in the mRNA expression of the pore-forming Cldn2 in the small intestine of T1D patients vs controls [15]. In ileal epithelium, mRNA levels of the sealing Cldn4 were decreased in NOD mice at diabetes onset compared to younger NOD mice [37].

Occludin (Ocln) is a transmembrane protein that ensures occlusion between the paracellular gap of the enterocytes (Fig. 3). Even though Ocln is not crucial for tight junction assembly [68]*, it is still able to occlude the paracellular flux of larger molecules when present [69]*. Ocln mRNA levels were found to be unchanged in the small intestine of T1D patients vs controls [15], in the colon of NOD mice vs BALB/c and B6 mice [67] and also at the protein level in the small intestine of BBDP vs BBDR and Wistar rats [32]. Ocln mRNA levels were decreased in colon of female vs male NOD mice [67] and in ileal epithelium of NOD mice at diabetes onset compared to younger NOD mice [37].

Myosin (Myo)-IXB is a motor protein with the ability to move on actin, as well as functioning as a Rho GTPase-activating protein (GAP) recruited to sites with actin polymerization [70]*. Myo-IXB knock-down in a Caco-2 BBe intestinal epithelial cell model resulted in a severely disrupted ZO-1 protein localization and increased permeability [71]*. It suggests the need for Myo-IXB in proximity to the tight junction (Fig. 3). Myo-IXB mRNA levels were unchanged in the small intestine of T1D patients vs controls [15] (Table 7).

Measurements of the different tight junction proteins is common in the evaluation of intestinal permeability, but results can be difficult to interpret because research groups use different panels of permeability markers. Only one study examined tight junction protein expression in T1D patients and found no alterations in mRNA levels of ZO-1, Cldn1, Cldn2, Ocln and Myo-IXB in the small intestine [15]. Results from animal models are hard to synthesize, especially because the intestinal segments and ages vary, which was also seen in *ex vivo* permeability measurements (see Section 3). Still, Rouland et al. observed decreased levels of several tight junction protein at diabetes onset [37] in compliance with FD4 (Table 1) and zonulin (Table 5) results strengthening the hypothesis that intestinal permeability is increased at diabetes-onset.

5.2. Animal models - genetic modifications and interventions

In the group of scaffolding ZO proteins, no difference was reported for ZO-1 mRNA expression in the ileum of NOD mice subjected to HFD *in utero* [43] (Table 8). This study did not register any difference in ileal expression of ZO-2 and ZO-3 mRNA either. XOS diet did not change the ZO-1 mRNA expression in ileum or colon of NOD mice [45]. Decreased mRNA levels of ZO-1 in ileal epithelium was observed in $NOD^{Trac-/-}$ mice injected with BDC2.5 T cells, which also induced diabetes [37]. No change was found in the colonic protein levels of ZO-2 in long chain inulin-type fructans (ITF(l))-supplemented NOD mice, despite a decreased diabetes incidence [72]. On the other hand, IRT5 treatment in NOD mice resulted in increased ZO-1 mRNA levels in the small intestine [47]. Fingolimod treatment resulted in increased protein levels of both ZO-1 and ZO-2 in the colon of NOD mice [46] and NOD mice on low-methoxyl pectin (LMP) diet had increased mRNA levels of ZO-2 in

Table 8

Molecular permeability in animal models - genetic modifications and interventions. The table depicts whether paracellular (para) permeability (perm) was changed (↑ increased, ↔ unchanged, ↓ decreased), which method was used to identify the change (↑ significantly increased, ↔ unchanged, ↓ significantly decreased), what organism was used and which groups that were compared and at what age. The "intervention" column denotes which treatment the animals were subjected to. "Incidence effect" is the observed effect of the intervention on diabetes incidence either in numbers, if available, as delayed diabetes onset or as accelerated diabetes onset ("-" marks if none of these diabetes endpoints were evaluated). "Tissue" refers to where the permeability outcome was examined at mRNA or protein level. ABX = broad-spectrum antibiotics, BBDP=Biobreeding Diabetes-Prone, BBDR=Biobreeding Diabetes-Resistant, Cldn = claudin, HC = hydrolyzed casein, HFD = high fat diet, IRT5 = Immune Regulation and Tolerance 5, ITF(l) = long chain inulin-type fructans, LMP = low-methoxyl pectin, Myo-IXB = myosin-IXB, NOD=Non-Obese Diabetic, Ocln = occludin, p-MLC2 = phosphorylated myosin light chain 2, XOS = xylooligosaccharide, ZO = zonula occludens.

		Method	Organism	Group comparison (age)	Intervention	Incidence effect	Tissue	Ref.
Para perm	↑	↓ ZO-1	Mouse	$NOD^{Trac-/-}$ (8–10 weeks)	BDC2.5 T cells	0%–~70%	Ileal epithelium (mRNA)	[37]
		↓ ZO-2	Mouse	NOD (11 weeks)	ABX	No change	Cecum (protein)	[74]
		↓ Cldn1	Mouse	NOD (11 weeks)	ABX	No change	Cecum (protein)	[74]
		↑ Cldn2	Mouse	HFD *in utero* - vs control (16 weeks)	HFD (*in utero)*	–	Ileum (protein)	[43]
				NOD (24 weeks)	ITF(l)	40%–10%	Colon (protein)	[72]
		↓ Ocln	Mouse	$NOD^{MR1-/-}$ vs $NOD^{MR1+/-}$ (15 weeks)	–	Accelerate	Ileum epithelium (mRNA)	[36]
		↑ p-MLC2	Mouse	HFD *in utero* - vs control (16 weeks)	HFD (*in utero*)	–	Ileum (protein)	[43]
	↔	↔ ZO-1	Mouse	NOD (13 weeks)	XOS	Delayed	Ileum, colon (mRNA)	[45]
				HFD *in utero* - vs control NOD (16 weeks)	HFD (*in utero*)	–	Ileum (mRNA)	[43]
		↔ ZO-2	Mouse	HFD *in utero* - vs control NOD (16 weeks)	HFD (*in utero*)	–	Ileum (mRNA)	[43]
				NOD (24 weeks)	ITF(l)	40%–10%	Colon (protein)	[72]
		↔ ZO-3	Mouse	HFD *in utero* - vs control NOD (16 weeks)	HFD (*in utero*)	–	Ileum (mRNA)	[43]
		↔ Cldn1	Rat	BBDP diabetic (73–100 days) vs BBDP non-diabetic (140 days)	HC diet	~95%–~55%	Ileum (mRNA)	[41]
			Mouse	NOD (22 weeks)	LMP diet	~70%–~38%	Colon (protein)	[73]
				NOD (16 weeks)	IRT5	~80%–~45%	Small intestine (mRNA)	[47]
		↔ Cldn2	Rat	BBDP (HC diet) and BBDR vs BBDP (21–50 days)	HC diet	~95%–~55%	Ileum (mRNA)	[41]
				BBDP (70–150 days)	Amino acid mix	Delayed	Ileum (mRNA)	[40]
				BBDP (70–150 days)	HC diet (Pancase S)	~85%–~60%	Ileum (mRNA)	[40]
				BBDP (70–150 days)	HC diet (Nutramigen)	~85%–~45%	Ileum (mRNA)	[40]
			Mouse	HFD *in utero* - vs control NOD (16 weeks)	HFD (*in utero*)	–	Ileum (mRNA)	[43]
		↔ Cldn4	Mouse	$NOD^{MR1-/-}$ vs $NOD^{MR1+/-}$ (15 weeks)	–	Accelerate	Ileum epithelium (mRNA)	[36]
				$NOD^{Trac-/-}$ (8–10 weeks)	BDC2.5 T cells	0%–~70%	Ileal epithelium (mRNA)	[37]
		↔ Ocln	Rat	BBDP (HC diet) and BBDR vs BBDP (21–50 days)	HC diet	~95%–~55%	Ileum (mRNA)	[41]
				BBDP (HC diet) and BBDR vs BBDP (51–70 days)	HC diet	~95%–~55%	Ileum (mRNA)	[41]
				BBDP (70–150 days)	Amino acid mix	Delayed	Ileum (mRNA)	[40]
				BBDP (70–150 days)	HC diet (Pancase S)	~85%–~60%	Ileum (mRNA)	[40]
				BBDP (70–150 days)	HC diet (nutramigen)	~85%–~45%	Ileum (mRNA)	[40]
			Mouse	NOD (13 weeks)	XOS diet	Delayed	Ileum, colon (mRNA)	[45]
				NOD (22 weeks)	LMP diet	~70%–~38%	Cecum, colon (protein)	[73]
				NOD (15 weeks)	Acetate-yielding diet	~65%–~30%	Colon (mRNA)	[67]
				NOD (16 weeks)	IRT5	~80%–~45%	Small intestine (mRNA)	[47]
				$NOD^{Trac-/-}$ (8–10 weeks)	BDC2.5 T cells	0%–~70%	Ileal epithelium (mRNA)	[37]
		↔ Myo-IXB	Rat	BBDP (21–50 days)	HC diet	~95%–~55%	Ileum (mRNA)	[41]
				BBDP (70–150 days)	Amino acid mix	Delayed	Ileum (mRNA)	[40]
				BBDP (70–150 days)	HC diet (Pancase S)	~85%–~60%	Ileum (mRNA)	[40]
				BBDP (70–150 days)	HC diet (nutramigen)	~85%–~45%	Ileum (mRNA)	[40]
		↔ CK2	Mouse	HFD *in utero* - vs control NOD (16 weeks)	HFD (*in utero*)	–	Ileum (protein)	[43]
	↓	↑ ZO-1	Mouse	NOD (16 weeks)	IRT5	~80%–~45%	Small intestine (mRNA)	[47]
				NOD (12 weeks)	Fingolimod	~58%–25%	Colon (protein)	[46]
		↑ ZO-2	Mouse	NOD (22 weeks)	LMP diet	~70%–~38%	Cecum (mRNA)	[73]
				NOD (11 weeks)	LMP diet	~90%–~50%	Cecum (mRNA)	[74]
				NOD (12 weeks)	Fingolimod	~58%–25%	Colon (protein)	[46]
		↑ Cldn1	Rat	BBDP (21–50 days)	HC diet	~95%–~55%	Ileum (mRNA)	[41]
				BBDP (51–70 days)	HC diet	~95%–~55%	Ileum (mRNA)	[41]
				BBDP (70–150 days)	Amino acid mix	Delayed	Ileum (mRNA)	[40]
				BBDP (70–150 days)	HC diet (pancase S)	~85%–~60%	Ileum (mRNA)	[40]
				BBDP (70–150 days)	HC diet (nutramigen)	~85%–~45%	Ileum (mRNA)	[40]
			Mouse	NOD (22 weeks)	LMP diet	~70%–~38%	Cecum (mRNA, protein)	[73]
				NOD (11 weeks)	LMP diet	~90%–~50%	Cecum (protein)	[74]
		↓ Cldn2	Rat	BBDP (HC diet) and BBDR vs BBDP (51–70 days)	HC diet	~95%–~55%	Ileum (mRNA)	[41]

(*continued on next page*)

Table 8 (*continued*)

	Method	Organism	Group comparison (age)	Intervention	Incidence effect	Tissue	Ref.
	↑ Ocln	Mouse	NOD (15 weeks)	Butyrate-yielding diet	~65%–~40%	Colon (mRNA)	[67]
			NOD (24 weeks)	ITF(l)	40%–10%	Colon (protein)	[72]
			NOD (12 weeks)	Fingolimod	~58%–25%	Colon (protein)	[46]
	↓ Myo-IXB	Rat	BBDR and BBDP (HC diet) vs BBDP (51–70 days)	HC diet	~95%–~55%	Ileum (mRNA)	[41]

cecum [73,74]. All of these interventions resulted in decreased incidence levels. Aberration of the microbiota in NOD mice by treatment with broad-spectrum antibiotics (ABX) resulted in decreased protein levels of ZO-2 in cecum but did not change diabetes incidence [74] (Table 8).

ABX treatment of NOD mice resulted in a decrease of the sealing Cldn1 protein in cecum [74] (Table 8). Ileal mRNA levels of Cldn1 was unchanged in diabetic vs non-diabetic BBDP rats on HC diet [41] and also NOD mice on LMP diet showed unchanged colonic protein levels of Cldn1 [73]. Unchanged Cldn1 mRNA levels were also observed in the small intestine of IRT5-treated NOD mice [47]. On the other hand, feeding the HC diets or the amino acid mixture diet to BBDP rats, resulted in increased mRNA levels of ileal Cldn1 at different ages along with decreased diabetes incidence or delayed diabetes onset [40,41] (Table 8). NOD mice on LMP diet had increased mRNA and/or protein levels of Cldn1 in cecum [73,74]. Finally, Cldn1 protein levels in cecum was decreased in NOD mice administered ABX [74]. The pore-forming Cldn2 protein levels were increased in the ileum of NOD mice subjected to HFD *in utero* [43] and also in colon of NOD mice fed an ITF(l) diet [72] (Table 8). On the contrary, Cldn2 mRNA levels in ileum was unchanged in BBDP rats on different HC diets at several ages [40,41] and in NOD mice subjected to HFD *in utero* [43]. BBDP rats on HC diet, however, had ileal mRNA levels of Cldn2 comparable to BBDR rats and lower than BBDP rats on normal chow [41]. mRNA levels of the sealing Cldn4 in ileal epithelium was also examined and found to be unchanged in $NOD^{MR1-/-}$ mice [36] and in BDC2.5 T cell-treated $NOD^{Trac-/-}$ mice [37] (Table 8).

Ocln mRNA levels in the ileal epithelium of $NOD^{MR1-/-}$ mice were decreased [36]. The HC and amino acid mix diets did not affect Ocln mRNA levels in ileum of BBDP rats at different ages [40,41]. Ocln mRNA levels in ileal epithelium of BDC2.5 T cell-treated $NOD^{Trac-/-}$ mice were also unchanged [37]. In NOD mice, the same was evident for colonic Ocln mRNA levels when fed an acetate-yielding diet [67], in ileum and colon when fed an XOS diet [45] and in the small intestine when treated with IRT5 [47]. Protein levels of Ocln in cecum and colon was also unchanged in NOD mice on LMP diet [73]. Ocln mRNA levels were increased in the colon of NOD mice fed a butyrate-yielding diet, which also decreased the diabetes incidence [67]. Similar was observed for Ocln protein levels of NOD mice administered LTF(l) diet [72] or treated with fingolimod [46] (Table 8).

In BBDP rats on HC diet, the mRNA levels of Myo-IXB in ileum were lowered in an age-dependent manner [41]. On the other hand, unaltered levels of Myo-IXB in ileum was observed on different HC diets and on amino acid mix diet [40,41] (Table 8).

Phosphorylation of myosin light chain (MLC)-2 causes contraction of the perijunctional actomyosin ring in proximity to the tight junction complex, resulting in opening of the junction [75]* (Fig. 3). The protein levels of p-MLC-2 were found increased in ileum of NOD mice subjected to HFD *in utero* [43]. In the same study, the protein level of casein kinase (CK)-2 was examined. The CK2 activity is important for normal intestinal epithelial cell homeostasis [76]* but protein levels of CK2 was found unchanged in ileum of NOD mice subjected to HFD *in utero* [43] (Table 8).

As stated earlier, the SCFA butyrate can improve the intestinal barrier function by modulating tight junction proteins (Section 3.2). Therefore, measuring these proteins in experiments that are aiming at changing the microbiota homeostasis through SCFA production is reasonable. Besides the effects seen on permeability, both fiber-diets (XOS, LMP, butyrate- and acetate-yielding diet, ITF(l)) and microbiota-modulating treatments (Fingolimod, IRT5) had immunomodulatory and anti-diabetogenic effects. Increased levels of Tregs where observed in both MLN [45,46,73], PLN [46,47,67,73], pancreas [72–74] and different intestinal tissue segments [47,72,74]. Subsequently, increased levels of the anti-inflammatory cytokines, IL-10 [67,72] and TGF-β [73] and decreased levels of the pro-inflammatory mediators IL-17 [72], IL-1β [46,72–74], TNF-α [73] and IL-6 [73] were observed. Thus, microbiota composition, SCFA levels and intestinal permeability are intertwined with the immune response that leads to T1D but the causality remains to be determined. Since the microbiota is most abundant in the colon (thereafter Ileum) [77]*, measuring permeability marker levels specifically in the colon is a clever way to estimate alterations in permeability caused by fiber fermentation and SCFA production. However, there is a need for an *in vivo* method for estimating the colonic permeability, as the oral FD4 permeability method (see Section 2.) only estimates small intestinal permeability.

As seen in this section, measuring the levels of different tight junction (or related) proteins is quite common in rodent T1D intervention studies. Table 8 demonstrates that the expression (mRNA or protein) of

Table 9
Blood markers in humans and animal models. The table depicts whether the barrier function was changed (↑ worsened, ↔ unchanged), which method was used to identify the change (↑ significantly increased, ↔ unchanged), what organism was used and which groups that were compared and at what age. "Tissue" refers to where the permeability outcome was examined. B6=C57BL/6, CCK-18 = cytokeratin 18 caspase-cleaved fragment, I-FABP = intestinal fatty-acid binding protein, LPS = lipopolysaccharide, NOD=Non-Obese Diabetic, T1D = Type 1 Diabetes.

		Method	Organism	Group comparison (age)	Tissue	Ref.
Barrier function	↑	↑ I-FABP	Human	T1D vs controls	Serum (protein)	[78]
				T1D vs controls	Plasma (protein)	[79]
		↑ LPS	Human	T1D vs controls	Serum	[8]
			Mouse	NOD vs B6 (5 weeks)	Plasma	[81]
		↑ Peptidoglycan	Human	T1D vs controls	Plasma	[79]
		↑ cCK-18	Human	T1D vs controls	Serum (protein)	[78]
		↑ 16s DNA	Mouse	NOD at diabetes onset vs NOD (9–11 weeks) and B6 (age-matched)	Liver	[37]
	↔	↔ LPS	Mouse	NOD vs B6 (3 weeks)	Plasma	[81]
		↔ 16s DNA	Mouse	NOD (9–11 weeks) vs NOD (6 weeks)	Liver	[37]

Table 10
Blood markers in animal models - genetic modifications and interventions. The table depicts whether the barrier function was changed (↑ worsened, ↔ unchanged, ↓ improved), which method was used to identify the change (↑ significantly increased, ↔ unchanged, ↓ significantly decreased), what organism was used and which groups that were compared and at what age. The "intervention" column denotes which treatment the animals were subjected to. "Incidence effect" is the observed effect of the intervention on diabetes incidence either in numbers, if available or as delayed diabetes onset ("-" marks if none of these diabetes endpoints were evaluated). "Tissue" refers to where the permeability outcome was examined. HFD = high fat diet, LMP = low-methoxyl pectin, LPS = lipopolysaccharide, MLN = mesenteric lymph nodes, NOD=Non-Obese Diabetic, PLN = pancreatic lymph node, SNase = Staphylococcal nuclease.

		Method	Organism	Group comparison (age)	Intervention	Incidence effect	Tissue	Ref.
Barrier function	↑	↑ 16s DNA	Mouse	$NOD^{MR1-/-}$ vs $NOD^{MR1+/-}$ (15 weeks)	–	Accelerate	PLN	[36]
	↔	↔ LPS	Mouse	NOD (12 weeks)	HFD	–	Serum	[43]
				NOD (5, 13, 15, 17, 19 weeks)	SNase	Delayed	Serum	[66]
	↓	↓ LPS	Mouse	NOD (15 weeks)	Acetate-yielding diet	~65%–~30%	Serum	[67]
				NOD (15 weeks)	Butyrate-yielding diet	~65%–~40%	Serum	[67]
				NOD (22 weeks)	LMP diet	~70%–~38%	Serum	[73]
				NOD (7, 9, 11 weeks)	SNase	Delayed	Serum	[66]
		↓ 16s DNA	Mouse	NOD (7 weeks)	XOS diet	Delayed	MLN	[45]

tight junction (or related) proteins are often unchanged despite interventions that reduce the diabetes incidence. Yet, when the expression of a tight junction protein is altered, there is typically consistency between the effect seen on the diabetes incidence and the change in expression. It suggests that these measurements have biological relevance. Accordingly, molecular permeability assessment of individual proteins should be supplemented with functional studies.

6. Blood markers

The intestinal barrier function is sometimes assessed by measuring blood markers of intestinal damage or bacterial translocation. Elevated levels can indicate both increased paracellular permeability but also a profound damage to the intestine allowing bacterial components to enter the bloodstream.

6.1. Humans and animal models

T1D patients have increased serum/plasma levels of the intestinal fatty-acid binding protein (I-FABP), which is an indicator of enterocyte damage [78–80*] (Table 9). Additionally, the epithelial apoptosis marker, cytokeratin 18 caspase-cleaved fragment (cCK-18), was increased in serum from T1D patients [78]. Serum or plasma levels of lipopolysaccharide (LPS) and peptidoglycan was also increased in T1D patients [8,79] while an age-dependent increase in LPS plasma levels was found in NOD mice compared to B6 mice [81]. NOD mice at diabetes onset versus NOD mice before onset and age-matched B6 mice had higher levels of bacterial DNA (16S rRNA) in the liver [37] (Table 9).

Despite the few studies, there is consistently observed increased levels of several blood markers for intestinal damage or bacterial translocation in T1D patients suggesting an aberrant intestinal barrier function in T1D patients. This suggest that alterations in transcellular permeability in T1D should be further investigated as discussed in Section 2.1. Again, it seems that the level of bacterial translocation is highest at diabetes onset [37], thus increased permeability at diabetes onset has been observed both when using FD4 (Table 1), zonulin (Table 5), molecular markers (Table 7) and blood markers (Table 9).

6.2. Animal models - genetic modifications and interventions

Serum LPS levels were decreased in SNase-treated NOD mice at 7, 9 and 11 weeks of age but not at earlier nor later ages [66] (Table 10). LPS levels were also decreased in NOD mice on both acetate- and butyrate-yielding diets [67] and on LMP diet [73]. Serum LPS levels were unchanged in NOD mice on HFD [43]. Presence of bacterial DNA (16s rRNA) were decreased in MLN of NOD mice on XOS diet [45] while increased in PLN of $NOD^{MR1-/-}$ mice [36] (Table 10).

From these intervention studies it becomes clear that the level of bacterial translocation is alterable. Accordingly, interventions that decreased the diabetes incidence or delayed diabetes onset also decreased bacterial translocation and vice versa [36,45,66,67,73]. Again, there seems to be specific ages were these alterations are most prominent in NOD mice.

7. Conclusion

This review shows that paracellular permeability of especially the small intestine is increased in both T1D patients and animal models of T1D at both the pre-diabetic and diabetic stage. *In vivo* tests are considered the gold standard for evaluation of small intestinal permeability while *ex vivo* permeability, zonulin and blood markers appears to be good supplements. Molecular markers, on the other hand, are difficult to interpret and should not stand alone when evaluating intestinal permeability. In animal models, the intestinal permeability varies considerably with age but is also modifiable, as different interventions can alter it. Interestingly, interventions that accelerate diabetes development or reduce the age of diabetes onset typically also increase intestinal permeability, while the opposite is observed with interventions that decrease diabetes incidence or delay diabetes onset. Furthermore, several studies suggest that intestinal permeability is increased at diabetes onset in animal models. The link between diabetes development and intestinal permeability strongly suggest that increased intestinal permeability indeed is a causal factor in T1D. This link should be a focus area in the development of a future preventive strategy or cure of T1D.

Author contributions

Mia Øgaard Mønsted: conceptualization, methodology, investigation, writing – original draft, writing – review and editing, visualization. Nora Dakini Falck: methodology, investigation, writing – review and editing. Kristina Pedersen: writing – review and editing. Karsten Buschard: writing – review and editing. Laurits Juulskov Holm: writing – review and editing. Martin Haupt-Jorgensen: conceptualization, methodology, writing – review and editing, supervision.

Fundings

This work was supported by Kirsten og Freddy Johansens Fond.

Declaration of competing interest

The authors declare no competing interests. Figures were created with BioRender.com.

References

[1] F. Pociot, Å. Lernmark, Genetic risk factors for type 1 diabetes, Lancet 387 (2016) 2331–2339.

[2] P. Onkamo, S. Väänänen, M. Karvonen, J. Tuomilehto, Worldwide increase in incidence of Type I diabetes - the analysis of the data on published incidence trends, Diabetologia 42 (1999) 1395–1403.

[3] The DIAMOND Project Group, Incidence and trends of childhood Type 1 diabetes worldwide 1990–1999, Diabet. Med. 23 (2006) 857–866.

[4] C.C. Patterson, V. Harjutsalo, J. Rosenbauer, A. Neu, O. Cinek, T. Skrivarhaug, et al., Trends and cyclical variation in the incidence of childhood type 1 diabetes in 26 European centres in the 25 year period 1989 – 2013: a multicentre prospective registration study, Diabetologia 62 (2019) 408–417.

[5] J. Kaprio, J. Tuomilehto, M. Koskenvuo, K. Romanov, A. Reunanen, J. Eriksson, et al., Concordance for Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland, Diabetologia 35 (1992) 1060–1067.

[6] K. Kyvik, A. Green, H. Beck-nielsen, Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins, BMJ 311 (1995) 913–917.

[7] M.J. Redondo, L. Yu, M. Hawa, T. Mackenzie, D.A. Pyke, G.S. Eisenbarth, et al., Heterogeneity of Type I diabetes: analysis of monozygotic twins in Great Britain and the United States, Diabetologia 44 (2001) 354–362.

[8] I. Leiva-Gea, L. Sánchez-Alcoholado, B. Martin-Tejedor, D. Castellano-Castillo, I. Moreno-Indias, A. Urda-Cardona, et al., Gut microbiota differs in composition and functionality between children with type 1 diabetes and MODY2 and healthy control subjects: a case-control study, Diabetes 41 (2018) 2385–2395.

[9] M. Murri, I. Leiva, J.M. Gomez-zumaquero, F.J. Tinahones, F. Cardona, F. Soriguer, et al., Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study, BMC Med. 11 (2013).

[10] L.C. Stene, S. Oikarinen, H. Hyöty, K.J. Barriga, J.M. Norris, G. Klingensmith, et al., Enterovirus infection and progression from islet autoimmunity to type 1 diabetes: the Diabetes and Autoimmunity Study in the Young (DAISY), Diabetes 59 (2010) 3174–3180.

[11] S. Oikarinen, M. Martiskainen, S. Tauriainen, H. Huhtala, J. Ilonen, R. Veijola, et al., Enterovirus RNA in blood is linked to the development of type 1 diabetes, Diabetes 60 (2011) 276–279.

[12] L. Krogvold, B. Edwin, T. Buanes, G. Frisk, O. Skog, M. Anagandula, et al., Detection of a low-grade enteroviral infection in the islets of langerhans of living patients newly diagnosed with type 1 diabetes, Diabetes 64 (2015) 1682–1687.

[13] M. Oikarinen, S. Tauriainen, T. Honkanen, S. Oikarinen, K. Vuori, K. Kaukinen, et al., Detection of enteroviruses in the intestine of type 1 diabetic patients, Clin. Exp. Immunol. 151 (2008) 71–75.

[14] E. Bosi, L. Molteni, M.G. Radaelli, L. Folini, I. Fermo, E. Bazzigaluppi, et al., Increased intestinal permeability precedes clinical onset of type 1 diabetes, Diabetologia 49 (2006) 2824–2827.

[15] A. Sapone, L. De Magistris, M. Pietzak, M.G. Clemente, A. Tripathi, F. Cucca, et al., Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives, Diabetes 55 (2006) 1443–1449.

[16] R. Paroni, I. Fermo, L. Molteni, L. Folini, M. Rocco, A. Mosca, et al., Lactulose and mannitol intestinal permeability detected by capillary electrophoresis, J. Chromatogr. B 834 (2006) 183–187.

[17] M. Secondulfo, D. Iafusco, R. Carratù, A. Sapone, M. Generoso, R. De Rosa, et al., Ultrastructural mucosal alterations and increased intestinal permeability in non-celiac, type I diabetic patients, Dig. Liver Dis. 36 (2004) 35–45.

[18] D. Schuppan, E.G. Hahn, Celiac disease and its link to type 1 diabetes mellitus, J. Pediatr. Endocrinol. Metab. 14 (2001) 597–605.

[19] T. Not, A. Tommasini, G. Tonini, E. Buratti, M. Pocecco, C. Tortul, et al., Undiagnosed coeliac disease and risk of autoimmune disorders in subjects with Type I diabetes mellitus, Diabetologia 44 (2001) 151–155.

[20] M. Westerholm-Ormio, O. Vaarala, P. Pihkala, J. Ilonen, E. Savilahti, Immunologic activity in the small intestinal mucosa of pediatric patients with type 1 diabetes, Diabetes 52 (2003) 2287–2295.

[21] E. Badami, C. Sorini, M. Coccia, V. Usuelli, L. Molteni, A.M. Bolla, et al., Defective differentiation of regulatory FoxP3+ T cells by small-intestinal dendritic cells in patients with type 1 diabetes, Diabetes 60 (2011) 2120–2124.

[22] J. Paronen, P. Klemetti, J.M. Kantele, E. Savilahti, J. Perheentupa, H.K. Åkerblom, et al., Glutamate decarboxylase-reactive peripheral blood lymphocytes from patients with IDDM express gut-specific homing receptor α4β7-integrin, Diabetes 46 (1997) 583–588.

[23] A. Hänninen, M. Salmi, O. Simell, S. Jalkanen, Mucosa-associated (β7-integrin (high)) lymphocytes accumulate early in the pancreas of NOD mice and show aberrant recirculation behavior, Diabetes 45 (1996) 1173–1180.

[24] Y. Mullen, Development of the Nonobese diabetic mouse and contribution of animal models for understanding, Pancreas 46 (2017) 455–466.

[25] T. Damci, I. Nuhoglu, G. Devranoglu, Z. Osar, M. Demir, H. Ilkova, Increased intestinal permeability as a cause of fluctuating postprandial blood glucose levels in type 1 diabetic patients, Eur. J. Clin. Invest. (2003) 397–401.

[26] C. Maffeis, A. Martina, M. Corradi, S. Quarella, N. Nori, S. Torriani, et al., Association between intestinal permeability and faecal microbiota composition in Italian children with beta cell autoimmunity at risk for type 1 diabetes, Diabetes Metab. Res. Rev. (2016) 700–709.

[27] R. Carratù, M. Secondulfo, L. de Magistris, D. Iafusco, A. Urio, M.G. Carbone, et al., Altered intestinal permeability to mannitol in diabetes mellitus type I, J. Pediatr. Gastroenterol. Nutr. 28 (1999) 264–269.

[28] M. Kuitunen, T. Saukkonen, J. Ilonen, H.K. Åkerblom, Intestinal permeability to mannitol and lactulose in children with type 1 diabetes with the HLA-DQB1 * 02 allele, Autoimmunity 35 (2002) 365–368.

[29] P.J. Landrigan, J.L. Sly, M. Ruchirawat, E.R. Silva, X. Huo, F. Diaz-Barriga, et al., Health consequences of environmental exposures: changing global patterns of exposure and disease, Ann. Glob. Heal. 82 (2016) 10–19.

[30] J.E. Harbison, A.J. Roth-Schulze, L.C. Giles, C.D. Tran, K.M. Ngui, M.A. Penno, R. L. Thomson, et al., Gut microbiome dysbiosis and increased intestinal permeability in children with islet autoimmunity and type 1 diabetes: a prospective cohort study, Pediatr. Diabetes (2019) 574–583.

[31] T. Orban, J.M. Sosenko, D. Cuthbertson, J.P. Krischer, J.S. Skyler, R. Jackson, et al., Pancreatic islet autoantibodies as predictors of type 1 diabetes in the diabetes prevention trial-type 1, Diabetes Care 32 (2009) 2269–2274.

[32] J. Neu, C.M. Reverte, A.D. Mackey, K. Liboni, L.M. Tuhacek-tenace, M. Hatch, et al., Changes in intestinal morphology and permeability in the BioBreeding rat before the onset of type 1 diabetes, J. Pediatr. Gastroenterol. Nutr. 40 (2005) 589–595.

[33] J.B. Meddings, J. Jarand, S.J. Urbanski, J. Hardin, D.G. Gall, Increased gastrointestinal permeability is an early lesion in the spontaneously diabetic BB rat, Am. J. Physiol. 276 (1999) 951–957.

[34] A.S. Lee, D.L. Gibson, Y. Zhang, H.P. Sham, B.A. Vallance, J.P. Dutz, Gut barrier disruption by an enteric bacterial pathogen accelerates insulitis in NOD mice, Diabetologia 53 (2010) 741–748.

[35] C. Sorini, I. Cosorich, M. Lo, L. De Giorgi, F. Facciotti, R. Lucianò, et al., Loss of gut barrier integrity triggers activation of islet-reactive T cells and autoimmune diabetes, Proc. Natl. Acad. Sci. 116 (2019) 15140–15149.

[36] O. Rouxel, J. Da Silva, L. Beaudoin, I. Nel, C. Tard, L. Cagninacci, et al., Cytotoxic and regulatory roles of mucosal-associated invariant T cells in type 1 diabetes, Nat. Immunol. (2017) 1321–1331.

[37] M. Rouland, L. Beaudoin, O. Rouxel, L. Bertrand, L. Cagninacci, A. Saffarian, et al., Gut mucosa alterations and loss of segmented filamentous bacteria in type 1 diabetes are associated with inflammation rather than hyperglycaemia, Gut (2021) 1–13, 0.

[38] P. Pozzilli, A. Signore, A.J.K. Williams, P.E. Beales, NOD mouse colonies around the world- recent facts and figures, Immunol. Today. 14 (1993) 193–196.

[39] P. Rausch, M. Basic, A. Batra, S.C. Bischoff, M. Blaut, T. Clavel, et al., Analysis of factors contributing to variation in the C57BL/6J fecal microbiota across German animal facilities, Int. J. Med. Microbiol. 306 (2016) 343–355.

[40] J.T.J. Visser, N.A. Bos, L.F. Harthoorn, F. Stellaard, J. Rozing, E.A.F. van Tol, Potential mechanisms explaining why hydrolyzed casein-based diets outclass single amino acid-based diets in the prevention of autoimmune diabetes in diabetes-prone BB rats, Diabetes Metab. Res. Rev. 28 (2012) 505–513.

[41] J.T.J. Visser, K. Lammers, A. Hoogendijk, M.W. Boer, S. Brugman, A. Zandvoort, et al., Restoration of impaired intestinal barrier function by the hydrolysed casein diet contributes to the prevention of type 1 diabetes in the diabetes-prone BioBreeding rat, Diabetologia (2010) 2621–2628.

[42] T. Watts, T. Gerarduzzi, R. Zielke, I. Berti, A. Sapone, A. Fasano, et al., Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabetic-prone rats, Proc. Natl. Acad. Sci. 102 (2005) 2916–2921.

[43] Y. Xue, H. Wang, M. Du, M.-J. Zhu, Maternal obesity induces gut inflammation and impairs gut epithelial barrier function in non-obese (NOD) mice, J. Nutr. Biochem. 25 (2014) 758–764.

[44] L. Kjer-Nielsen, O. Patel, A.J. Corbett, J. Le Nours, B. Meehan, L. Liu, et al., MR1 presents microbial vitamin B metabolites to MAIT cells, Nature (2012) 1–9, 0.

[45] C.H.F. Hansen, C.S. Larsen, H.O. Petersson, L.F. Zachariassen, A. Vegge, C. Lauridsen, et al., Targeting gut microbiota and barrier function with prebiotics to alleviate autoimmune manifestations in NOD mice, Diabetologia 62 (2019) 1689–1700.

[46] L. Jia, M. Zhang, H. Liu, J. Sun, L. Pan, Early-life fingolimod treatment improves intestinal homeostasis and pancreatic immune tolerance in non-obese diabetic mice, Acta Pharmacol. Sin. (2021) 1–10, 0.

[47] T.K. Kim, J.-C. Lee, S.-H. Im, M.-S. Lee, Amelioration of autoimmune diabetes of NOD mice by immunomodulating probiotics, Front. Immunol. 11 (2020) 1–10.

[48] L.L. Clarke, A guide to Ussing chamber studies of mouse intestine, Am. J. Physiol. Gastrointest. Liver Physiol. 296 (2009) 1151–1166.

[49] I. Hadjiyanni, K.K. Li, D.J. Drucker, Glucagon-like peptide-2 reduces intestinal permeability but does not modify the onset of type 1 diabetes in the Nonobese diabetic mouse, Endocrinology 150 (2009) 592–599.

[50] D.J. Morrison, T. Preston, Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism, Gut Microb. 7 (2016) 189–200.

[51] N. Li, M. Hatch, C.H. Wasserfall, Ã.M. Douglas-escobar, M.A. Atkinson, Ã.D. A. Schatz, et al., Butyrate and type 1 diabetes mellitus: can we fix the intestinal leak? Gastroenterology 51 (2010) 414–417.

[52] H.J. Galipeau, N.E. Rulli, J. Jury, X. Huang, R. Araya, J.A. Murray, et al., Sensitization to gliadin induces moderate enteropathy and insulitis in Nonobese diabetic-DQ8 mice, J. Immunol. 187 (2011) 4338–4346.

[53] M.A. Benjamin, D.M. Mckay, P. Yang, H. Cameron, M.H. Perdue, Glucagon-like peptide-2 enhances intestinal epithelial barrier function of both transcellular and paracellular pathways in the mouse, Gut 47 (2000) 112–119.

[54] W. Wang, S. Uzzau, S.E. Goldblum, A. Fasano, Human zonulin , a potential modulator of intestinal tight junctions, J. Cell Sci. 113 (2000) 4435–4440.

[55] A. Fasano, Intestinal permeability and its regulation by zonulin: diagnostic and therapeutic implications, Clin. Gastroenterol. Hepatol. 10 (2012) 1096–1100.

[56] A. Fasano, Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer, Physiol. Rev. 91 (2011) 151–175.
[57] R. El Asmar, P. Panigrahi, P. Bamford, I. Berti, T. Not, G.V. Coppa, et al., Host-dependent zonulin secretion causes the impairment of the small intestine barrier function after bacterial exposure, Gastroenterology 123 (2002) 1607–1615.
[58] T. Vorobjova, H. Raikkerus, L. Kadaja, I. Talja, Circulating zonulin correlates with density of enteroviruses and tolerogenic dendritic cells in the small bowel mucosa of celiac disease patients, Dig. Dis. Sci. 62 (2017) 358–371.
[59] M. Ajamian, D. Steer, G. Rosella, P.R. Gibson, Serum zonulin as a marker of intestinal mucosal barrier function: may not be what it seems, PloS One 14 (2019) 1–14.
[60] L. Scheffler, A. Crane, H. Heyne, A. Tönjes, D. Schleinitz, C.H. Ihling, et al., Widely used commercial elisa does not detect precursor of haptoglobin2 , but recognizes properdin as a potential second member of the zonulin family, Front. Endocrinol. 9 (2018) 1–11.
[61] M. Simpson, An exploration of Glb1 homologue antibody levels in children at increased risk for type 1 diabetes mellitus, Pediatr. Diabetes 10 (2009) 1–16.
[62] W.C. Joesten, A.H. Short, M.A. Kennedy, Spatial variations in gut permeability are linked to type 1 diabetes development in non-obese diabetic mice, BMJ Open Diab. Res. Care. (2019) 1–9.
[63] ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US), Identifier: NCT03569007, Study to Evaluate the Efficacy and Safety of Larazotide Acetate for the Relief of CeD Symptoms; 2018 June 26, 2000 Feb 29 [cited 2021 May 20]; [about 3 pages]. Available from: https://clinicaltrials.gov/ct2/show/NCT03569007.
[64] Y. Wang, Y. Xiao, L. Zhong, D. Ye, J. Zhang, Y. Tu, et al., Increased neutrophil elastase and proteinase 3 and augmented NETosis are closely associated with b -cell autoimmunity in patients with type 1 diabetes, Diabetes 63 (2014) 4239–4248.
[65] J. Lang, X. Wang, K. Liu, D. He, P. Niu, R. Cao, et al., Oral delivery of staphylococcal nuclease by Lactococcus lactis prevents type 1 diabetes mellitus in NOD mice, Appl. Microbiol. Biotechnol. 101 (2017) 7653–7662.
[66] Y. Liang, X. Wang, D. He, Q. You, T. Zhang, W. Dong, et al., Ameliorating gut microenvironment through staphylococcal nuclease- mediated intestinal NETs degradation for prevention of type 1 diabetes in NOD mice, Life Sci. 221 (2019) 301–310.
[67] E. Mariño, J.L. Richards, K.H. Mcleod, D. Stanley, Y.A. Yap, J. Knight, et al., Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes, Nat. Immunol. 18 (2017) 552–564.
[68] M. Saitou, M. Furuse, H. Sasaki, M. Fromm, H. Takano, T. Noda, et al., Complex phenotype of mice lacking occludin , a component of tight junction strands, Mol. Biol. Cell. 11 (2000) 4131–4142.
[69] R. Al-sadi, K. Khatib, S. Guo, D. Ye, M. Youssef, T. Ma, Occludin regulates macromolecule flux across the intestinal epithelial tight junction barrier, Am. J. Physiol. Liver Physiol. 300 (2011) 1054–1064.
[70] Boom Frank van den, H. Düssmann, K. Uhlenbrock, M. Abouhamed, M. Bähler, The myosin IXb motor activity targets the myosin IXb RhoGAP domain as cargo to sites of actin polymerization, Mol. Biol. Cell. 18 (2007) 1507–1518.
[71] S.K. Chandhoke, M.S. Mooseker, A role for myosin IXb , a motor – RhoGAP chimera , in epithelial wound healing and tight junction regulation, Mol. Biol. Cell. 23 (2012) 2468–2480.
[72] K. Chen, H. Chen, M.M. Faas, B.J. de Haan, J. Li, P. Xiao, et al., Specific inulin-type fructan fibers protect against autoimmune diabetes by modulating gut immunity , barrier function , and microbiota homeostasis, Mol. Nutr. Food Res. 61 (2017).
[73] C. Wu, L. Pan, Y. Luo, W. Niu, X. Fang, W. Liang, et al., Low methoxyl pectin protects against autoimmune diabetes and associated caecal dysfunction, Mol. Nutr. Food Res. 63 (2019).
[74] C. Wu, L. Pan, W. Niu, X. Fang, W. Liang, J. Li, et al., Modulation of gut microbiota by low methoxyl pectin attenuates type 1 diabetes in non-obese diabetic mice, Front. Immunol. 10 (2019) 1–13.
[75] Y. Jin, A.T. Blikslager, The regulation of intestinal mucosal barrier by myosin light chain kinase/Rho kinases, Int. J. Mol. Sci. 21 (2020).
[76] S. Koch, C.T. Capaldo, R.S. Hilgarth, B. Fournier, C.A. Parkos, A. Nusrat, Protein kinase CK2 is a critical regulator of epithelial homeostasis in chronic intestinal inflammation, Mucosal Immunol. 6 (2013) 136–145.
[77] R.M. Shah, E.J. McKenzie, M.T. Rosin, S.R. Jadhav, S.V. Gondalia, D. Rosendale, et al., An integrated multi-disciplinary perspective for addressing challenges of the human gut microbiome, Metabolites 10 (2020) 1–35.
[78] I. Hoffmanová, D. Sánchez, V. Hábová, M. Anděl, L. Tučková, Serological markers of enterocyte damage and apoptosis in patients with celiac disease, autoimmune diabetes mellitus and diabetes mellitus type 2, Physiol. Res. (2015) 537–546, 8408.
[79] Y. Duan, R. Prasad, D. Feng, E. Beli, S.L. Calzi, A.L.F. Longhini, et al., Bone marrow-derived cells restore functional integrity of the gut epithelial and vascular barriers in a model of diabetes and ACE2 deficiency, Circ. Res. (2019) 969–988.
[80] M.M.A.L. Pelsers, W.T. Hermens, J.F.C. Glatz, Fatty acid-binding proteins as plasma markers of tissue injury, Clin. Chim. Acta. 352 (2005) 15–35.
[81] R. Emani, C. Alam, S. Pekkala, S. Zafar, M.R. Emani, Peritoneal cavity is a route for gut-derived microbial signals to promote autoimmunity in non-obese diabetic mice, Scand. J. Immunol. (2014) 102–109.