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ORIGINAL ARTICLE

NOD alleles at *Idd1* and *Idd2* loci drive exocrine pancreatic inflammation

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Abstract

Non-obese diabetic (NOD) mice spontaneously develop autoimmune diabetes and have enabled the identification of several loci associated with diabetes susceptibility, termed insulin-dependent diabetes (*Idd*). The generation of congenic mice has allowed the characterization of the impact of several loci on disease susceptibility. For instance, NOD.B6-*Idd1* and B6.NOD-*Idd1* congenic mice were instrumental in demonstrating that susceptibility alleles at the MHC locus (known as *Idd1*) are necessary but not sufficient for autoimmune diabetes progression. We previously showed that diabetes resistance alleles at the *Idd2* locus provide significant protection from autoimmune diabetes onset, second to *Idd1*. In search of the minimal genetic factors required for T1D onset, we generated B6.*Idd1*.*Idd2* double-congenic mice. Although the combination of *Idd1* and *Idd2* is not sufficient to induce diabetes onset, we observed immune infiltration in the exocrine pancreas of B6.*Idd2* mice, as well as an increase in neutrophils and pancreatic tissue fibrosis. In addition, we observed phenotypic differences in T-cell subsets from B6.*Idd1*.*Idd2* mice relative to single-congenic mice, suggesting epistatic interaction between *Idd1* and *Idd2* in modulating T-cell function. Altogether, these data show that *Idd1* and *Idd2* susceptibility alleles are not sufficient for autoimmune diabetes but contribute to inflammation and immune infiltration in the pancreas.

Keywords Autoimmune diabetes · Type 1 diabetes · Exocrine pancreas · B6 congenic strains · NOD susceptibility alleles · *Idd1* and *Idd2* loci

Introduction

Autoimmune or type 1 diabetes (T1D) is characterized by a break in immune tolerance that leads to the destruction of pancreatic β cells, resulting in insulin deficiency (Jeker et al. 2012). Susceptibility to this complex disease involves multiple loci, referred to as insulin-dependent diabetes (*Idd*) in mice and insulin-dependent diabetes mellitus (*IDDM*) in humans (Steck and Rewers 2011; Wicker et al. 1995). The non-obese diabetic (NOD) mouse model which spontaneously develops autoimmune diabetes has helped reveal over 40 loci linked to autoimmune diabetes susceptibility (Kachapati et al. 2012; Ridgway et al. 2008). Among those, the *Idd1* locus coincides with the MHC locus (Chen et al. 2018; Mullen 2017; Todd et al. 1987; Wicker et al. 1995). Engineering of a congenic mouse strain bearing NOD susceptibility alleles at the *Idd1* locus, namely, B6.NOD-*Idd1* (B6.*Idd1*) mice, also referred to as B6.$H2^{g7}$, revealed that the NOD *Idd1* locus is not sufficient to promote the development of autoimmune diabetes (Chen et al. 2018; Koarada et al. 2004; Podolin et al. 1993; Wicker et al. 1995; Yui et al. 1996). Yet B6.*Idd1* mice exhibit pancreatic infiltration of immune cells and altered T-cell proportions (Koarada et al. 2004; Rajasekaran et al. 2013; Yui et al. 1996).

NOD congenic strains bearing C57-derived loci have allowed for precise characterization of their implication in autoimmune diabetes susceptibility. Notably, the *Idd1* locus is the only one conferring full protection against overt diabetes, whereas others offer partial protection (Hill et al. 2000; Hunter et al. 2007; Wicker et al. 1992, 1994). We have recently shown that NOD mice congenic for *Idd2* are significantly protected from diabetes, second to the NOD.*Idd1* congenic mouse (Lombard-Vadnais et al. 2022). Several examples of interactions between different loci have

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also been described in the context of autoimmune diabetes (Fraser et al. 2010; Hollis-Moffatt et al. 2005; Ikegami et al. 2004; Morin et al. 2006). For example, NOD mice bearing the combination of *Idd3* with either *Idd5* or *Idd10* are highly protected from diabetes (Robles et al. 2003; Wicker et al. 2004, 1994). Hence, we wondered if the combination of NOD-derived *Idd1* and *Idd2*, the two loci conferring the highest diabetes protection, was sufficient to induce overt diabetes in the diabetes-resistant B6 mouse.

In the context of T1D, T cells are the most abundant immune cells present in the pancreas that infiltrate and actively participate in β-cell destruction (Foulis et al. 1991; Itoh et al. 1993; Knight et al. 2013; Willcox et al. 2009). T cells are critical in the development of autoimmune diabetes in mice. Indeed, T cell-deficient NOD mice never progress to overt diabetes (Mora et al. 1999; Serreze et al. 1994; Shizuru et al. 1988; Verdaguer et al. 1997), and transfer of diabetogenic T cells to non-diabetic animals is sufficient to rapidly induce disease (Berry and Waldner 2013; Chen et al. 2013; Haskins 2005; Kurts et al. 1997; Wicker et al. 1986). These observations have established T cells as central mediators in T1D development.

In this study, we took advantage of single and double-congenic mouse models to assess the impact of susceptibility alleles at *Idd1* and *Idd2* on diabetes development. We generated a single-congenic model where B6 mice bear the NOD-derived *Idd2* locus. These B6.NOD-*Idd2* mice will hereafter be referred to as B6.*Idd2*. We also generated double-congenic B6.NOD-*Idd1/Idd2* (B6.*Idd1.Idd2*) mice. While both models are resistant to autoimmune diabetes, susceptibility alleles at both the *Idd1* and *Idd2* loci increase immune infiltration in the exocrine pancreas and impact the $CD8^+$ T-cell phenotype. Moreover, the *Idd2* locus was associated with an increased proportion of neutrophils as well as pancreatic fibrosis. These observations suggest a break in immune tolerance in *Idd2* congenic mice, leading to an inflammatory response in the pancreas.

Materials and methods

Mice

C57BL/6 J (B6; #000664), B6.NOD-(*D17Mit21-D17Mit10*)/LtJ (B6.*Idd1*; #003300), and NOD/ShiLtJ (NOD; #001976) mice were purchased from The Jackson Laboratory. The congenic B6.*Idd2* strain was obtained by backcrossing B6 X NOD F1 mice to B6 mice for eight generations. Each progeny was genotyped by PCR at markers D9Mit4, D9Mit259, D9Mit328, D9Mit330, and D9Mit323, and mice bearing NOD alleles at all markers were backcrossed to B6 mice. The last generation was genotyped with additional markers (D9Mit11, D9Mit120, D9Mit182, D9Mit205, and D9Mit296) to more precisely define the congenic region (see Fig. 1A). The B6.*Idd1.Idd2* double-congenic strain was obtained by intercrossing B6.$H2^{g7}$ (B6.*Idd1*) and B6.*Idd2* mice and selecting for mouse homozygous for NOD alleles at both *Idd1* and *Idd2* loci. Due to possible genetic recombinations during intercrossing, the genotype of the B6.*Idd1.Idd2* strain was confirmed for all the markers listed in Fig. 1A. All strains were maintained at the Maisonneuve-Rosemont Hospital animal house facility. Eight- to 12-week-old males and females were used for phenotypical analysis and 30- to 36-week-old mice were used for diabetes incidence and pancreas analysis. When no differences in immunological phenotypes were observed between males and females (not shown), the data were pooled. A significant sex difference in histopathological quantification of pancreatic fibrotic regions was observed. The Maisonneuve-Rosemont Hospital ethics committee, overseen by the Canadian Council for Animal Protection, approved the experimental procedures (protocol #2021–2356).

Diabetes incidence study

Diabetes incidence was monitored daily for overt signs of diabetes (wet cage, hunched posture) and every 2 weeks for urine glucose level using Diastix (Bayer, Toronto, ON, Canada), from 10 to 30 weeks of age. At the end of the study, the absence of overt diabetes was confirmed by measuring blood glucose levels (diabetes is confirmed for values > 12 mmol/l). At culling, the pancreas was collected and conserved in formalin for at least 48 h before being sent for paraffin embedding.

Histology

To characterize pancreatic infiltration, H&E staining was performed on 6 µm pancreatic sections from paraffin-embedded specimens for 2 consecutive sections per slide. Replicates of four slides per pancreas were stained, each representing a different depth of the organ. Histopathological evaluation was performed on samples with blinded identity. Slides were scored for infiltration according to this scale: 0 = non-infiltration, 1 = peri-insulitis, 2 = less than 50% of insulitis, 3 = more than 50% insulitis, 4 = complete insulitis, and E = exocrine infiltration (Hillhouse et al. 2013). To identify fibrosis regions, Masson's trichrome staining was performed on 6 µm pancreatic sections from paraffin-embedded specimens for two non-consecutive sections per slide. All images were acquired using an automated microscope (Axio Imager 2, Zeiss). Image analysis was performed using open-source Fiji software with the Trainable Weka Segmentation plugin.

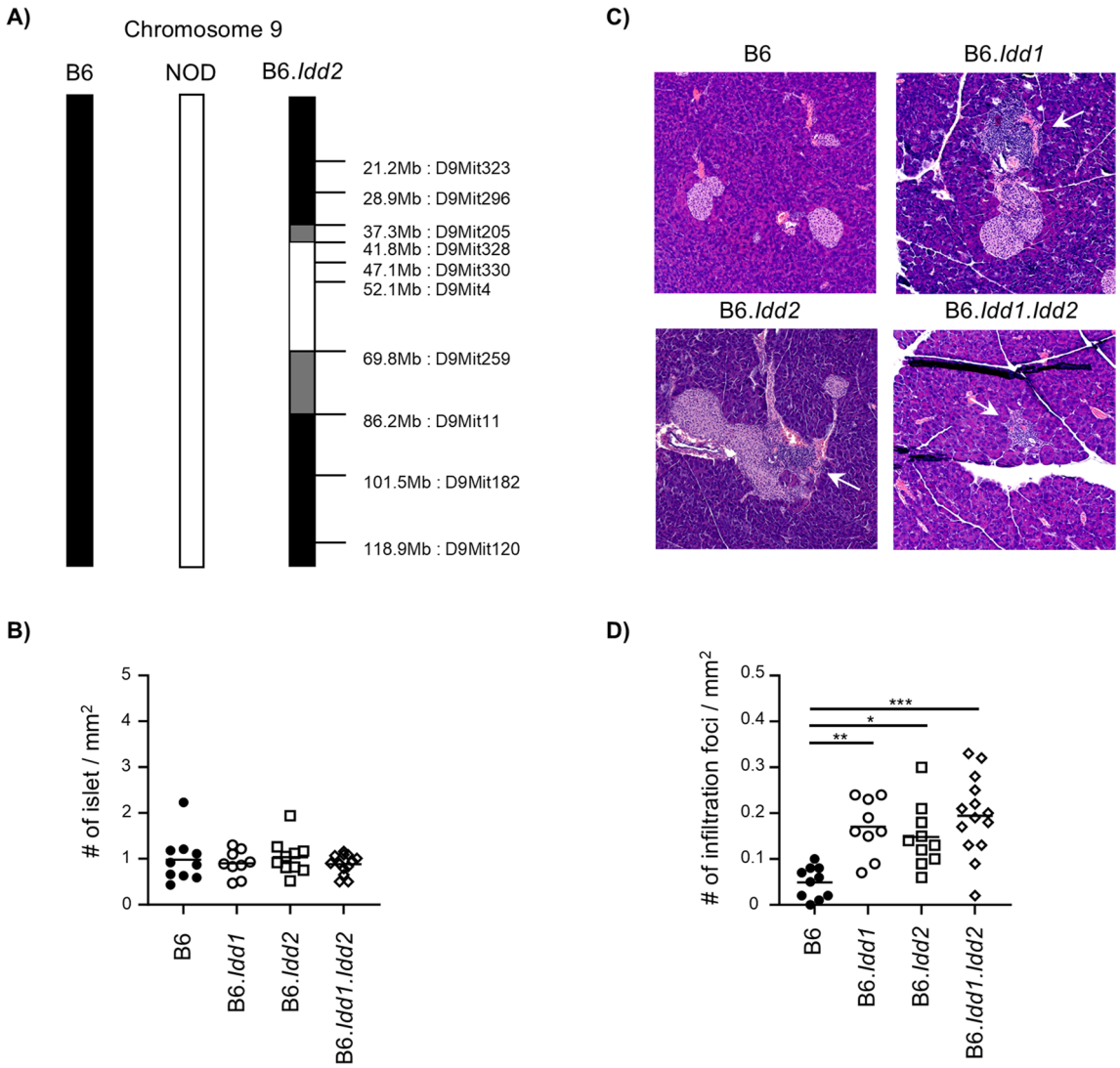

Fig. 1 NOD alleles at the *Idd1* and *Idd2* loci accentuate immune infiltration in the exocrine pancreas. **A** Representation of the chromosome 9 of the B6.*Idd2* mouse. The NOD-derived interval in the B6.*Idd2* strain is delimited based on Mit markers, as indicated. B6 alleles were confirmed at D9Mit205 and D9Mit11, and NOD alleles were confirmed at D9Mit328 and D9Mit259. Grey areas represent genomic regions of uncertain origin. B6, black; NOD, white. **B** Compilation of the number of islets per mm^2 of pancreatic tissue in 30- to 36-week-old male and female B6, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1*.*Idd2* mice. **C** Representative H&E staining of pancreatic slices from 30- to 36-week-old male and female B6, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1*.*Idd2* mice. **D** Compilation of the number of infiltration foci per $mm.^2$ of tissue from pancreatic slices of 30- to 36-week-old male and female B6, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1*.*Idd2* mice. $N > 9$; **P*-value < 0.05; ***P*-value < 0.01; ****P*-value < 0.001

Genotyping

Genomic DNA was isolated from ear punches of B6.*Idd2* and B6.*Idd1*.*Idd2* mice using the M-Fast PCR Genotyping Kit (ZmTech Scientifique). Genetic markers were used to delimit the interval on the mouse chromosome 9, namely, D9Mit323 (21.2 Mb), D9Mit296 (28.9 Mb), D9Mit205 (37.3 Mb), D9Mit328 (41.8 Mb), D9Mit330 (47.1 Mb), D9Mit4 (52.1 Mb), D9Mit259 (69.8 Mb), D9Mit11 (86.2 Mb), D9Mit182 (101.5 Mb), and D9Mit120 (118.9 Mb). B6 and NOD mice were used as controls. Marker location (in Mb) was determined using the National Center for Biotechnology Information Build m37.

Flow cytometry

Eight to 12-week-old mice were analyzed. Spleens, skin-draining lymph nodes (sdLNs), and pancreatic lymph nodes (pLNs) were pressed through a 70 µm strainer (Thermo Fischer Scientific). Spleen suspensions were treated with a solution of NH_4Cl to lyse red blood cells. Single-cell suspensions were stained with a combination of the antibodies

listed in Table 1, at 4 °C for 30 min. Data were collected on an LSRFortessa (BD Biosciences) and analyzed with the FlowJo software (BD Biosciences). Gating strategies are shown in Supplementary Fig. 1.

Statistics

Data for the various experiments were tested for significance using a one-way ANOVA followed by Tukey's multiple comparisons test. The minimal significance threshold was set at 0.05.

Results

NOD alleles at the *Idd1* and *Idd2* loci accentuate infiltration of the exocrine pancreas

Genetic studies of NOD mice have identified over 40 loci contributing to diabetes susceptibility (Kachapati et al. 2012; Ridgway et al. 2008). We have previously showed that C57BL/10 alleles at the *Idd2* locus confer significant autoimmune diabetes resistance in TCR-transgenic and non-transgenic NOD mice (Collin et al. 2014; Lombard-Vadnais et al. 2022). To determine whether the NOD *Idd2* locus is sufficient to drive overt autoimmune diabetes, we generated B6.*Idd2* congenic mice, bearing homozygous NOD alleles between D9Mit328 and D9Mit259 (Fig. 1A). We monitored diabetes incidence in B6 and B6.*Idd2* mice up until 30 weeks of age. Similar to B6 mice, no diabetes symptoms or hyperglycemia were observed in any B6.*Idd2* mouse (not shown). As significant pancreatic immune infiltration (insulitis) can be observed in non-diabetic NOD mice or NOD congenic (including NOD.*Idd2* mice (Lombard-Vadnais et al. 2022)), we collected the pancreas of mice at the end of the diabetes incidence study and quantified insulitis by histology. Consistent with the absence of progression to diabetes in B6.*Idd2* mice, we did not observe any change in islet number relative to B6 mice (Fig. 1B). In addition, we did not observe any signs of insulitis, with all islets remaining free of immune infiltration. However, we observed the presence of immune cell foci outside of the islets, in the acini and surrounding blood vessels (Fig. 1C). NOD alleles at *Idd2* therefore promote the infiltration of immune cells in the exocrine but not the endocrine pancreas (Fig. 1C, D).

Diabetes susceptibility results from the combination of several loci. The *Idd1/IDDM1* locus, coding for MHC molecules, is responsible for the highest genetic susceptibility to autoimmune/type 1 diabetes in mice and humans (Todd et al. 1987, 2007; Wicker et al. 1995). To determine if the combination of susceptibility alleles at *Idd1* and *Idd2* would induce diabetes onset, we generated B6.*Idd1*.*Idd2* double-congenic mice by intercrossing B6.*Idd1* and B6.*Idd2* mice. Similar to B6.*Idd2* mice, B6.*Idd1* and B6.*Idd1*.*Idd2* mice did not develop overt diabetes, displayed a normal number of pancreatic islets, and were free of insulitis (Fig. 1B, C).

Table 1 Key resource table

Antibodies				
Antigen	Clone	Fluorochrome	Company	Catalog number
B220	RAE-6B2	Pacific Blue	BioLegend	103227
B220	RAE-6B2	PerCP-Cy5.5	BioLegend	103233
CD3e	145-2C11	PE	BioLegend	100307
CD4	RMA4-5	BV786	BD Biosciences	563727
CD8a	53–6.7	BUV395	BD Biosciences	563686
CD8b	YTS156.7.7	A700	BioLegend	126617
CD11b	M1/70	FITC	BioLegend	101206
CD44	IM7	APC-Cy7	BioLegend	103027
CD45	30-F11	PE-Cy7	BioLegend	103113
CD49b	DX5	PE-Cy7	eBioscience	25–597181
CD62L	MEL-14	FITC	BioLegend	104405
CD69	H1.2F3	PE	BioLegend	104508
Ly-6G	A18	APC-Cy7	BioLegend	127623
TCRb	H57-597	BV711	BioLegend	109243
Viability dye			ThermoFisher	L34968
Software				
FlowJo_v10			BD Biosciences	https://www.flowjo.com
GraphPad Prism 9/10			GraphPad	https://www.graphpad.com

As for B6.*Idd2* mice, we observed immune cell infiltration in the exocrine pancreas of B6.*Idd1* and B6.*Idd1.Idd2* mice (Fig. 1D). Together, these results show that the NOD *Idd2* locus, even in combination with the *Idd1* locus, is not sufficient to induce diabetes on the B6 background. Yet both the *Idd1* and *Idd2* loci promote the infiltration of immune cells in the exocrine pancreas.

Increased LN cellularity in double-congenic mice

To investigate the changes in the immune system of B6.*Idd1* and B6.*Idd2* mice leading to pancreatic infiltration, we examined cells from the spleen and LNs of 8- to 12-week-old mice. Interestingly, we observed an increased cellularity in LNs from the double-congenic mice, for both skin-draining LNs (sdLNs) and pancreatic LNs (pLNs), relative to B6 and single-congenic mice (Fig. 2). In contrast, the cellularity of the spleen was not affected (Fig. 2).

T-cell alterations in congenic mice

We next aimed to uncover how immune cells were affected in the LNs of B6.*Idd1.Idd2* mice. As T cells are the most abundant cells in the LNs and pancreatic infiltrate during diabetes progression (Foulis et al. 1991; Itoh et al. 1993; Willcox et al. 2009), we focused our investigation on T-cell subsets (Fig. S1). Expectedly, and consistent with previous reports (Dong et al. 2021; Koarada et al. 2004), we observed an increased proportion of $CD4^+$ T cells and a decrease of $CD8^+$ T cells in mice bearing the NOD *Idd1* locus (B6.*Idd1* and B6.*Idd1.Idd2*) (Fig. 3A, B). $CD4^+$ and $CD8^+$ T-cell proportions were similar between B6 and B6.*Idd2* mice. Considering the degree of immune cell infiltration in the exocrine pancreas, we exploited the congenic models to assess T-cell activation levels. We observed similar expression of the early activation marker CD69 between all strains, for both $CD4^+$ and $CD8^+$ T cells (Fig. 4A, B). We then assessed the distribution of naive and antigen-experienced cells using CD62L and CD44 markers. Only minimal differences were observed for $CD4^+$ T cells, with a small reduction in naïve cells ($CD62L^+CD44^-$) in the sdLNs of B6.*Idd1* mice, relative to B6 mice (Fig. 5A, B). This decrease in naïve cells in the sdLNs of B6.*Idd1* mice was also observed for $CD8^+$ T cells (Fig. 5A, D). In addition, we observed a modest increase in effector memory $CD8^+$ T cells ($CD62L^-CD44^+$) in the sdLNs B6.*Idd1.Idd2* mice relative to B6.*Idd2* mice (Fig. 5E). Finally, we observed a decrease in central memory ($CD62L^+CD44^+$) $CD8^+$ T cells in the LNs of B6.*Idd1* and B6.*Idd1.Idd2* mice, relative to B6 mice (Fig. 5F). While this decrease was significant in the sdLNs of both B6.*Idd1* and B6.*Idd1.Idd2* mice, the difference was more pronounced in double-congenic mice. Similarly, a significant decrease was observed in pLNs, but only in double-congenic mice relative to B6.*Idd2* mice. Together, these data suggest that *Idd1* has an impact on T-cell subset distribution. Moreover, genetic interactions between *Idd1* and *Idd2* loci influence the proportion of effector memory T-cell subsets.

The *Idd2* locus promotes pancreatic fibrosis

Overall, the data show that *Idd1* and *Idd2* loci have an impact on T-cell phenotypes and promote immune infiltration in the exocrine pancreas. Next, we further investigated the impact of *Idd2*-driven infiltration in pancreatic tissue. Histology analysis of aged mice with Masson's trichrome staining revealed the presence of fibrotic regions in the exocrine pancreas of all strains, including B6 mice (Fig. 6A, B). Quantification of the fibrotic regions revealed a particularly high level of fibrosis in the pancreas of B6.*Idd2* mice, relative to B6 mice and the other congenic strains (Fig. 6B). Neutrophils are important mediators of fibrosis, and neutrophil-derived inflammation in the pLNs and pancreas contributes to diabetes development in NOD mice (Diana et al. 2013; Ding et al. 2021; Herrero-Cervera et al. 2022). Thus, we quantified neutrophils in the LNs of 8- to 12-week-old congenic mice. While

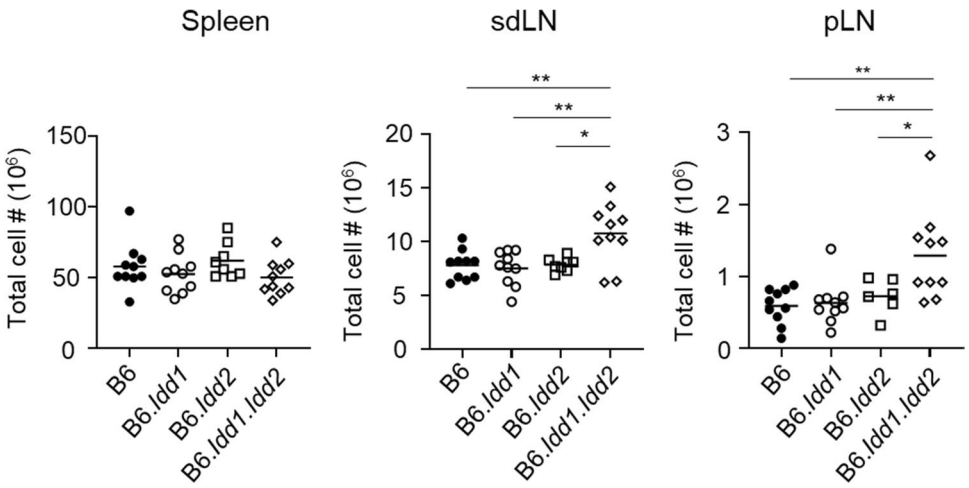

Fig. 2 Synergistic effect of NOD-derived *Idd1* and *Idd2* loci on LN cellularity. Absolute numbers of cells in the spleen, sdLNs, and pLNs of 8- to 12-week-old male and female B6, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1.Idd2* mice. $N > 5$; *P-value < 0.05; **P-value < 0.01

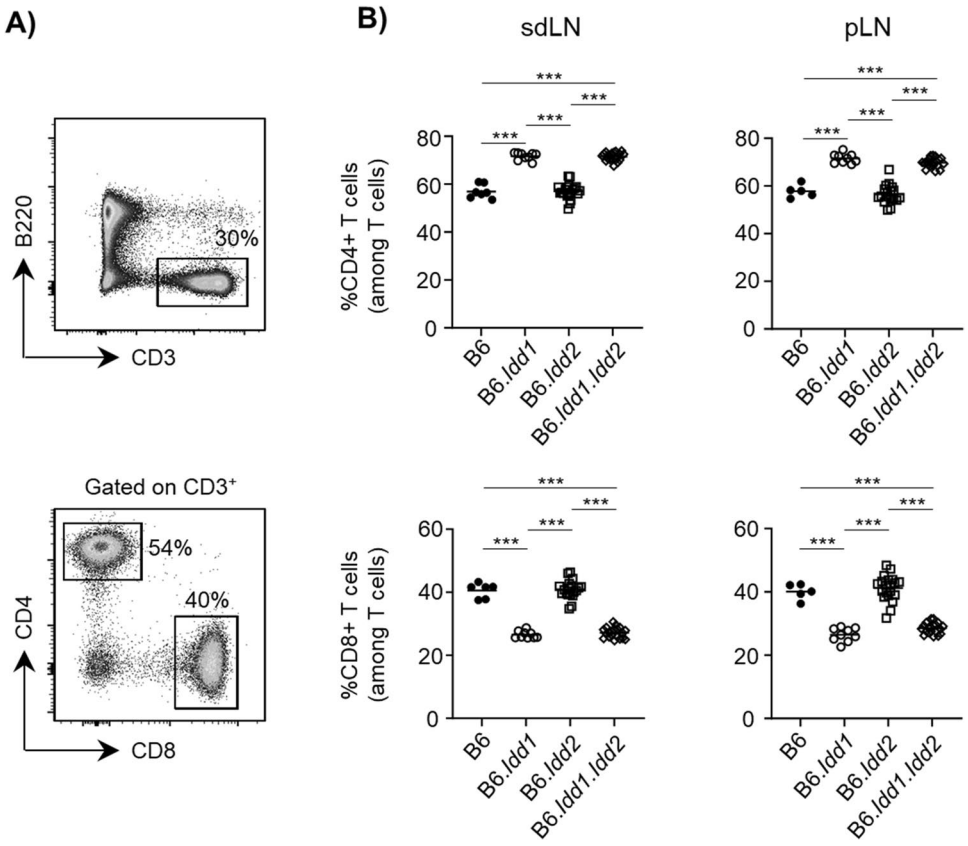

Fig. 3 NOD alleles at the *Idd1* but not the *Idd2* locus influence $CD4^+$ and $CD8^+$ T-cell proportions. **A** Representative flow cytometry profiles of $CD4^+$ and $CD8^+$ T cells in the LNs of a 10-week-old mouse. **B** Compilation of $CD4^+$ T-cell (top panels) and CD8.$^+$ T-cell (bottom panels) proportions among total T cells in sdLNs and pLNs of 8- to 12-week-old male and female B6, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1*.*Idd2* mice. $N > 5$. ***P-value < 0.01

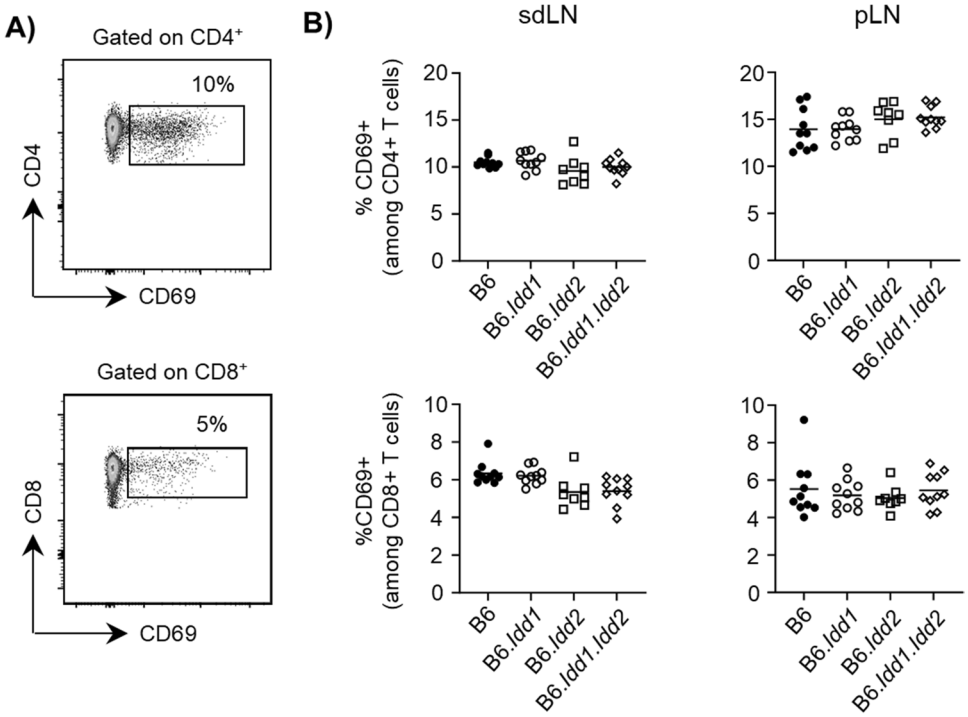

Fig. 4 No impact of *Idd1* or *Idd2* on CD69 expression in T cells. **A** Representative flow cytometry profiles of CD69 expression on $CD4^+$ and $CD8^+$ T cells in the sdLNs of a 12-week-old mouse. **B** Compilation of the percentage of CD69-expressing $CD4^+$ (top panels) and CD8.$^+$ (bottom panels) T cells in the sdLNs and pLNs of 8- to 12-week-old male and female B6, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1*.*Idd2* mice. $N > 7$

proportions of neutrophils were lower in the sdLNs of all congenic mice, relative to B6 mice (Fig. 6C), B6.*Idd2* mice displayed an increase of neutrophils in pLNs relative to the two other congenic strains (Fig. 6C). As female NOD mice are more prone to develop autoimmune diabetes (Pozzilli et al. 1993), we separated the data by sex. Strikingly, elevated fibrosis was only observed in the

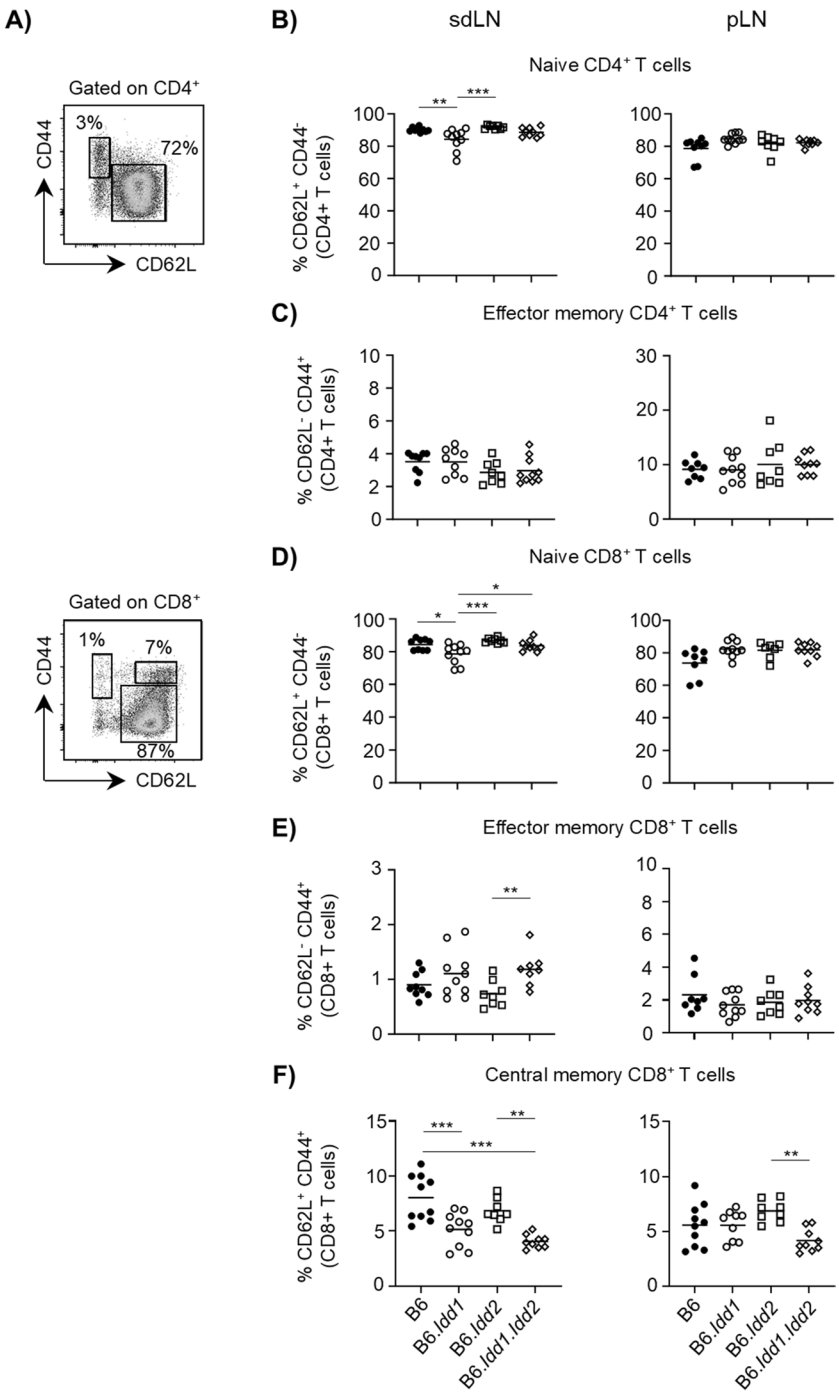

Fig. 5 NOD alleles at the *Idd1* and *Idd2* loci determine the distribution of memory T-cell subsets. **A** Representative flow cytometry profiles showing expression of CD62L and CD44 on $CD4^+$ and $CD8^+$ T cells in the sdLNs of a 10-week-old mouse. Compilation of **B** naïve and **C** effector memory $CD4^+$ T cells among total $CD4^+$ T cells and of **D** naïve, **E** effector memory, and **F** central memory $CD8^+$ T cells among total $CD8.^+$ T cells in the sdLNs and pLNs of 8- to 12-week-old male and female B6, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1.Idd2* mice. $N > 7$. **P*-value < 0.05; ***P*-value < 0.01; ****P*-value < 0.001

pancreas of female B6.*Idd2* mice, with male mice showing a similar amount of fibrosis relative to the other strains (Fig. 7A). No sex bias was observed for neutrophil proportions in the LNs (Fig. 7B). Together, these data suggest that inflammation in the exocrine pancreas of female B6.*Idd2* mice may lead to fibrosis. It also suggests that a higher abundance of neutrophils in pLNs, where priming of diabetogenic T cells takes place (Gagnerault et al. 2002), may contribute to this establishment of fibrosis.

Discussion

Over 40 loci have been linked to autoimmune diabetes susceptibility (Kachapati et al. 2012; Ridgway et al. 2008). The NOD mouse and related congenic strains have been crucial tools for the investigation of the implication of these loci in autoimmune diabetes. Here, we generated two novel congenic mice on the diabetes-resistant B6

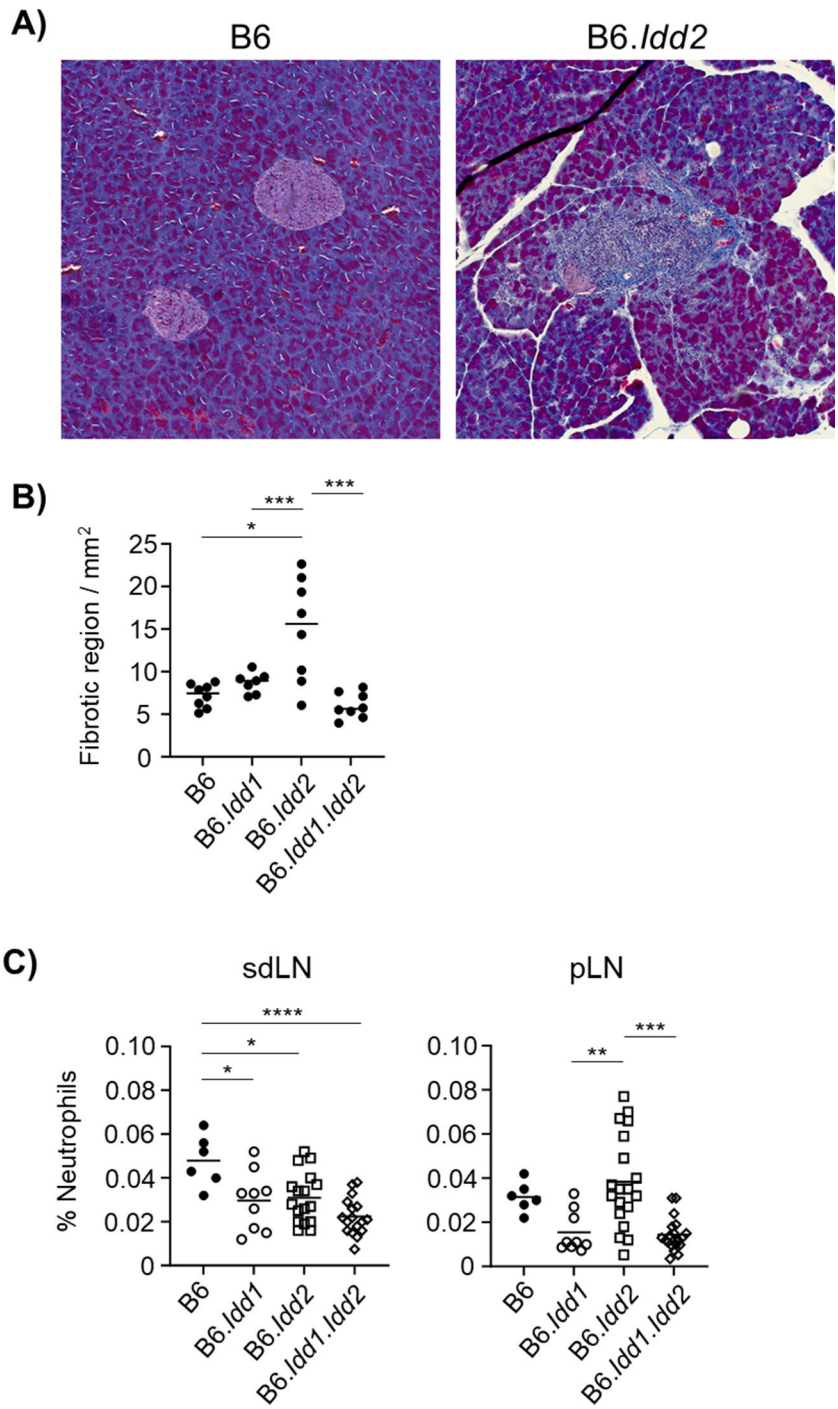

Fig. 6 NOD-derived *Idd2* locus promotes pancreatic fibrosis. **A** Representative Masson's trichrome staining of pancreatic slices showing absence of fibrosis in B6 mice (left panel) and a fibrotic area in B6.*Idd2* mice (right panel). **B** Quantification of fibrotic area from pancreatic slices of 30- to 36-week-old male and female B6, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1.Idd2* mice. **C** Compilation of neutrophil proportions among live cells in the sdLNs and pLNs of 8- to 12-week-old male and female B6, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1.Idd2* mice. $N > 5$. **P*-value < 0.05; ***P*-value < 0.01; ****P*-value < 0.001

background, named B6.*Idd2* and B6.*Idd1.Idd2*. Similar to B6.*Idd1* mice, the newly generated congenic strains do not progress to diabetes. Yet a break in immune tolerance can be observed in both strains, with immune infiltration detected in the exocrine pancreas. In the case of B6.*Idd2* female mice, this immune cell infiltration was accompanied by the presence of extensive fibrosis. B6.*Idd1.Idd2* mice also presented with altered $CD8^+$ T-cell phenotypes, with an increase of effector memory cells. Finally, and linked to the increased pancreatic fibrosis in 30- to 36-week-old mice, we observed an accumulation of neutrophils in the pLNs of 8 to 12-week-old B6.*Idd2* mice. Taken together, the data show that the *Idd2* locus, either alone or in combination with *Idd1*, can influence pancreatic infiltration, T-cell memory phenotypes, neutrophil abundance, and fibrosis.

Histology analysis revealed that NOD alleles at the *Idd2* locus are sufficient to promote immune infiltration in the

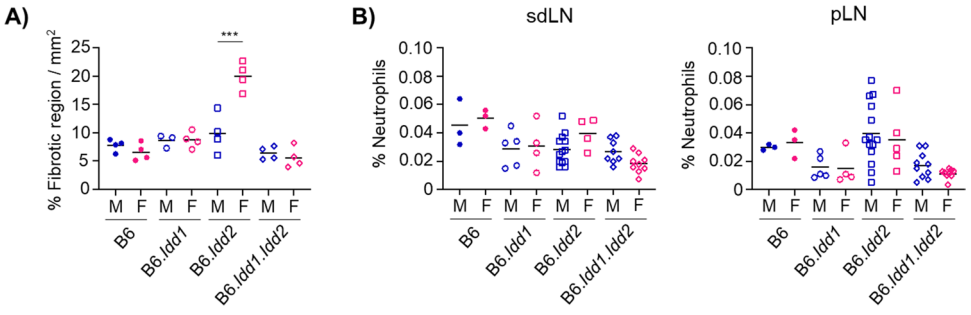

Fig. 7 Sex bias in the level of pancreatic fibrosis in B6.*Idd2* mice. **A** Compilation of fibrotic area on pancreatic slices from 30- to 36-week-old male and female B6, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1.Idd2* mice, separated by sex (blue = male mice; pink = female mice). **B** Compilation of neutrophil proportions among live cells in the sdLNs and pLNs of 8- to 12-week-old male and female B6, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1.Idd2* mice, separated by sex. ***P-value < 0.001

pancreas of B6 mice. This is consistent with our previous observation in NOD.*Idd2* mice, where B6 alleles at *Idd2* were sufficient to significantly attenuate insulitis (Lombard-Vadnais et al. 2022). Immune infiltration has also been observed in the pancreas of B6.*Idd1* congenic mice (Rajasekaran et al. 2013; Yui et al. 1996). The infiltration was described as peri-insulitis, with most immune cells accumulating around the vessels and islets, with extremely rare intra-islet infiltration (Rajasekaran et al. 2013; Yui et al. 1996). Our analysis of B6.*Idd1* mice confirmed these observations. Similarly, B6.*Idd2* and B6.*Idd1.Idd2* mice both displayed infiltration exclusively to the exocrine pancreas, with no sign of intra-islet infiltration. Thus, the results suggest that the *Idd2* locus is not sufficient to cause insulitis. Consistent with the absence of intra-islet infiltration and normal islet numbers, B6.*Idd1*, B6.*Idd2*, and B6.*Idd1.Idd2* congenic mice were resistant to diabetes, indicating that NOD alleles at *Idd2*, alone or in combination with NOD alleles at *Idd1*, are not sufficient for autoimmune diabetes progression. Additional *Idd* loci are therefore required to induce a loss of tolerance toward β cells and subsequent autoimmune diabetes progression.

As autoimmune diabetes is a T-cell-mediated disease, we investigated T-cell phenotypes in the different congenic mouse strains. As previously reported (Dong et al. 2021; Koarada et al. 2004), we observed a significant impact of *Idd1* on the CD4/CD8 T-cell ratio, leading to an increased ratio in both B6.*Idd1* and B6.*Idd1.Idd2* mice. In contrast, the *Idd2* locus did not impact $CD4^+$ and $CD8^+$ T-cell proportion relative to B6 mice. This was somewhat surprising, as the *Idd2* locus has previously been linked to $CD4^+$ T-cell frequency (Pearce 1998; Pearce et al. 1995). However, this linkage was observed in F1 mice obtained from the breeding of NON mice to NOD mice, resulting in a majority NOD-derived genetic background, suggesting that the impact of *Idd2* alleles on T-cell proportion may be driven by genetic epistasis to other NON or NOD alleles. Still, in terms of T-cell phenotypes, the most striking difference between the congenic strains was observed for $CD8^+$ T cells, with a reduction of central memory cells and an increase of effector memory cells in the LNs of B6.*Idd1.Idd2* mice. In autoimmune diabetes, a high number of self-reactive $CD8^+$ T cells exhibit an effector memory phenotype, and their abundance positively correlates with insulitis severity (Chee et al. 2014). In addition, these cells can traffic between the peripheral LNs and the pancreas (Chee et al. 2014). The higher frequency of effector memory T cells in the LNs of the B6.*Idd1.Idd2* double-congenic mice may indicate an increased propensity to generate autoantigen-specific $CD8^+$ T cells. Altogether, *Idd2*, in combination with *Idd1*, influences memory T-cell distribution and leads to pancreatic immune infiltration.

In addition to T cells, neutrophils play notable roles in diabetes development. Through NETosis and cytokine release, they accelerate tissue damage, promote autoreactive T-cell expansion, and recruit other immune cells to the pancreas (Diana et al. 2013; Fu et al. 2021; Hatanaka et al. 2006; Petrelli et al. 2022; Rosales 2018). Consistently, neutrophil depletion or inhibition of NET formation significantly reduces diabetes incidence in NOD mice (Diana et al. 2013; You et al. 2021). We observed an increase in neutrophil proportion in B6.*Idd2* mice, relative to other congenic mice, specifically in pLNs. As neutrophils tend to swarm toward sites of inflammation, this suggests a unique and ongoing inflammatory response in the pancreas of B6.*Idd2* mice relative to the other strains. Still, the increased proportion of neutrophils in the pLNs was modest in 8- to 12-week-old B6.*Idd2* mice. Considering that neutrophil response peaks at 2 weeks of age in the pancreas of NOD mice (Diana et al. 2013), it is possible that neutrophil abundance in the pLNs of B6.*Idd2* mice is highest at an earlier time point. A longitudinal study would need to be conducted to gain more insight into the dynamics of pancreatic inflammation and neutrophil trafficking in the congenic mice. In addition to neutrophils, we also observed pronounced fibrosis in the pancreas of older B6.*Idd2* mice, particularly in female mice.

Yet there were no sex differences in the proportion of neutrophils, at least in 8- to 12-week-old mice. Interestingly, the increased neutrophil abundance and increase in fibrosis were not observed in B6.*Idd1*.*Idd2* double-congenic mice, suggesting that alleles at the *Idd1* locus limit the pancreatic inflammation driven by the *Idd2* locus. Additional studies are required to understand the sex-driven differences in fibrotic phenotype observed in 30-week-old B6.*Idd2* mice. Taken together, the NOD-derived *Idd2* locus therefore promotes the establishment of pancreatic fibrosis, in addition to driving immune cell infiltration in the exocrine tissue. Overall, this study reveals that various aspects of the immune system are influenced by NOD-derived alleles at the *Idd2* locus, ultimately leading to pancreatic inflammation and fibrosis.

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DV: investigation and writing—review and editing.

FLV: investigation, conceptualization, writing (review and editing), and supervision.

SL: funding acquisition, conceptualization, visualization, writing (review and editing), and supervision.

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Data availability Upon reasonable request, the data is available by contacting the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

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