ARTICLE

F. Maurano · G. Mazzarella · D. Luongo · R. Stefanile ·
R. D'Arienzo · M. Rossi · S. Auricchio · R. Troncone

Small intestinal enteropathy in non-obese diabetic mice fed a diet containing wheat

Abstract *Aims/hypothesis:* A deranged mucosal immune response and dietary factors may play an important role in the pathogenesis of type 1 diabetes. The aims of our work were to look for the presence of small intestinal enteropathy in non-obese diabetic (NOD) mice in relation to the presence of wheat proteins in the diet, and to assess their role in the risk of developing diabetes. *Methods:* Female NOD mice were fed a standard or gluten-free diet or a gluten-free diet with the addition of wheat proteins (MGFD). Small intestine architecture, intraepithelial CD3$^+$ infiltration, epithelial expression of H2-IA, mRNA for IFN-gamma and IL-4 were assessed. *Results:* NOD mice fed a standard diet showed reduced villous height, increased intraepithelial infiltration by CD3$^+$ cells and enhanced expression of H2-IA and IFN-gamma mRNA when compared with mice on the gluten-free diet. The cumulative diabetes incidence at 43 weeks of age was 65% in the latter and 97% in the former ($p<0.01$). Mice on MGFD also showed increased epithelial infiltration and a higher incidence of diabetes. *Conclusions/ interpretation:* Mice fed a wheat-containing diet showed a higher incidence of diabetes, signs of small intestinal enteropathy and higher mucosal levels of proinflammatory cytokines.

F. Maurano (✉) · G. Mazzarella · D. Luongo · R. Stefanile ·
R. D'Arienzo · M. Rossi
Institute of Food Science, CNR,
via Roma 52 A/C,
83100 Avellino, Italy
e-mail: maurano@isa.cnr.it
Tel.: +39-0825-299506
Fax: +39-0825-781585

D. Luongo · R. Stefanile · S. Auricchio · R. Troncone
European Laboratory for the Study of
Food Induced Diseases (ELFID),
Naples, Italy

S. Auricchio · R. Troncone
Department of Pediatrics,
University Federico II,
Naples, Italy

Introduction

Type 1 diabetes is considered to be an organ-specific autoimmune disease precipitated in genetically susceptible individuals. Environmental factors such as food antigens and viruses have also been associated with type 1 diabetes. The gut immune system seems to provide the link between the environmental risk factors and type 1 diabetes. In humans, glutamate decarboxylase-reactive T cells express gut-associated homing receptor [1] and T cells derived from human diabetic pancreas show mucosal homing properties [2]; signs of inflammation in structurally normal intestine of patients with type 1 diabetes have also been described [3]. Very interestingly, a close association between coeliac disease and type 1 diabetes has been reported [4]; this finding further supports the notion that oral tolerance mechanisms are disturbed in these patients. In fact, we have recently shown that a subset of type 1 diabetic patients reacts with lymphocyte infiltration to rectal instillation of gliadin [5] and that in type 1 diabetes the small intestinal mucosa shows signs of T-cell activation when challenged in vitro with gliadin [3]. It has also been suggested that the incidence of type 1 diabetes in coeliac patients is related to the duration of exposure to gluten-containing diet [6].

The non-obese diabetic (NOD) mouse is a very well-studied model of autoimmune diabetes [7]. NOD mice develop insulitis, an early infiltration of leucocytes into the pancreas that leads to inflammatory lesions within the islets. Compelling evidence of gut immune system involvement in diabetes is also provided by this model: lymphocytes expressing mucosal adhesion molecules were found to infiltrate the islets [8–10], and mesenteric lymphocytes derived from 3-week-old NOD mice have been shown to transfer

diabetes [11, 12]. In NOD mice, but also in BioBreeding (BB) rats, another well-characterised animal model of diabetes, it has been clearly shown that diet modifies the incidence of the disease [13–15]; wheat flour and soy bean proteins remain the major identified diabetogenic food components [16, 17]. More recently, it was shown that a gluten-free diet delayed and in some cases prevented diabetes onset in NOD mice that had never been exposed to gluten [18].

The aims of our work were to look for the presence of enteropathy in NOD mice in relation to the presence of gluten in the diet, and to reassess the role of dietary gluten in the development of diabetes.

Materials and methods

Diets In a first series of experiments, mice fed the gluten-free diet (GFD) or standard diet (SD) were compared. As the GFD and SD differed in both soy and wheat proteins, we subsequently used a modified GFD (MGFD), adding only wheat proteins to the GFD, to specifically analyse the influence of wheat proteins on histology and diabetes risk. The composition of the different diets, obtained from Mucedola (Settimo Milanese, Milan, Italy), is given in Table 1. The total protein content of GFD, MGFD and SD was 19.9, 19.8 and 20.9%, respectively. The gliadin content, assessed using an in-house competitive ELISA, was estimated to be 59, 1,148 and 1,616 mg/kg in GFD, MGFD and SD, respectively. The caloric value was 18.02 kJ/kg for GFD, 17.60 kJ/kg for MGFD and 16.76 kJ/kg for SD. All diets were equally supplemented with vitamins (A, B1, B2, B6, B12, C, D3, E, K3, nicotinic acid) and had the same content of other elements, such as sodium, magnesium, copper, iron, manganese and zinc.

Animals Female NOD mice were purchased at the age of 3–4 weeks from Charles River (Calco, Milan, Italy). The mice were fed GFD ($n=34$) or SD ($n=31$). In the second series of experiments, which specifically analysed the effects of wheat proteins, female NOD mice weaned on GFD were used; their progeny were split into two groups and weaned on GFD ($n=20$) or MGFD ($n=26$) respectively. All mice were maintained under strict pathogen-free conditions and had free access to drinking water.

Diabetes assessment Mice were assessed for diabetes by blood glucose measurement using a Glucometer One-Touch (Ortho Clinic Diagnostic, Milan, Italy) at weekly intervals, starting at 12 weeks of age. A diagnosis of diabetes was made after two sequential measurements of blood glucose levels higher than 11.1 mol/l [19]. Mice were killed within 1 week of diagnosis and the pancreas and small intestine evaluated. All surviving mice were killed at 43 weeks of age.

Immunohistochemistry and microscopic evaluation Fragments of proximal jejunum and pancreas were embedded in OCT compound (Tissue Tek; Miles Laboratories, Elkhart, IN, USA). Cryostat sections (5 μm thick), fixed in acetone for 30 min at 4°C, were individually incubated for 60 min at room temperature with the primary antibodies rat anti-mouse CD3 (1:400) (Serotec, Düsseldorf, Germany) and rat anti-mouse class anti H-2IA (IgG2b) (1:5) (Serotec). After washing in triethanolamine-buffered saline (100 mmol/l Tris–HCl, 150 mmol/l NaCl), slides were incubated with biotinylated rabbit anti-rat (Dako, Milan, Italy), followed by streptavidin–peroxidase conjugate (Dako). Peroxidase activity was demonstrated by incubation in 3-amino-9-ethylcarbazole (Dako) containing hydrogen peroxide. Sections were finally counterstained with Mayer's haematoxylin solution (Dako) and mounted.

Table 1 Composition of the diets used in this study

Ingredients (g/100 g)	Gluten-free diet (GFD)	Modified gluten-free diet+3.5% protein from cereals (MGFD)	Standard diet 4RF21 certificate (SD)
	Calculated values %	Calculated values %	Calculated values %
Total protein	18.63	18.44	19.45
Protein from			
Cereals (wheat and corn)	–	–	8.32
Wheat	–	3.57	–
Potato starch	0.21	0.11	–
Peanuts	8.57	5.02	–
Milk	9.44	9.44	–
Soya	–	–	7.83
Fish	–	–	2.21
Lucerne (alfalfa)	–	–	0.67
Whey	–	–	0.06
Amino acids	1.64*	1.64*	1.78*
Lysine	0.98	0.97	0.97
Methionine	0.47	0.50	0.47
Cysteine	0.26	0.30	0.39
Threonine	0.66	0.67	0.72
Tryptophan	0.20	0.24	0.28
Arginine	1.19	1.01	1.09
Histidine	0.43	0.44	0.50
Isoleucine	0.91	0.91	0.84
Leucine	1.46	1.49	1.57
Phenylalanine	0.85	0.85	0.89
Tyrosine	0.61	0.59	0.65
Valine	1.00	1.01	0.96
Glycine	0.50	0.47	0.87
Serine	0.73	1	0.91
Amino acids, total	10.25	10.25	11.11
Protein*	1.64	1.64	1.78
Crude fat	3.85	3.98	2.58
Fibre	6.18	6.76	5.53
Ash	6.03	6.08	6.75
Gross energy [kJ/kg]	18.02	17.60	16.76

*Percent (%) from protein

Fig. 1 Jejunal mucosa archi-tecture and intraepithelial infil-tration in NOD mice on the gluten-free diet (*filled bars*; *n*=34) or standard diet (*open bars*; *n*=31). Values are means± SD. *IEL*, intraepithelial lym-phocytes. ***$p<0.001$, *t*-test for unpaired data
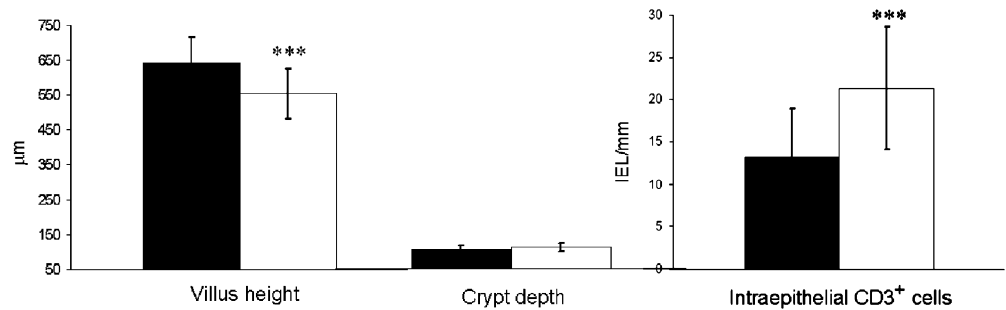
The villus height and crypt depth of 20 villi were mea-sured with an ocular micrometer; 20–30 individual mea-surements were made on each slide, and average crypt depths and villus heights were calculated. The density of intraepithelial cells expressing CD3 was determined by counting the number of stained cells per millimetre of ep-ithelium. Epithelial intestine expression of H2-IA was evaluated in terms of staining intensity and graded on an arbitrary scale: no expression, 0; weak expression 1; mod-erate expression, 2; strong expression, 3. The insulitis score was evaluated using the following scale: 0, no insulitis; 1, mild infiltration of islets; 2, moderate infiltration of islets; 3, severe infiltration of islets. Both H2-IA expression and insula infiltration were analysed independently in a blinded manner by two observers.

mRNA cytokine analysis The IFN-gamma and IL-4 mRNA levels in the small intestine and in vitro culture of lymph node cells were assessed by semiquantitative RT-PCR, as described [20]. The products of PCR were analysed on a 2% agarose gel stained with Vistra Green (Amersham Interna-tional, Little Chalfont, UK). Fluorescence scanning and quantitative analysis of detected bands were carried out using a Storm 860 system and ImageQuant software (Mo-lecular Dynamics, Sunnyvale, CA, USA). Results were expressed as the ratio (transcripts produced in the presence/transcripts produced in the absence of antigen), after nor-malisation to beta-actin mRNA levels.

Specific anti-gliadin responses Mesenteric lymph node and spleen cells were collected, passed through a stainless steel wire mesh, washed and cultured (5×10^5 cells/well) in 0.2 ml culture medium in 96-well flat-bottomed tissue culture plates at 37°C for 72 h in the absence or presence of gliadin (Sigma, St Louis, MO, USA) at 25 μg/well. Sixteen hours before collection, the cultures were pulsed with 0.037 MBq/well [^{3}H]-thymidine. Results were expressed as the stimu-lation index, calculated by dividing the mean counts per minute of triplicate cultures containing antigen by the mean counts per minute of cells cultured with medium alone.

Serum was collected and anti-gliadin IgG antibodies were assayed by ELISA as previously described [21].

Statistical analysis Data were edited, corrected and as-sessed as a descriptive analysis by using the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA). Results for all the experiments were expressed as means± SD; statistically significant differences ($p<0.05$) in the ex-amined groups were evaluated with the chi square test and *t*-test for unpaired data (two-sample analysis). The cumu-lative diabetes incidence was determined by Kaplan–Meier estimation.

Results

Morphology of jejunum and immunohistochemical anal-ysis The architecture of the small intestine showed sig-nificant differences among groups of diabetic NOD mice fed different diets. In NOD mice fed SD, villus height was 551.0 ± 69.2 μm compared with 639.0 ± 74.5 μm in mice fed
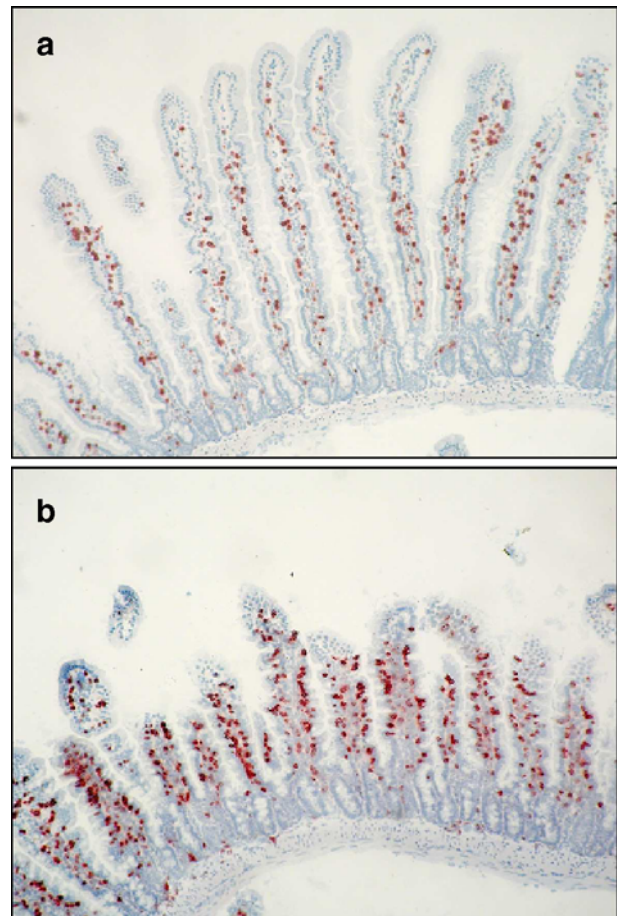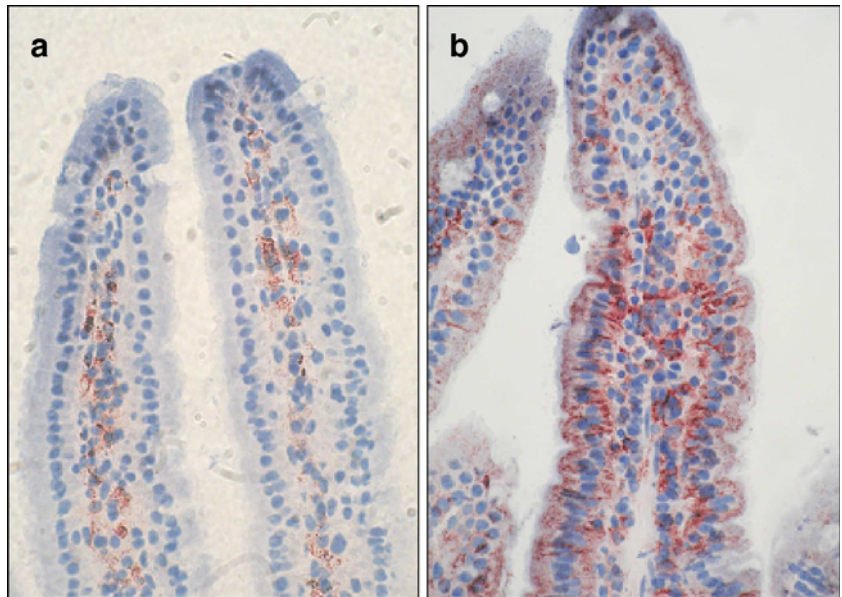

Fig. 2 Jejunal architecture and CD3$^+$ lymphocyte infiltration in the jejunum of NOD mice on the gluten-free diet (**a**) and standard diet (**b**)

Fig. 3 H2-IA expression on jejunal epithelial cells of NOD mice on the gluten-free diet (**a**) and standard diet (**b**)

GFD ($p<0.001$); by contrast, no significant differences were found for crypt depth (SD vs GFD, 107.2±10.7 vs 102.4±9.7; p=NS) (Fig. 1). A significant difference in the number of intraepithelial CD3$^+$ cells was also observed (SD vs GFD, 21.5±7.2 vs 12.8±6.2 CD3$^+$ cells/mm epithelium; $p<0.001$; Figs. 1 and 2). Interestingly, the H-2IA molecule was not found to be expressed in the villous epithelium in any of the NOD mice fed GFD enterocytes, but was expressed there in 38% of those on SD ($p<0.01$; Fig. 3). In the latter group, 15, 25 and 60% showed weak, moderate and strong epithelial expression, respectively.

As GFD and SD differed in both soy and wheat proteins, we used a modified gluten-free diet (MGFD), adding only wheat proteins to the GFD, to specifically analyse the influence of wheat proteins. Significant differences in small intestinal architecture were again found when mice from GFD colonies fed GFD or MGFD mice were compared. No significant differences were found for villus height (MGFD vs GFD, 667.3±75.8 vs 668.0±78.9; p=NS) and crypt depth (MGFD vs GFD, 105.2±8.5 vs 104.0±8.8; p=NS), but a significant difference in the number of intraepithelial CD3$^+$ cells was again observed (MGFD vs GFD, 18.0±6.6 vs 14.6±4.1 CD3$^+$ cells/mm epithelium; $p<0.05$) (Fig. 4).

Cytokine mRNA To assess if the infiltrative pattern observed and the enhanced expression of class II histocompatibility molecules on the epithelium in NOD mice fed wheat proteins could be attributed to greater expression of proinflammatory cytokines, IFN-gamma and IL-4 transcript levels were examined. IFN-gamma transcript levels were higher in diabetic NOD mice on SD than in those on GFD (SD vs GFD, 0.16±0.05 vs 0.07±0.04 densitometric units, $p<0.01$; Fig. 5), while IL-4 expression did not differ between the two groups (Fig. 5). The level of cytokine mRNA showed no significant difference when GFD and MGFD were compared.

Specific anti-gliadin responses The gliadin-specific in vitro cell proliferation responses were measured in mesenteric lymph node cells isolated from all groups of mice. No evidence was found of primed T-cell-mediated responses; similarly, levels of mRNAs for IFN-gamma and IL-4 measured in mesenteric lymph node 24 h after gliadin stimulation were unchanged. Finally, no differences were noted in serum anti-gliadin titres between NOD mice fed different diets (data not shown).

Incidence of diabetes The cumulative diabetes incidence at 43 weeks of age was 65% (n=34) and 97% (n=31) in GFD and SD mice respectively ($p<0.01$). These observations represent cumulative data from three experiments involving 20, 20 and 25 mice respectively. In all experiments a trend towards a 'protective' effect of GFD was observed. Also, the survival curve showed a highly significant dif-

Fig. 4 Jejunal mucosa architecture and intraepithelial infiltration in NOD mice on the gluten-free diet (*filled bars*; n=20) or the modified gluten-free diet (*open bars*; n=26). Mice were from a colony reared on the gluten-free diet. Values are means±SD. *IEL*, intraepithelial lymphocytes. *$p<0.05$, t-test for unpaired data
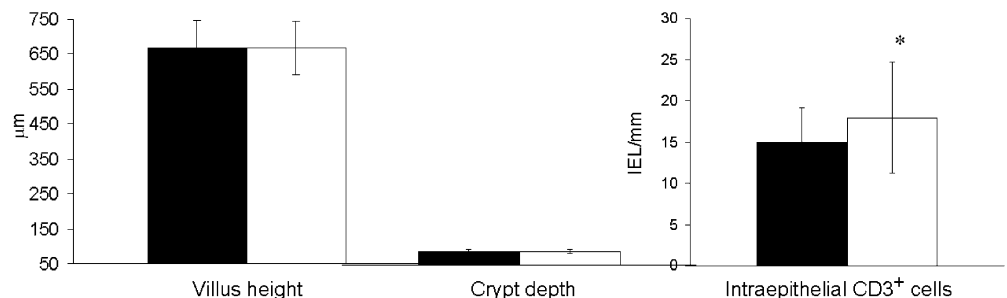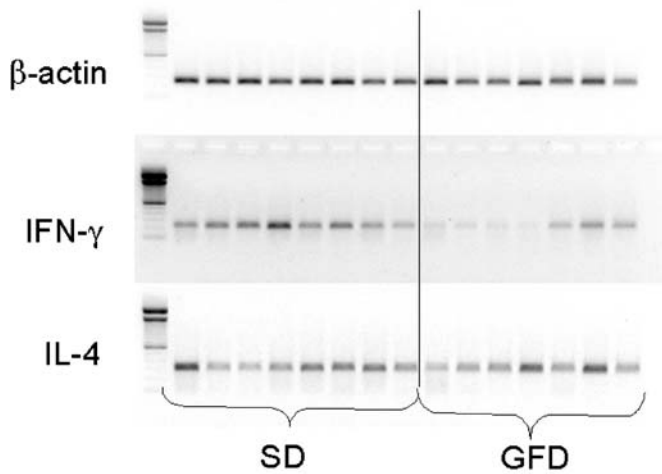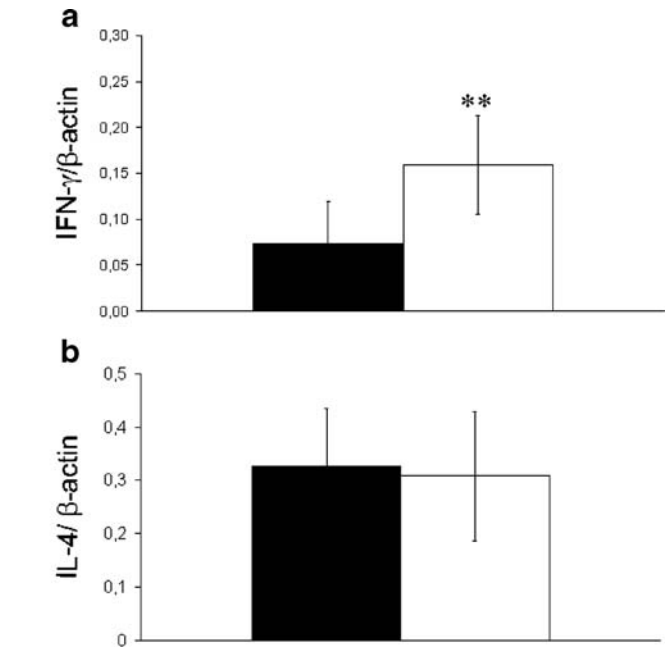
Fig. 5 Interferon-gamma and IL-4 mRNA expression in jejunal fragments from diabetic NOD mice. Increased expression of IFN-gamma mRNA in 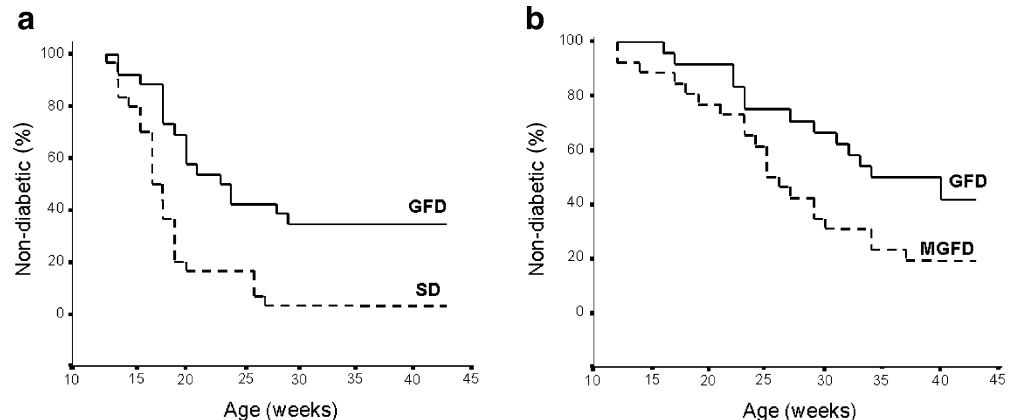NOD mice on the standard diet (*open bars*; *n*=8) compared with NOD mice on the gluten-free diet (*filled bars*; *n*=7). RNA was extracted from tissue and cytokines were measured using semiquantitative RT-PCR. Values are means±SD. **$p<0.01$

ference between the two examined groups ($p<0.001$); the relative risk was estimated to be 0.68 (95% confidence interval, 0.51–0.90; Fig. 6). Blood glucose levels in diabetic mice ranged between 11.1 mmol/l and 33.3 mmol/l, whilst no significant differences in body weights were observed between diabetic mice fed GFD and SD. The median age of disease onset was 28 weeks and 19 weeks for GFD and SD respectively ($p<0.001$). The histological evaluation of the haematoxylin and eosin-stained pancreatic sections revealed severe terminal stages of insulitis in diabetic animals; the severity of insulitis in diabetic animals was not related to the diet regimen (insulitis score, GFD vs SD, 2.38 vs 2.50).

In the second series of experiments, mice from the GFD colony were split into two groups and weaned on GFD and MGFD diets. The incidence of diabetes in mice born to GFD dams and weaned on GFD was lower than in mice weaned on MGFD (54 vs 84%; $p<0.05$).

Discussion

The present study shows that NOD mice fed a wheat protein-containing diet have histological signs of enteropathy. Furthermore, it demonstrates that the presence of wheat proteins in the diet of NOD mice favours the spontaneous development of diabetes.

Evidence that experimental type 1 diabetes may be related to antigens encountered via the gut lumen was first obtained in studies performed in BB rats [17]. In NOD mice, Funda et al. [18] showed that, in animals that had never been exposed to cereal proteins, a GFD (a diet lacking wheat and oat proteins) delayed and, to a large extent, prevented diabetes. Recently, delayed exposure to wheat and barley was found to reduce diabetes incidence in NOD mice [22]. In our first series of experiments we compared NOD mice from a colony reared on a normal diet and weaned on a normal or wheat-free diet. Compared with the standard diet, the wheat-

Fig. 6 Cumulative incidence of diabetes in NOD mice on different diets. **a** Gluten-free (GFD) vs standard diet (SD) ($p<0.01$). **b** GFD vs modified gluten-free diet (MGFD) (from GFD colony) ($p<0.05$)

free diet was also a soy-free diet, with wheat proteins mainly replaced by peanuts and milk proteins. The results, in terms of fewer animals developing diabetes and late onset of the disease, were very clear, but a diabetogenic effect of soy or a protective effect of peanuts and milk could not be excluded. In the subsequent series of experiments, more stringent conditions were introduced; instead of an SD, an MGFD was used, represented by a GFD with the addition of only wheat proteins. Again, a diabetogenic effect of the MGFD was noted, although it was less marked than in the first series of experiments. This smaller effect could have been due to the lower content of wheat proteins. The most relevant of our observations was the finding of enteropathy in NOD mice on a standard diet; the enteropathy, as defined by altered small intestinal architecture and increased intraepithelial infiltration, was quite mild. However, the alterations noted were of the same extent as in other experimental models of immune-mediated enteropathy [23]; adult mice are more resistant to villous atrophy than rats or mice in their first 2 weeks of life. Again, the degree of enteropathy was less in mice fed MGFD compared with those fed SD; in the former the main finding was the increased intraepithelial infiltration. The reasons for the difference could have been the lower content of wheat protein compared with the SD, but also the lack of soy proteins in the MGFD. The intraepithelial infiltration by $CD3^+$ cells and the enhanced expression of class II histocompatibility molecules on the intestinal epithelium suggest local immunological dysregulation; in particular, H2-IA overexpression indicates greater synthesis of mRNA for IFN-gamma. These observations are in keeping with those recently published by Flohè et al. [24]; they found a shift towards a proinflammatory cytokine milieu in the gut of NOD mice after feeding a cereal-based diet compared with a hypoallergenic semipurified diet: upregulation for mRNAs for IFN-gamma and TNF-alpha was reported, together with an increase in the inflammatory marker iNOS. More recently, NOD mice fed a wheat- and barley-free diet were found to show an altered cytokine profile in the gut or pancreas [22]. It is unclear if the effect observed is a direct consequence of immunomodulation by wheat proteins; alternatively, it could be a consequence of changes in the gut flora.

Among wheat proteins, gluten remains the best candidate for being responsible for such changes, but we found no evidence for a gliadin-specific immune response in cells obtained from mesenteric lymph nodes. On the other hand, it has been reported that addition of gliadin to a wheat- and barley-free diet did not completely restore diabetogenicity [22]. Finally, it should be not forgotten that other wheat proteins have been implicated in the pathogenesis of diabetes; antibodies to G1b1, a wheat storage globulin, have been found in the serum of diabetic patients but not in age-, sex- and HLA-matched controls [25].

In conclusion, we have confirmed that wheat proteins exert a diabetogenic effect in NOD mice. Most importantly, we have shown that NOD mice show a mild enteropathy, which is dependent on their diet, most probably on the presence of wheat proteins in it.

References

1. Paronen J, Klemetti P, Kantele J-M et al (1997) Glutamate–decarboxylase-reactive peripheral blood lymphocytes from patients with IDDM express gut specific homing receptor $\alpha_4\beta_7$-integrin. Diabetes 46:583–588
2. Hänninen A, Jalkanen S, Salmi M, Toikkanen S, Nikolakaros G, Simell O (1992) Macrophages, T cell receptor usage, and endothelial cell activation in the pancreas at the onset of insulin-dependent diabetes mellitus. J Clin Invest 90:1901–1910
3. Auricchio R, Paparo F, Maglio M et al (2004) In vitro deranged intestinal immune response to gliadin in type 1 diabetes. Diabetes 53:1680–1683
4. Holmes G-K (2002) Coeliac disease and type 1 diabetes mellitus—the case for screening. Arch Dis Child 87:495–498
5. Troncone R, Franzese A, Mazzarella G et al (2003) Gluten sensitivity in a subset of children with insulin dependent diabetes mellitus. Am J Gastroenterol 98:590–595
6. Ventura A, Magazzu G, Greco L (1999) Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease. Gastroenterology 117:297–303
7. Delovitch TL, Singh B (1997) The nonobese diabetic mouse as a model of autoimmune diabetes: immune dysregulation gets the NOD (Review). Immunity 7:727–738
8. Yang XD, Sytwu HK, McDewitt HO, Michie SA (1997) Involvement of β7 integrin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in the development of diabetes in nonobese diabetic mice. Diabetes 46:1542–1547
9. Hanninen A, Jaakkola I, Jalkanen S (1998) Mucosal addressin is required for the development of diabetes in nonobese diabetic mice. J Immunol 160:6018–6025
10. Hanninen A, Salmi M, Simell O, Jalkanen S (1996) Mucosa-associated (beta 7-integrin^{high}) lymphocytes accumulate early in the pancreas of NOD mice and show aberrant recirculation behaviour. Diabetes 45:173–180
11. Hanninen A, Jaakkola I, Jalkanen S (1996) High prevalence of diabetogenic lymphocytes in gut-associated lymphoid tissue of young NOD mice. Scand J Immunol 43:735–738
12. Jaakkola I, Jalkanen S, Hanninen A (2003) Diabetogenic T cells are primed both in pancreatic and gut-associated lymph nodes in NOD mice. Eur J Immunol 33:3255–3264
13. Scott FW, Trick KD (1983) Dietary modification of spontaneous diabetes in the BB Wistar rat. Proc Can Fed Biol Soc 26:222
14. Elliot RB, Reddy SN, Bibby NJ, Kida K (1988) Dietary prevention of diabetes in the non-obese diabetic mouse. Diabetologia 31:62–64
15. Scott FW, Cloutier EH, Kleeman R et al (1997) Potential mechanisms by which certain foods promote or inhibit the development of spontaneous diabetes in BB rats: dosage, timing, early effect on islet area, and switch in infiltrate from Th1 to Th2 cells. Diabetes 46:589–598
16. Scott FW, Marliss EB (1990) Conference summary: diet as an environmental factor in development of insulin-dependent diabetes mellitus. Can J Physiol Pharm 69:311–319
17. Hoorfar J, Buschard K, Dagnes-Hansen F (1993) Prophylactic nutritional modification of the incidence of diabetes in autoimmune nonobese (NOD) mice. Br J Nutr 69:597–607
18. Funda DA, Kaas A, Bock T, Tlaskalova-Hogenova, Bushard K (1999) Gluten-free diet prevents diabetes in NOD mice. Diabetes/Metab Res Rev 15:323–327

19. Trembleau S, Penna G, Gregori S, Chapman HD, Serreze D, Magram J, Adorini L (1999) Pancreas-infiltrating Th1 cells and diabetes develop in IL-12-deficent nonobese diabetic mice. J Immunol 163:2960–2968

20. Senger S, Luongo D, Maurano F et al (2003) Intranasal administration of a recombinant α-gliadin down-regulates the immune response to wheat gliadin in DQ8 transgenic mice. Immunol Lett 88:127–134

21. Rossi M, Maurano F, Caputo N et al (1999) Intravenous or intranasal administration of gliadin is able to down-regulate the specific immune response in mice. Scand J Immunol 50:177–182

22. Schmid S, Koczwara K, Schwinghammer S, Lampasona V, Ziegler AG, Bonifacio E (2004) Delayed exposure to wheat and barley proteins reduces diabetes incidence in non-obese diabetic mice. Clin Immunol 111:108–118

23. Strobel S, Ferguson A (1986) Modulation of intestinal and systemic immune response to a fed protein antigen, in mice. Gut 27:829–837

24. Flohè SB, Wasmuth HE, Kerad JB et al (2003) A wheat-based, diabetes-promoting diet induces a Th1-type cytokine bias in the gut of NOD mice. Cytokine 21:149–154

25. MacFarlane AJ, Burghardt KM, Kelly J, Simell T, Simell O, Altosaar I, Scott FW (2003) A type 1 diabetes-related protein from wheat (Triticum aestivum). cDNA clone of a wheat storage globulin, Glb1, linked to islet damage. J Biol Chem 278:54–63