Immune Reactivity to a Glb1 Homologue in a Highly Wheat-Sensitive Patient With Type 1 Diabetes and Celiac Disease

MAJID MOJIBIAN, MLD[1,2]
HABIBA CHAKIR, PHD[1,2]
AMANDA J. MACFARLANE, PHD[1,2]
DAVID E. LEFEBVRE, BSC[1,2]
JOHN R. WEBB, PHD[2]
CLAIRE TOUCHIE, MD[3]
JACOB KARSH, MD[3]
JENNIFER A. CROOKSHANK, MSC[1]
FRASER W. SCOTT, PHD[1,2]

In type 1 diabetes, the environmental factors that induce the patient's immune system to attack and destroy the insulin-secreting β-cells are not known. There is evidence that some cases of type 1 diabetes could be wheat related (1,2). Exposure to wheat protein was a risk factor for development of type 1 diabetes autoantibodies in high-risk infants (1,2), and as many as 2–8% of type 1 diabetic patients have celiac disease (3), also known as gluten-sensitive enteropathy. Some studies in animals also suggest that wheat could be involved (4–7). Recently, a wheat protein, WP5212, with 80% amino acid homology to wheat storage globulin-1 (Glb1), was identified as the first candidate diabetes-related wheat protein in diabetes-prone rats (5). We report the case of a highly wheat-sensitive patient with type 1 diabetes/celiac disease who displayed strong antibody and T-cell responses to this protein.

A 28-year-old Caucasian female, with a 10-year history of type 1 diabetes, presented early in 1999 with diarrhea followed by oral ulcerations and severe lip swelling (Fig. 1*A*). At diagnosis, antigliadin IgG, anti-tissue transglutaminase IgA, and endomysial antibodies were present. The diarrhea and lip swelling improved initially with a gluten-free diet, but the symptoms recurred within a few months with worsening of the oral ulcerations, lip swelling, and continued diarrhea. Despite 6 months of a standard gluten-free diet, a duodenal biopsy showed mild villous atrophy. Colonoscopy and small bowel follow-through were normal. Biopsy of the oral lesion showed noncaseating granulomas, which were negative for IgG, IgA, IgM, C3, fibrinogen, and albumin with no histological evidence of eosinophilia. The patient was evaluated by a clinical allergist. Skin prick tests for several foods, including wheat, were all negative, and there was no evidence of IgE-mediated food allergy. In December 2001, the mucosa of her lower lip was extensively ulcerated with the formation of a fistula in the lower right lip area. She was having 7–10 bowel movements in a 24-h period with nocturnal incontinence. Magnetic resonance imaging ruled out the presence of Melkersson-Rosenthal syndrome. The patient initiated a strict specified carbohydrate, cereal-free diet (8). In a period of weeks the diarrhea resolved with an average of two bowel movements a day and healing of her mouth ulcerations. Over the next few months, her lips returned to a normal level, and her condition has remained stable for the last 3 years.

RESEARCH DESIGN AND METHODS

— The case of this 28-year-old female with type 1 diabetes and celiac disease was studied. The patient had the diabetes-associated HLA types DRB1*0401, DRB1*0301, and DQB1*0302, as well as the celiac-associated HLA types DRB1*0301, DQB1*0201, and HLA DR53. Control subjects ($n = 8$) with no significant medical history ranging in age from 24 to 40 years were investigated. The study was approved by the Ottawa Hospital Research Ethics Board, and informed consent was obtained.

Probing wheat proteins to detect antibody reactivity in serum

Wheat protein–expressing clones identified previously were screened with serum from the type 1 diabetic/celiac disease patient or healthy control subjects as described (5).

Primary reactivity of peripheral blood mononuclear cells to wheat gluten protein components

Analyses of the patient's blood were performed on samples obtained on two occasions during clinical remission. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient. Cells were labeled with 2 μmol/l 5,6-carboxyfluorescein succinimidyl ester and incubated at 37°C in 5% CO_2. Cells were cultured with various concentrations of chymotrypsin-treated, heat-inactivated wheat gluten protein (3.2–12.4 μg/ml), gliadin 10 μg/ml (Sigma), fractionated Glb1 bound to nitrocellulose (Glb1) 10 μg/ml, ovalbumin 1 μmol/l, Tetanus toxoid 2.7 LF/ml, and phytohemaglutinin 5 μg/ml. After 2 days, 10 IU/ml interleukin (IL)-2 was added to each well. On day 8, proliferation was assessed using a 5,6-carboxyfluorescein succinimidyl ester–based flow cytometric assay, with results expressed as cell division index (9) and the concentration of Th1 or Th2 cytokines, IFN-γ and IL-5, respectively, analyzed in supernatants using enzyme-linked immunosorbent assay.

From [1]Molecular Medicine, Ottawa Health Research Institute, Ottawa, Ontario, Canada; the [2]Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Ontario, Canada; and the [3]Department of Medicine, The Ottawa Hospital, Ottawa, Ontario, Canada.

Address correspondence and reprint requests to Fraser W. Scott, Molecular Medicine, Ottawa Health Research Institute, 501 Smyth Rd., Ottawa ON, K1H 8L6. E-mail: fscott@ohri.ca.

Received for publication 18 November 2005 and accepted in revised form 21 January 2006.

Abbreviations: Glb1, wheat storage globulin-1; IFN-γ, γ-interferon; IL, interleukin; PBMC, peripheral blood mononuclear cell.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

DOI: 10.2337/dc05-2260

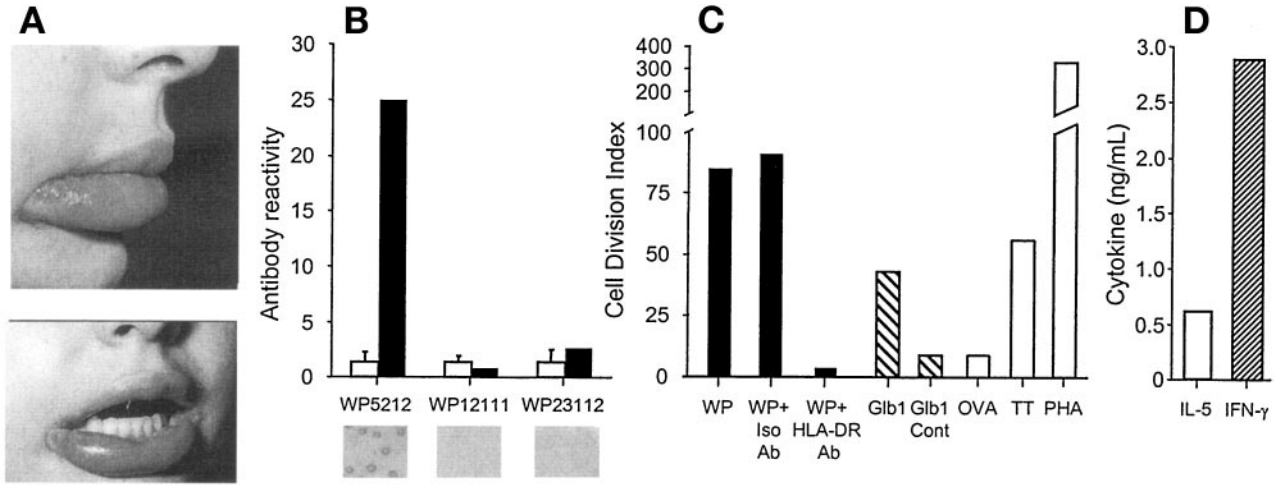

Figure 1—*Wheat-sensitive patient with Glb1 antibodies and strong wheat protein–induced T-cell reactivity.* A*: A highly wheat-sensitive patient with type 1 diabetes and celiac disease developed swelling of the lip and ulceration of the cheek when following a standard celiac diet.* B*: Even after several years on a much stricter diet, there was strong antibody reactivity to the Glb1 clone WP5212 (pictured below the graph), as further illustrated in the densitometric analysis (graph). Black bars are patient results, open bars are age-matched healthy subjects (mean ± SD,* n = *8). Antibody reactivity: mean intensity/pixel* × 10^3 *expressed relative to a negative clone, WPCON.* C*: CD3*$^+$ *T-cells showed strong proliferation in response to 6 μg/ml wheat gluten proteins that were blocked by adding antibodies against HLA-DR but not by adding the isotype (Iso) antibody (filled bars). T-cells also responded to Glb1 but not to Glb1 negative control (hatched bars). There was no response to an irrelevant food antigen, chicken ovalbumin (OVA), whereas response was strong to the positive control recall antigen tetanus toxoid (TT) and the T-cell mitogen phytohemaglutinin (PHA) (open bars). Cell division index was calculated as described by Mannering et al. (9).* D*: Cytokine response of patient PBMC to Glb1 measured by enzyme-linked immunosorbent assay.*

RESULTS

Antibody reactivity to Glb1 protein in the patient with type 1 diabetes/celiac disease

The patient showed strong antibody reactivity to Glb1 (clone WP5212) in comparison with control subjects (Fig. 1*B*). On dilution of the serum, antibody specificity increased relative to a control protein, WPCON, whereas that of control subjects remained low (data not shown). Antibody reactivity to two non–diabetes-related wheat proteins from the wheat cDNA library, WP12111 and WP23112, was low at all dilutions for both the patient and control subjects.

Stimulation of PBMC from the patient with type 1 diabetes/celiac disease but not in control subjects

$CD3^+$ T-cells from the type 1 diabetic/celiac disease patient proliferated in response to an extract of partially digested wheat gluten proteins Fig. 1*C*) and to gliadin, whereas cells from control subjects did not (data not shown). $CD3^+$ T-cells also proliferated in response to Glb1, and cells secreted high concentrations of the Th1 cytokine, γ-interferon (IFN-γ), relative to the Th2 cytokine, IL-5 (Fig. 1*D*). Response to the dietary antigen ovalbumin was low and similar to the negative controls, with strong responses to the recall antigen, tetanus toxoid, and phytohemaglutinin. The addition of HLA DR antibody blocked the $CD3^+$ T-cell response to wheat gluten proteins.

CONCLUSIONS — Immune reactivity to wheat usually decreases when celiac patients are placed on a gluten-free diet and it reappears only after subsequent rechallenge. The type 1 diabetic/celiac disease patient in our study showed unusually high sensitivity to cereals, which was present even when following a standard gluten-free diet (Fig. 1*A*). During this time, she experienced inflammation, ulceration of the mouth, and severe diarrhea. These symptoms only resolved when she adopted a strict, specified carbohydrate, cereal-free diet (8). Despite maintaining this strict diet for more than 3 years, her immune response to wheat persisted and was characterized by strong wheat gluten protein and Glb1-specific T-cell proliferation. She displayed very strong antibody response to Glb1 (Fig. 1*B* and *C*). The disappearance of the symptoms despite the persistence of humoral and cellular immune reactivity to wheat suggests the beneficial effect of the strict wheat-free diet was due to avoidance of specific immunogenic dietary proteins.

PBMC and wheat gluten protein–specific $CD3^+$ T-cells from the type 1 diabetic/celiac disease patient proliferated in response to wheat gluten protein and Glb1 (Fig. 1*C*). PBMCs from healthy control subjects did not respond to these antigens. This difference in T-cell reactivity was not explained by differences in the type or proportion of T-cells, which were similar in the patient and control subjects (data not shown). The Th1-biased phenotype was further confirmed at the transcriptional level using RT-PCR or gene microarrays (SuperArray), documenting increased expression of IFN-γ, tumor necrosis factor-α, and IL-12Rβ2 mRNA (data not shown), the last being a specific marker for Th1 cells. This profile was similar to celiac disease patients rechallenged with wheat despite the fact that this patient carefully maintained a strict cereal-free diet. The wheat gluten protein–responsive $CD3^+$ T-cells from the patient were mostly $CD4^+$ (~95%) with a low percentage of $CD8^+$ (~3%). The fact that HLA DR antibody blocked $CD3^+$ T-cell response to wheat gluten protein suggests that wheat gluten protein antigens were presented to T-cells mainly on class II HLA-DR. Thus, during the period of clinical remission the patient displayed a high concentration of Glb1 antibodies and a strong Th1-predominant HLA-DR–related recall response to a mixture of wheat proteins.

Because this patient has both type 1 diabetes and celiac disease, we cannot at-

tribute her immune reaction to Glb1 to one disease or the other. However, it should be noted that Glb1 was discovered as a diabetes-related protein by screening a wheat library with sera from overt diabetic rats (5). Furthermore, wheat globulins such as Glb1 are not generally considered celiac antigens (10). Preliminary results from an ongoing study in our laboratory suggest that a subset of patients with type 1 diabetes also displays immune reactivity to wheat gluten proteins and Glb1 (11,12). The strong immune reactivity to Glb1 in this patient suggests that further study is required to determine whether Glb1 plays any role in the pathogenesis of type 1 diabetes and/or celiac disease and whether immune reactivity against this protein is a characteristic of type 1 diabetic patients at high risk for developing celiac disease.

Acknowledgments— These studies were funded by the Juvenile Diabetes Research Foundation and Canadian Institutes of Health Research. A.J.M. and D.E.L. received Ontario Graduate Scholarships, and M.M. received a scholarship from Canadian Blood Services.

We thank Dr. Hubert Kolb and Dr. Markus Walz of the German Diabetes Center, Düsseldorf, for providing the viral clone of WP5212 (Glb1) and for helpful discussions. We are grateful to the volunteers, particularly the patient with type 1 diabetes/celiac disease whose generous support is much appreciated.

References

1. Ziegler AG, Schmid S, Huber D, Hummel M, Bonifacio E: Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. *JAMA* 290:1721–1728, 2003
2. Norris JM, Barriga K, Klingensmith G, Hoffman M, Eisenbarth GS, Erlich HA, Rewers M: Timing of initial cereal exposure in infancy and risk of islet autoimmunity. *JAMA* 290:1713–1720, 2003
3. Schuppan D, Hahn EG: Celiac disease and its link to type 1 diabetes mellitus. *J Pediatr Endocrinol Metab* 14 (Suppl 1):597–605, 2001
4. Scott FW, Sarwar G, Cloutier HE: Diabetogenicity of various protein sources in the diet of the diabetes-prone BB rat. *Adv Exp Med Biol* 246:277–285, 1988
5. MacFarlane AJ, Burghardt KM, Kelly J, Simell T, Simell O, Altosaar I, Scott FW: 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, 2003
6. Hoorfar J, Buschard K, Dagnaes-Hansen F: Prophylactic nutritional modification of the incidence of diabetes in autoimmune non-obese diabetic (NOD) mice. *Br J Nutr* 69:597–607, 1993
7. Maurano F, Mazzarella G, Luongo D, Stefanile R, D'Arienzo R, Rossi M, Auricchio S, Troncone R: Small intestinal enteropathy in non-obese diabetic mice fed a diet containing wheat. *Diabetologia* 48:931–937, 2005
8. Gottschall E: *Breaking the Vicious Cycle, Intestinal Health Through Diet.* Baltimore, MD, Kirkton Press, 1994
9. Mannering SI, Morris JS, Jensen KP, Purcell AW, Honeyman MC, van Endert PM, Harrison LC: A sensitive method for detecting proliferation of rare autoantigen-specific human T cells. *J Immunol Methods* 283:173–183, 2003
10. Sollid LM: Coeliac disease: dissecting a complex inflammatory disorder. *Nat Rev Immunol* 2:647–655, 2002
11. MacFarlane A, Scott FW: Diabetes-associated antibodies to clone WP5212, a Glb1-like wheat storage globulin (Abstract). *FASEB J* 19 (Suppl. S):A1465–A1466, 2005
12. Mojibian M, Lefebvre DE, Chakir H, Scott FW: T-cell reactivity to wheat proteins in human type 1 diabetes (Abstract). *Diabetes* 54 (Suppl. 1):A79, 2005