Gluten-sensitive Enteropathy

IMMUNOGLOBULIN G HEAVY-CHAIN (Gm) ALLOTYPES AND THE IMMUNE RESPONSE TO WHEAT GLIADIN

JOSEPH B. WEISS, RALEIGH K. AUSTIN, MOSES S. SCHANFIELD, and MARTIN F. KAGNOFF, *Department of Medicine, University of California at San Diego, M-023-D, La Jolla, California 92093; Immunohematology Laboratory, Blood Services, American Red Cross, Bethesda, Maryland 20814*

ABSTRACT Anti-gliadin antibody was measured by radioimmunoassay in 30 Caucasians with gluten-sensitive enteropathy (GSE). 22 GSE patients maintained on a gluten-free diet for 1.5 to 20 yr (mean duration 76 mo) had elevated serum concentrations of IgG antigliadin antibody. Among GSE patients on a gluten-free diet, antigliadin antibody was seen only in those having the chromosome 14-encoded IgG immunoglobulin heavy chain allotype marker G2m(n). IgG antigliadin antibody was found in GSE patients with G2m(n) regardless of whether the HLA-B8 and/or -DR3 major histocompatibility complex antigens that occur frequently in GSE were present. No patient lacking G2m(n) had significant levels of antigliadin antibody. The association between antigliadin antibody and the immunoglobulin heavy chain allotype marker G2m(n) in GSE patients likely reflects the presence of Gm^n-linked variable region genes or Gm^n-linked genes that regulate variable region gene expression.

INTRODUCTION

Gluten-sensitive enteropathy (GSE)[1] (celiac disease, nontropical sprue) is a disease that results in injury to the small intestinal mucosa and is precipitated by the ingestion of wheat gluten and similar proteins in rye, barley, and possibly oats. Exclusion of these proteins from the diet results in clinical and histological improvement. The exact mechanisms causing mucosal damage and subsequent malabsorption are unknown. However, several reports suggest that immune mechanisms are important in the pathogenesis of GSE (1–5).

The major histocompatibility complex (MHC)-encoded antigens, HLA-B8 and/or -DR3 are found in ~65–90% of GSE patients of Northern European origin (6–10) and linkage studies in families show significant segregation of these antigens with disease (11). However, 20–30% of subjects in unselected healthy populations carry the same HLA-B8 and/or -DR3 antigens and yet <0.2% of these individuals develop clinical disease (11). Thus, additional genetic loci or environmental determinants must also influence susceptibility to GSE (12–14).

Antibodies to gluten or gliadin can be detected in many GSE patients, but their role, if any, in the pathogenesis of this disease is unknown (15–18). We recently reported that antibody responses to gliadin in mice are associated with genes that map to two separate genetic regions; the MHC (H-2) on chromosome 17 and the immunoglobulin heavy chain allotype (IgC_H) locus on chromosome 12 (19).

In this study, we sought to determine whether IgG heavy chain allotype markers (Gm markers) were associated with the production of antibody to gliadin. This report describes a striking association between the IgG2 heavy chain allotype marker G2m(n) and IgG antigliadin antibody (GAb) that was revealed in GSE patients maintained on a gluten-free diet.

METHODS

Subjects. The patient population consisted of 30 unrelated Caucasians with GSE (22 males and 8 females) and 28 unrelated healthy controls (21 males and 7 females) matched for ethnic background and geographic origin. Ages of the patients and controls ranged from 17 to 81. The diagnosis of GSE was based on clinical evidence of malabsorption, a

Received for publication 23 November 1982 and in revised form 17 February 1983.

[1] *Abbreviations used in this paper:* GAb, antigliadin antibody; GARG, goat anti-rabbit globulin; GSE, gluten-sensitive enteropathy; MHC, major histocompatibility complex; RT, room temperature.

small bowel biopsy compatible with GSE, clinical and/or biopsy improvement on a gluten-free diet, and clinical and/or biopsy abnormalities on rechallenge with a gluten-containing diet. Sera from patients reported in this study were obtained 18 mo or more after initiation of a gluten-free diet (mean duration 76 mo, range 18 mo to 20 yr), at which time patients were asymptomatic. When serial serum samples were available in a given patient, the serum obtained furthest from initiation of a gluten-free diet was used.

Sera were typed for IgG (Gm) allotypes using a hemagglutination-inhibition procedure as described before (20, 21). Notation follows the World Health Organization recommendation on human allotypic markers (22). Typing was performed for the following allotype markers: G1m(a,x,f), G2m(n), G3m(b0,b1,b3,b5,c3,s,t,g). Because of the lack of a measurable allele, it cannot be determined whether sera having the Gm(f;b) phenotype and IgG2 marker G2m(n) are homozygous or heterozygous for G2m(n). We have used the term G3m(b) to refer to individuals having b0,b1,b3, and b5.

All specimens were typed for 13 A locus (A1,2,3,9,10, 11,28,29,30,31,32,33,34), 17 B locus (B5,7,8,12,13,14,15,16, 17,18,21,22,27,35,37,40,53) and 7 DR locus (1,2,3,4,5,6,7) antigens as described before (20).

Gliadin. Whole gliadin derived from unbleached flour from bread wheat variety Scout 66 was prepared as follows. 20 g of flour was extracted with 200 ml of 55% ethanol at 40°C for 60 min. After centrifugation at 19,000 *g* for 10 min, 600 ml of 1.5% NaCl was added to the supernatant to precipitate gliadins from wheat albumins and other nonstorage proteins, and the mixture was centrifuged at 25,000 *g* for 50 min. The precipitate was washed several times with 1.5% NaCl, dissolved in 0.01 M acetic acid, dialyzed against H_2O, and then lyophilized. To remove glutenins and further diminish any albumins that might remain after the precipitation step, the gliadin was further purified by chromatography on Sephadex G-100 using 0.01 M acetic acid, pH 3.2, as eluant. Polyacrylamide gel electrophoresis in pH 3.2 aluminum lactate buffer showed no evidence of albumins moving faster than gliadins, indicating that the preparative approach was effective in separating gliadins from albumins (23, 24).

Antisera. Rabbit anti-human IgG, IgA, and IgM (Miles Yeda, Ltd., Rehovot, Israel) were tested and shown to be specific for human IgG, IgA, and IgM respectively by radioimmunoassay (RIA). Human IgG, IgA, and IgM were the generous gift of Dr. Hans Spiegelberg, Scripps Clinic and Research Foundation, La Jolla, CA.

^{125}I-Goat anti-rabbit globulin (GARG) prepared as described previously (25) was adsorbed with human gamma globulin coupled to Sepharose 4B before use.

RIA. Antibody responses were measured by a modification of a solid-phase RIA (25). Briefly, 96-well Flex-vinyl U-bottom microtiter Dyna-tech plates (Cooke Laboratory Products Division, Dynatech Laboratories, Inc., Alexandria, VA) were coated with whole-gliadin (0.1 mg/ml) for 2 h at 37°C and then quenched with 1% bovine serum albumin (BSA). Control plates were coated with 1% BSA. Plates could be stored at 4°C in phosphate-buffered saline (PBS) containing 0.02% sodium azide for up to 2 mo before use.

For the assay, serial dilutions of test sera were added to gliadin and control BSA-coated plates and the plates were incubated at 37°C for 15 min and at room temperature (RT) for 2 h. After rinsing with PBS, appropriate dilutions of rabbit anti-human class specific antisera were added to each well (anti-IgG 1:80,000, anti-IgA 1:5,000, anti-IgM 1:12,000 in PBS containing BSA 10 mg/ml) and the plates were reincubated for 15 min at 37°C and 2 h at RT. The plates were rinsed with PBS, after which 0.05 ml ^{125}I-GARG containing $25–30 \times 10^3$ cpm was added to each well and the plates were incubated for an additional 15 min at 37°C and 2 h at RT. Wells were aspirated, rinsed, and counted in an automatic gamma counter. Specific binding to gliadin was obtained by subtracting background counts in BSA-coated wells from counts on gliadin-coated wells. All assays were done in triplicate.

Standard curve. Affinity-purified IgG GAb for use in the standard curve was prepared from the serum of GSE patients. Briefly, serum globulins twice precipitated with 50% saturated ammonium sulfate were dialyzed against 0.015 M potassium phosphate buffer, pH 8.0, and then loaded onto a 20-ml DEAE cellulose column (DE 52, Whatman, Inc., Clifton, NJ) equilibrated with the same buffer. The IgG fraction eluted using 0.015 M potassium phosphate buffer, pH 8.0, was reprecipitated with 50% saturated ammonium sulfate and dialyzed overnight against PBS before affinity purification by chromatography on an AH-Sepharose 4B-whole-gliadin column (25). IgG affinity-purified GAb was shown by RIA to be free of contaminating IgA and IgM.

IgG GAb levels > 8.5 μg/ml were deemed positive. This value was chosen arbitrarily, based on the arithmetic mean + 3 SD of GAb concentrations in the control group.

Statistical analysis. *P* values for 2 × 2 tables calculated using Fisher's exact test were corrected for the number of comparisons made. Student's *t* test was used to determine the significance of differences between IgG GAb concentrations in groups of GSE patients and controls.

RESULTS

IgG GAb levels in the 30 GSE patients on a gluten-free diet and controls are shown in Fig. 1. 22 of 30 GSE patients but none of the 28 controls had IgG GAb >8.5 μg/ml. The striking association between the IgG heavy chain allotype marker G2m(n) and IgG GAb in GSE patients can be seen in Fig. 2. Concentrations of IgG GAb were significantly elevated in patients having G2m(n) regardless of whether the HLA antigens -B8 or -DR3 were present. Although IgG GAb concentrations were initially two- to threefold higher when measured in several GSE patients having G2m(n) before initiation of a gluten-free diet, determinations on serial samples available at two to four intervals over a 2-yr period while on a gluten-free diet indicated that elevated IgG GAb concentrations remained consistently elevated. In two GSE patients having HLA-B8 and -DR3, but lacking G2m(n), sera were available both at the time of diagnosis and at intervals for the subsequent 2 yr. In both patients, GAb levels were elevated at the time of diagnosis (79.5 and 37.0 μg/ml), but declined to normal (2.4 and 2.1 μg/ml) within 6 mo of beginning a gluten-free diet. Table I lists GAb concentrations in our GSE patients and controls according to Gm phenotype. As shown, 22 of 24 GSE patients having the G2m(n) marker had elevated concentrations of IgG GAb, whereas IgG GAb was not increased above control values in any of the six GSE patients lacking G2m(n).

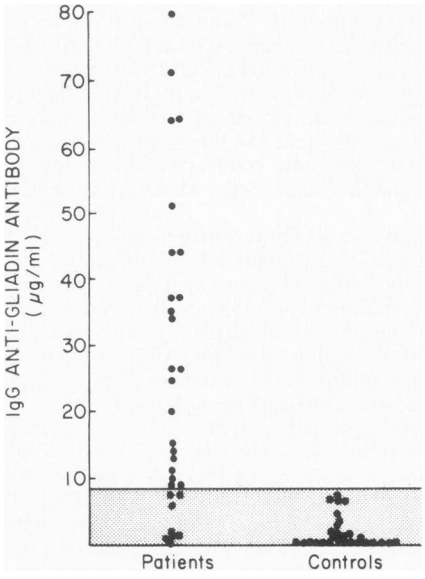

FIGURE 1 IgG GAb in GSE patients on a gluten-free diet and controls. Each point represents the data from a different subject. GAb concentrations >8.5 μg/ml (unshaded area) were considered positive as indicated in Methods.

IgG GAb levels were not elevated in 19 disease controls (12 patients with ulcerative colitis, two patients with infectious diarrheas, five patients with peptic ulcer disease) or in 10 additional unrelated healthy subjects (five with HLA-B8, -DR3, and G2m(n); five with G2m(n) but lacking HLA-B8 and -DR3) who were not ethnically matched to the GSE group. We have found two related apparently healthy controls (i.e., mother and son) with elevated IgG GAb levels (10.5 and 15.3 μg/ml respectively). Both had G2m(n) and the son was positive for HLA-B8. Although they denied symptoms suggesting GSE or other intestinal disease, no intestinal biopsies were taken to exclude subclinical disease. IgG GAb levels were increased in 14 of 98 Crohn's disease subjects with small intestinal involvement, but in those subjects there was no association between elevated GAb concentrations and Gm allotype. Serum IgG anti-BSA antibody titers did not differ significantly between GSE patients and controls ($P > 0.50$).

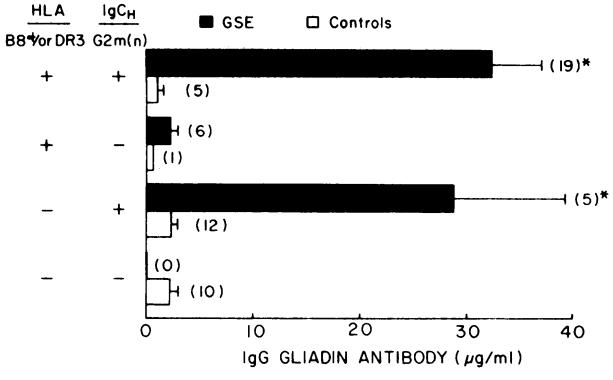

FIGURE 2 Association between IgG GAb and G2m(n) in GSE patients. Subjects are grouped according to whether they are positive or negative for HLA-B8 and/or -DR3 and the IgG heavy chain allotype marker G2m(n). Bars represent the arithmetic mean±SEM, with the number of subjects in each group indicated in brackets. Asterisks indicate statistically significant differences between GSE patients and controls within groups ($P < 0.005$). All GSE patients were on a gluten-free diet for >18 mo at the time of GAb determination. Not shown, deviation from a gluten-free diet was associated with a transient two- to threefold increase in IgG GAb in two patients having G2m(n) (one patient also had HLA-B8 but not -DR3; one patient lacked both HLA-B8 and -DR3).

IgA GAb levels were increased in 14 of 30 GSE patients on a gluten-free diet. Like IgG GAb, GSE patients with increased IgA GAb all had the G2m(n) allotypic marker. Although quantitative levels of serum IgA GAb were not assessed because of the lack of a highly purified IgA antigliadin standard, when expressed as counts per minute of ^{125}I-GARG bound per well, IgG and IgA GAb levels in individual patients correlated significantly ($r^2 = 0.53$; $P < 0.005$).

DISCUSSION

Our data demonstrate that IgG GAb are present in 73% of GSE patients maintained on a gluten-free diet for 1.5 to 20 yr. Persistently elevated IgG GAb titers were seen only in GSE patients having the chromosome 14-encoded IgG2 heavy chain allotype marker G2m(n). GAb has been reported in GSE patients before, although prior studies usually used less sensitive assay methods and measured antibody directed to determinants associated with peptic-tryptic digests of gluten or gliadin, or gliadins containing wheat albumins and other impurities (15–18, 26–30). We were fortunate to have a specific, sensitive, and reproducible RIA for GAb (25) and highly purified gliadin (23, 24).

The finding of persistently increased levels of IgG and, to a lesser extent, IgA GAb in our G2m(n)-positive GSE patients on a gluten-free diet could indicate that these patients are unknowingly ingesting gluten in quantities that are insufficient to activate disease. It seems less likely that the GAb was stimulated by host self-components that cross-react with gluten (i.e., autoimmune), since antibody was present in the absence of active disease and activation of disease required the ingestion of gliadin. This does not exclude the possibility that GAb is involved in mechanisms that damage the small intestine. GAb-mediated injury could be secondary to immune complex deposition in the intestine (i.e., GAb-gliadin immune complexes) (31, 32) or to

TABLE I

*IgG GAb in GSE Patients and Controls Having Different Gm Phenotypes**

Gm phenotype‡	GSE patients				Controls			
	With GAb		Without GAb		With GAb		Without GAb	
	n	*μg/ml*§	*n*	*μg/ml*	*n*	*μg/ml*	*n*	*μg/ml*
a;--;b,g	0	—	1	0.3	0	—	0	—
a,x;--;g	0	—	1	1.5	0	—	2	3.3±2.0
f;--;b	0	—	0	—	0	—	3	0.6±0.1
f;n;b	15	35.7±5.1	1	7.5	0	—	12	1.6±0.6
a,f;--;b,g	0	—	2	4.3±1.5	0	—	5	1.4±0.7
a,x,f;--;b,g	0	—	2	1.7±0.5	0	—	1	7.0
a,f;n;b,g	4	18.8±5.3	1	7.5	0	—	4	1.3±0.7
a,x,f;n;b,g	3	42.9±16.0	0	—	0	—	1	6.5
Total	22		8		0		28	

* GAb was present in 22 of 30 (73%) GSE patients and 0 of 28 controls ($P < 0.001$). Among GSE patients, GAb was significantly associated with phenotypes having the Gm marker G2m(n) ($P < 0.001$).

‡ b indicates positive for G3m(b0,1,3,5).

§ Data expressed as means±1 SE.

K-cell mediated antibody-dependent cytotoxic reactions (33), with GAb directed to gliadin determinants bound to components of the intestinal mucosa. In this regard, gliadin reportedly binds to reticulin (18, 34) and it has been postulated that GSE patients may have receptors for gliadin on intestinal epithelial cells (4, 13, 35).

We propose that the striking association between the IgG2 heavy chain allotype marker G2m(n) and GAb in GSE patients on a gluten-free diet reflects the presence of Gm^n-linked variable (V)-region genes or Gm^n-linked genes that regulate variable region gene expression. Such V-region genes would encode a variable region on the immunoglobulin heavy chain that in combination with light chains, determines the structure and specificity of GAb (19). On the basis of this hypothesis, GAb in GSE patients with G2m(n) on a gluten-free diet would be predicted to be of restricted heterogeneity. HLA-B8 and -DR3 positive GSE patients lacking G2m(n) also produced GAb while ingesting gluten. However, such antibody declined rapidly after initiation of a gluten-free diet and, like the GAb noted in some of our Crohn's disease subjects, may differ in its determinant specificity from GAb in GSE subjects having G2m(n). More detailed studies on the characteristics of GAb in these patient groups are currently in progress. This proposal does not exclude the possibility that other IgC_H-linked genes on chromosome 14 also could be important in immunoregulation (19).

HLA-B8 and -DR3 were present in >70% of our GSE patients, a finding noted also in earlier studies (6, 10). However, the same HLA antigens occur at a high frequency in several organ-specific diseases of presumed autoimmune origin (e.g., autoimmune chronic active hepatitis, myasthenia gravis, Grave's disease, insulin-dependent diabetes mellitus) (36–39). We would suggest that genes that code for HLA-B8 and -DR3 or closely linked genes are common in those diseases because they determine the host response (40) to environmental agents (e.g., viruses) that are critical in the initiation of these otherwise markedly different diseases. Disease susceptibility and organ specificity would be further determined by antigens unique for each disease (e.g., gliadin bound to intestinal mucosal structures in GSE, acetylcholine receptor in myasthenia gravis), the site of antigen encounter and V-region genes determining antibody specificity. We note that an association between IgC_H allotype markers and antiinsulin antibody in insulin-dependent diabetes mellitus (41), and associations between Gm markers and autoimmune chronic active hepatitis (42), myasthenia gravis (43), and Grave's disease (44, 45) recently have been described.

We previously reported an association between the Gm phenotype (f;n;b) and GSE in patients lacking HLA-B8 and -DR3 (20). Thus, both MHC and IgC_H-linked genes appear to determine susceptibility to GSE. Athough the effects of MHC genes predominate, in patients lacking HLA-B8 and -DR3 a significant association between IgC_H locus markers and GSE can be revealed. Finally, any hypothesis of the pathogenesis of GSE must take into account the fact that HLA-B8, -DR3, and G2m(n) occur in many apparently healthy individuals who do not have clinically evident GSE or GAb. This could suggest that other genes, perhaps coding for a receptor on intestinal mucosal structures that bind gliadin (4, 13), are necessary for the

expression of disease. However, given the lack of complete concordance for GSE in identical twins (6), we favor the additional possibility that an as yet unidentified environmental factor (e.g., a virus) plays a role in initiating GSE.

ACKNOWLEDGMENTS

We thank Dr. H. C. L. Johnson for his generous assistance during the conduct of this study, Dr. T. Lee for typing of human histocompatibility leukocyte antigens, Dr. R. J. Brown for allotyping, Dr. D. D. Kasarda and Dr. J. E. Bernardin for testing the purity of the gliadin preparation, and Dr. R. Langman for his helpful comments on the manuscript. Ms. D. Glazer is acknowledged for typing the manuscript.

This work was supported by National Institutes of Health grant AM 17276 and a grant from the National Foundation for Ileitis and Colitis, Inc. Dr. Weiss was supported by National Institutes of Health training grant AM 07202.

REFERENCES

1. Holmes, G. K. T., P. Asquith, P. L. Stokes, and W. T. Cooke. 1974. Cellular infiltrate of jejunal biopsies in adult coeliac disease in relation to gluten withdrawal. *Gut.* 15:278–283.
2. Lancaster-Smith, M., P. Kumar, R. Marks, M. L. Clark, and A. M. Dawson. 1974. Jejunal mucosal immunoglobulin in adult coeliac disease and dermatitis herpetiformis. *Gut.* 15:371–376.
3. Ferguson, A., T. T. MacDonald, J. P. McLure, and R. J. Holden. 1975. Cell-mediated immunity to gliadin within the small intestinal mucosa in coeliac disease. *Lancet.* I:895–897.
4. Strober, W. 1977. An immunological theory of gluten-sensitive enteropathy. *In* Perspectives in Coeliac Disease. B. McNicholl, C. F. McCarthy, and P. F. Fottrell, editors. University Park Press, Baltimore. 169–182.
5. Falchuk, Z. M. 1979. Update on gluten-sensitive enteropathy. *Am. J. Med.* 67:1085–1096.
6. Polanco, I., I. Biemond, A. van Leeuwen, I. Schreuder, P. Meera Khan, J. Guerrero, J. D'Amaro, C. Vazquez, J. J. van Rood, and A. S. Peña. 1981. Gluten-sensitive enteropathy in Spain: genetic and environmental factors. *In* The Genetics of Coeliac Disease. R. B. McConnell, editor. MTP Press, Lancaster, England. 211–230.
7. Falchuk, Z. M., G. N. Rogentine, and W. Strober. 1972. Predominance of histocompatibility antigen HLA-8 in patients with gluten-sensitive enteropathy. *J. Clin. Invest.* 51:1602–1605.
8. Stokes, P. L., G. K. T. Holmes, P. Asquith, P. Mackintosh, and W. T. Cooke. 1972. Histocompatibility antigens associated with adult coeliac disease. *Lancet.* II:162–164.
9. Ek, J., D. Albrechtsen, B. G. Solheim, and E. Thorsby. 1978. Strong association between HLA-Dw3-related B cell alloantigen-DRw3 and coeliac disease. *Scand J. Gastroenterol.* 13:229–233.
10. Braun, W. E. 1979. HLA and Disease: A Comprehensive Review. CRC Press, Inc., West Palm Beach, FL.
11. Albert, E. D., K. Harms, R. Wank, I. Steinbauer-Rosenthal, and S. Scholz. 1973. Segregation analysis of HLA antigens and haplotypes in 50 families of patients with coeliac disease. *Transplant. Proc.* 5:1785–1789.
12. Van Rood, J. J., J. P. Van Hooff, and J. J. Keuning. 1975. Disease predisposition, immune responsiveness, and the fine structure of the HLA-A supergene. *Transplant. Rev.* 22:75–104.
13. Mann, D. L., D. L. Nelson, S. I. Katz, L. D. Abelson, and W. Strober. 1976. Specific B-cell antigens associated with gluten-sensitive enteropathy and dermatitis herpetiformis. *Lancet.* I:110–111.
14. Pena, A. S., D. L. Mann, N. E. Hague, J. A. Heck, A. Van Leeuwen, J. J. Rood, and W. Strober. 1978. Genetic basis of gluten-sensitive enteropathy. *Gastroenterology.* 75:230–235.
15. Eterman, K. P., and T. E. W. Feltkamp. 1978. Antibodies to gluten and reticulin in gastrointestinal diseases. *Clin. Exp. Immunol.* 31:92–99.
16. Stern, M., K. Fischer, and R. Grüttner. 1979. Immunofluorescent serum gliadin antibodies in children with coeliac disease and various malabsorptive disorders. *Eur. J. Pediatr.* 130:165–172.
17. Signer, E., A. Burgin-Wolff, R. Berger, A. Birbaumer, and M. Just. 1979. Antibodies to gliadin as a screening test for coeliac disease. *Helv. Paediatr. Acta.* 34:41–52.
18. Unsworth, D. J., P. D. Manuel, J. A. Walker-Smith, C. A. Campbell, G. D. Johnson, and E. J. Holborow. 1981. New immunofluorescent blood test for gluten sensitivity. *Arch. Dis. Child.* 56:864–868.
19. Kagnoff, M. F. 1982. Two genetic loci control the murine immune response to A gliadin, a wheat protein that activates coeliac sprue. *Nature (Lond.).* 270:158–160.
20. Kagnoff, M. F., J. B. Weiss, R. J. Brown, T. Lee, and M. S. Schanfield. 1983. Immunoglobulin allotype markers in gluten-sensitive enteropathy. *Lancet.* I:952–953.
21. Schanfield, M. S. 1978. Genetic markers of human immunoglobulins. *In* Basic and Clinical Immunology, H. H. Fudenberg, D. D. Stites, J. L. Caldwell, and J. V. Wells, editors. Lange Medical Publishers, Los Altos, CA. 2nd edition. 59–65.
22. Review of the notation for the allotype and related markers of human immunoglobulins. 1976. *Eur. J. Immunol.* 6:599–601.
23. Kasarda, D. D. 1978. The relationship of wheat proteins to coeliac disease. *Cereal Foods World.* 23:240–262.
24. Kagnoff, M. F., R. K. Austin, H. C. L. Johnson, J. E. Bernardin, M. D. Dietler, and D. D. Kasarda. 1982. Coeliac sprue: correlation with murine T-cell responses to wheat gliadin components. *J. Immunol.* 129:2693–2697.
25. Trefts, P. E., and M. F. Kagnoff. 1981. Gluten-sensitive enteropathy. 1. The T-dependent anti-A-gliadin antibody response maps to the murine major histocompatibility locus. *J. Immunol.* 126:2249–2252.
26. Kenrick, K. G., and J. A. Walker-Smith. 1970. Immunoglobulins and dietary protein antibodies in childhood coeliac disease. *Gut.* 11:635–640.
27. Kivel, R. M., D. H. Kearns, and D. Liebowitz. 1964. Significance of antibodies to dietary proteins in the serums of patients with nontropical sprue. *N. Engl. J. Med.* 271:769–772.
28. Alarćon-Segovia, D., T. Herskovic, K. G. Wakim, P. A. Green, and H. H. Scudamore. 1964. Presence of circulating antibodies to gluten and milk fractions in patients with nontropical sprue. *Am. J. Med.* 36:485–499.
29. Taylor, K. B., S. C. Truelove, and R. Wright. 1964. Serological reactions to gluten and cow's milk proteins in gastrointestinal disease. *Gastroenterology.* 46:99–108.
30. Jonsson, J., and W. Schilling. 1981. Some characteristics of immunofluorescence tests for antibodies against gluten, using wheat grain sections or gliadin-coated Sepharose beads. *Acta Pathol. Microbiol. Scand.* 89:253–262.

31. Shiner, M., and J. Ballard. 1972. Antigen-antibody reactions in jejunal mucosa in childhood coeliac disease after gluten challenge. *Lancet.* I:1202–1205.
32. Scott, B. B., D. G. Scott, and M. S. Losowsky. 1977. Jejunal mucosal immunoglobulins and complement in untreated coeliac disease. *J. Pathol.* 121:219–223.
33. Hahn, W. V., M. F. Kagnoff, and L. H. Hatlen. 1978. Immune responses in human colon cancer. II. Cytotoxic antibody detected in patients' sera. *J. Natl. Cancer Inst.* 60:779–784.
34. Unsworth, D. J., G. D. Johnson, G. Haffenden, L. Fry, and E. J. Holborow. 1981. Binding of wheat gliadin in vitro to reticulin in normal and dermatitis herpetiformis skin. *J. Invest. Dermatol.* 76:88–93.
35. Rubin, W., A. S. Fauci, M. H. Sleisenger, and G. H. Jeffries. 1965. Immunofluorescent studies in adult coeliac disease. *J. Clin. Invest.* 44:475–485.
36. Mackay, I. R., and B. D. Tait. 1980. HLA associations with autoimmune-type chronic active hepatitis: identification of B8-DRw3 haplotype by family studies. *Gastroenterology.* 79:95–98.
37. Dawkins, R. 1980. Myasthenia gravis. *In* Histocompatibility Testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, CA. 662–667.
38. Thorsby, W., E. Svejgaard, J. M. Solem, and L. Kornstad. 1975. The frequency of major histocompatibility antigens (SD and LD) in thyrotoxicosis. *Tissue Antigens.* 6:54–55.
39. Svejgaard, A., P. Platz, and L. P. Ryder. 1980. Insulin-dependent diabetes mellitus. *In* Histocompatibility Testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, CA. 638–656.
40. Lawley, T. J., R. P. Hall, A. S. Fauci, S. I. Katz, M. I. Hamburger, and M. M. Frank. 1981. Defective Fc-receptor functions associated with the HLA-B8/DRw3 haplotype. Studies in patients with dermatitis herpetiformis and normal subjects. *N. Engl. J. Med.* 304:185–192.
41. Nakao, Y., T. Miyazaki, N. Arima, K. Okimoto, K. Tsuji, H. Matsumoto, N. Mizuno, A. Wakisaka, Y. Akazawa, and T. Fujita. 1981. IgG heavy-chain (Gm) allotypes and immune response to insulin in insulin-requiring diabetes mellitus. *N. Engl. J. Med.* 304:407–409.
42. Whittingham, S., J. D. Mathews, M. S. Schanfield, B. D. Tait, and I. R. Mackay. 1981. Interaction of HLA and Gm in autoimmune chronic active hepatitis. *Clin. Exp. Immunol.* 43:80–86.
43. Nakao, Y., T. Miyazaki, K. Ota, H. Matsumoto, H. Nishitani, T. Fujita, and K. Tsuji. 1980. Gm allotypes in myasthenia gravis. *Lancet.* I:677–680.
44. Uno, H., T. Sasazuki, H. Tamai, and H. Matsumoto. 1981. Two major genes, linked to HLA and Gm, control susceptibility to Graves' disease. *Nature (Lond.).* 292:768–770.
45. Farid, N. R., R. M. Newton, E. P. Noel, and W. H. Marshall. Gm phenotypes in autoimmune thyroid disease. *J. Immunogenet. (Oxf.).* 4:429–432.