POSSIBLE ROLE FOR A HUMAN ADENOVIRUS IN THE PATHOGENESIS OF CELIAC DISEASE

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Celiac disease in humans is characterized by small intestinal mucosal injury and malabsorption (1). Disease is activated by dietary exposure to wheat gluten and similar proteins in rye, triticale, barley, and possibly oats (2, 3). Wheat gluten is a mixture of gliadin and glutenin (3). It is the gliadin fraction of wheat gluten that is responsible for activating disease.

Genetic factors and immune mechanisms appear to be important in the pathogenesis of celiac disease (3, 4). The HLA serologic specificities HLA-B8, -DR3 and/or -DR7, and -DC3 have been reported in as many as 60–100% of celiac disease patients (5–11). However, <0.2% of individuals with those HLA specificities develop celiac disease (10), despite the dietary intake of wheat and other grains, and disease is not always concordant among monozygotic twins (11–14). Based on those observations, it has been postulated (15, 16) that additional environmental factors may be important in the pathogenesis of this disease.

The present studies examined known protein sequences for amino acid sequence homologies with A-gliadin, a wheat gliadin protein component with a molecular mass of 31,000 daltons that activates celiac disease (17) and whose primary amino acid sequence has been determined (18). Our search revealed a region of homology between A-gliadin and the 54 kilodalton (kD) early region E1b protein of human adenovirus type 12 (Ad12),[1] an adenovirus usually isolated from the human intestinal tract (19, 20). Antibody that reacts with the Ad12 E1b protein specifically cross-reacts with A-gliadin, a 119 amino acid cyanogen bromide peptide fragment of A-gliadin containing the region of shared sequence, and a synthetic heptapeptide of A-gliadin from within the region of sequence

[1] *Abbreviations used in this paper:* Ad12, adenovirus type 12: Bis, *bis N,N'*-methylene *bis*acrylamide; BHK, baby hamster kidney; BRK, baby rat kidney; BRK$_{p17}$, baby rat kidney cells transformed by a plasmid containing the left-hand 17% of the Ad12 genome; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; E1, early region 1; GARG, goat anti-rabbit gamma globulin; OVA, ovalbumin; NP-40, Nonidet P-40; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; RIA, radioimmunoassay; SDS, sodium dodecyl sulfate.

homology. These data suggest that antigenic determinants produced during intestinal viral infection may be important in the pathogenesis of celiac disease.

Materials and Methods

Proteins. A-gliadin, purified from "Scout 66" wheat, and three cyanogen bromide peptides of A-gliadin (CNI, CNII, CNIII) that span the entire molecule, were prepared as described before (21). CNI contains A-gliadin residues 1–127; CNII, residues 128–246; and CNIII, residues 247–266. A synthetic peptide having A-gliadin residues 211–217 (i.e., FRPSQQN) was prepared by solid phase synthesis at the peptide-oligonucleotide synthesis facility, University of California, San Diego (22). The proline residue corresponding to position 213 was radiolabeled with ^{14}C. For use as an immunogen, the synthetic peptide was coupled to ovalbumin (OVA) or bovine serum albumin (BSA) with glutaraldehyde (23). A synthetic decapeptide of cytochrome *b* (i.e., MTPMRKINPE) unrelated to the A-gliadin synthetic peptide was provided by Dr. R. Doolittle, University of California, San Diego (UCSD).

Cell Lines. Ad12 (strain Huie) transformed baby rat kidney (BRK) and baby hamster kidney (BHK) cells expressing the 54-kD Ad12 E1b protein and BRK cells transformed by a plasmid (pBR322) containing the left-hand 17% of the Ad12 genome (BRK$_{p17}$ cells) were provided by Dr. W. Topp, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY. BRK$_{p17}$ cells do not express the 54-kD Ad12 E1b protein or mRNA for that protein. Normal BHK cells were provided by Dr. J. Klein, UCSD. All cell lines were grown in monolayer cultures in complete tissue culture medium (RPMI 1640 supplemented with 2 mM L-glutamine, 10% fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Antiserum. Serum reactive with the 54-kD Ad12 E1b protein used in these studies were as follows: (*a*) Ad12 antitumor serum from rats bearing a tumor of pAd12RIC-transformed baby rat kidney cells provided by Drs. A. Zantema and A. J. Van der Eb, University of Leiden; (*b*) Ad12 antitumor serum from hamsters bearing a tumor of Ad12-transformed baby hamster kidney cells provided by Dr. W. Topp; (*c*) antisera to Ad12-transformed BRK or BHK cells prepared in our laboratory. Those sera were prepared by injecting rats with extracts of Ad12-transformed BRK cells and hamsters with extracts of Ad12-transformed BHK cells. Initial injections were in complete Freund's adjuvant (CFA) (10^7 cells/injection), followed by four to eight weekly injections of cell lysates in incomplete Freund's adjuvant or saline. Rat antisera to control BRK$_{p17}$ cells and hamster antisera to control BHK cells were prepared using the same protocol. Rabbit antisera to adenovirus type 5 E1b protein were a generous gift of Dr. A. J. Berk, University of California, Los Angeles. Rabbit antisera to A-gliadin and the synthetic A-gliadin heptapeptide were prepared by multiply injecting rabbits with A-gliadin or synthetic A-gliadin heptapeptide coupled to OVA. Initial injections in CFA were followed by biweekly injections in saline.

Monoclonal A-Gliadin Antibodies. Monoclonal antibodies to the synthetic A-gliadin peptide (residues 211–217) were prepared by fusing spleen cells from BALB/c mice immunized with the synthetic peptide coupled to OVA with the murine myeloma fusion line NS-1. Hybridoma tissue culture supernatants were screened by radioimmunoassay (RIA) as described below for activity against A-gliadin, CNI, CNII, CNIII, and the synthetic A-gliadin heptapeptide. Four hybridomas secreted antibody that was positive by RIA against A-gliadin, CNII, and the A-gliadin peptide (residues 211–217), but did not react with CNI or CNIII. These hybrids were cloned by limit dilution and subsequently used to produce ascites in BALB/c mice. Monoclonal antibody was purified from ascites on DEAE Affi-gel blue columns (Bio-Rad Laboratories, Richmond, CA) (24).

Cell Labeling and Preparation of Cell Extracts. Cell lines grown to ~80% confluency in 100-mm tissue culture dishes were labeled for 5 h at 37°C in 2 ml methionine-free Dulbecco's modified Eagle's medium supplemented with L-glutamine and 2% dialyzed fetal calf serum using 50 μCi/ml [^{35}S]methionine (sp act, >800 Ci/mmol; Amersham Corp., Arlington Heights, IL). Plates were washed three times with ice-cold phosphate-buffered saline (PBS), pH 7.0, after which cells were harvested and centrifuged. The

pellet was resuspended in lysis buffer (0.02 M Tris, pH 7.6, 0.15 M NaCl, 0.005 M EDTA, 0.5% Nonidet P-40 [NP-40]) to which phenylmethylsulfonyl fluoride (PMSF) (0.3 mg/ml final concentration) was added. Cell suspensions were then sonicated twice for 10 s using a sonifier (Branson Sonic Power Co., Danbury, CT) at a setting of 5. Equal volumes of salt buffer (0.02 M Tris, pH 7.6, 0.7 M NaCl, 0.005 M EDTA, 0.5% NP-40) were added and the viscosity of the sonicate was lowered by forcing the solution through a 23-gauge needle. Cellular debris was removed by centrifugation for 20 min at 15,000 g. Supernatants were immunoprecipitated as described below.

Immunoprecipitation. Formalin fixed *Staphylococcus aureus* strain Cowan I cells (Pansorbin; Calbiochem-Behring Corp., La Jolla, CA) were prepared in washing buffer (0.02 M Tris, pH 7.6, 0.5 M NaCl, 0.005 M EDTA, 1.0% NP-40, 5.0% sucrose). Aliquots of cell extracts were preadsorbed with 20 μl of normal rat serum and 150 μl of 10% (wt/vol) Staph A for 15 min at room temperature, after which the bacteria were pelleted. 20 μl of antisera, as indicated in the text, was added to the preadsorbed extracts, the immune reaction was incubated for 30 min at room temperature, and then 75 μl of 10% (wt/vol) Staph A was added. The mixture was incubated for an additional 15 min on ice, after which the bacteria were pelleted by centrifugation. Pellets were washed three times in washing buffer and once in Tris-EDTA buffer (0.02 M Tris, pH 6, 0.005 M EDTA). Antigen-antibody complexes were solubilized at 60°C for 10 min in sample buffer (0.08 M Tris, pH 6.8, 2% sodium dodecyl sulfate [SDS] [Bio-Rad Laboratories], 0.4 M 2-mercaptoethanol, 25% glycerol). After centrifugation, samples were counted and loaded on SDS-polyacrylamide slab gels.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-polyacrylamide gels were run according to Laemmli (25) using slab gels (1.5 mm thick × 110 mm long). The separation gel contained 10% acrylamide and 0.27% *bis* N,N'-methylene *bis*acrylamide (Bis). The stacking gel contained 3% acrylamide, 0.08% Bis. Samples were electrophoresed through the stacking gel at 15 mA, and through the separation gel at 30 mA (constant current) until the bromophenyl blue front reached the bottom of the gel. After electrophoresis, portions of the gel were stained with Coomassie Brilliant Blue R 0.25% and then destained with 40% methanol, 10% acetic acid. Gels subsequently were impregnated with 22% 2,5,diphenyloxazole (Mallinckrodt Chemical, Paris, KY) in dimethylsulfoxide and dried on a gel dryer (SE540; Hoeffer Scientific Instrument, San Francisco, CA). Dried gels were exposed at −70°C to flash sensitized Kodak XRP-1 film for 1-3 d. Molecular-weight protein standards were included in each experiment (Bio-Rad Laboratories).

Immunoblotting. Western immunoblotting was done as described by others (26). Briefly, proteins from SDS-polyacrylamide gels were electrophoretically transferred to nitrocellulose paper (BA85; Schleicher & Schuell, Inc., Keene, NH) using 20 mM Tris, 150 mM glycine, and 20% methanol as buffer at 7 V/cm overnight at 4°C. The nitrocellulose strips were soaked in 1% rabbit gamma globulin or normal mouse serum in buffer (5% BSA, 10 mM Tris, pH 7.4, 0.9% NaCl, 0.2% NP-40) at 37°C for 30 min to saturate additional protein binding sites and then rinsed in the same buffer. Blots subsequently were probed with ^{125}I-labeled affinity-purified rabbit antibody or mouse monoclonal antibody (2-5 × 10^5 cpm/ml) in a 20-ml vol for 90 min at room temperature with gentle rocking, after which they were washed in 10 mM Tris, pH 7.4, 0.9% NaCl, 0.2% NP-40 until counts returned to background levels. After blotting dry, nitrocellulose strips were exposed to Kodak XRP-1 film with an enhancing screen at −70°C for several hours overnight.

RIA. Solid phase RIA was performed as described before (27) using 96-U polyvinyl plates (2797; Costar, Data Packaging, Cambridge, MA). Briefly, wells were sensitized for 2 h at room temperature with 50 μl of protein (0.1 mg/ml) and then quenched with 0.2 ml of 1% BSA in PBS, pH 7.2. For the assay, 50 μl of test serum in titrated dilutions or hybridoma supernatants was added in triplicate to individual wells and the plates were allowed to incubate for 2 h at room temperature. Wells were then rinsed with PBS, pH 7.2, after which 50 μl of suitably diluted rabbit anti-mouse, rabbit anti-rat, or rabbit anti-hamster sera was added and the plates were incubated for an additional 2 h. Plates were again rinsed with PBS, after which 50 μl of purified ^{125}I-goat anti-rabbit globulin (GARG)

(2-3 × 10⁴ cpm) was added to each well. After a final 2-h incubation, plates were rinsed and individual wells were cut with a hot wire and counted in an automatic gamma counter.

Affinity Chromatography. Synthetic A-gliadin peptide (residues 211–217) OVA was coupled to cyanogen bromide-activated Sepharose 4B as described by others (28). Antibody was eluted from affinity columns with 1 M acetic acid and collected into 2 M Tris.

Computer Analysis. The amino acid sequence of A-gliadin was screened for identities with known protein structures in the UCSD data bank. The data base consisted of 1,498 protein sequences, including 1,081 entries from the Dayhoff Atlas of Protein Sequence and Structure (29) and 417 sequences from the UCSD Newatt Atlas (30). The latter contained sequence data for 139 nonenzyme eukaryotic proteins, 66 nonenzyme prokaryotic proteins, 109 enzymes, and 103 animal virus proteins. We selected a sliding segment approach adjusted to detect 8 or more amino acid identities over a 20-residue segment for the computer search since those boundaries usually exclude 95–99% of screened proteins. The SOAP-7 program was used to evaluate the hydrophilicity and hydrophobicity of proteins along their amino acid sequence (31).

Results

Sequence Homology Between A-Gliadin and the 54-kD E1b Protein of Ad12. Using the sliding segment boundaries selected to screen the data base, we expected to retrieve between 15 and 75 proteins with 8 or more amino acid identities with A-gliadin over a 20-residue segment. Table I lists the 11 proteins actually retrieved. Homology with A-gliadin for 10 of those proteins was not considered significant since the amino acid identities involved mainly glutamine or proline and included multiple gaps within the 20-residue segment. 2 of 10 proteins had

TABLE I
Proteins Retrieved from Data Base

Data Base	Number of proteins screened	Number of proteins retrieved (percent of proteins retrieved)	Proteins retrieved
Newatt			
Eukaryotic proteins	139	0 (0)	—
Prokaryotic proteins	66	0 (0)	—
Enzymes	109	1 (0.9)	Chicken sulfite oxidase, heme-binding domain*
Animal viruses	103	4 (3.9)	Ad12, early region E1b protein
			Ad2, fiber protein
			Sindbis virus, capsid structural protein
			Herpes simplex, 2.7-kb frame 2 Wagner protein
Dayhoff			
Atlas of protein sequence and structure	1,081	6 (0.5)	Statherin, human saliva‡
			Papain, papaya
			Tobacco mosaic virus dahlemense, coat protein
			Collagen alpha 1 chain, rat skin
			Beta casein, bovine
			Ferredoxin, taro
Total	1,498	11 (0.7)	

* Tetrapeptide identity with A-gliadin; LQQY.
‡ Tetrapeptide identity with A-gliadin; YQPQ.

tetrapeptide identities with A-gliadin. In one protein, two of four residues, and in the other, three of four residues were glutamine and/or proline (Table I). The glutamine and proline identities with A-gliadin were expected since 32/100 residues in A-gliadin are glutamine and 15/100 residues are proline (18).

The region of homology between A-gliadin and the early region E1b protein of human Ad12 (32, 33) appeared potentially important. As shown in Fig. 1, the region included 8 identities over a span of 12 amino acid residues, had an uninterrupted 5-residue identity that was not present in the other 1,498 protein sequences, and involved only 1 proline and glutamine residue in the E1b protein. Homology with Ad12 E1b occurred in domain V of A-gliadin, a domain that lacks repeating sequences with a high glutamine and proline content (18).

Examination of the hydropathic character (31) of A-gliadin and the Ad12 E1b protein indicated that, for both molecules, the region of homology was hydrophilic and likely to be located on the exterior of the molecule (Fig. 2). The region of homology between A-gliadin and the Ad12 E1b protein would not be predicted to have α-helix or β-sheet conformation when analyzed by the structure prediction method of Chou and Fasman (34).

Antisera to a Synthetic A-Gliadin Peptide (Residues 211–217) from within the Region of Sequence Homology Cross-reacts with the 54-kD Ad12 E1b Protein. The expression of the Ad12 E1b genes in the Ad12-transformed BRK and BHK cell lines was verified by immunoprecipitation of ^{35}S-labeled cell extracts with antitumor serum or antiserum to extracts of those cells. Fig. 3 shows autoradiograms of SDS-polyacrylamide gels used to separate the immunoprecipitated proteins. Both the Ad12-transformed BRK and BHK cell lines express the 54-kD E1b protein (Fig. 3). The 54-kD Ad12 E1b protein was not detected in control BRK$_{p17}$ cells or normal BHK cells immunoprecipitated with the same antisera and was not immunoprecipitated from Ad12-transformed BRK or BHK cells by normal rat or hamster serum or by serum from rats immunized with control BRK$_{p17}$ cells. Western immunoblotting documented reactivity between the 54-kD Ad12 E1b protein and affinity-purified rabbit (Fig. 4) or murine monoclonal antibody to the synthetic A-gliadin heptapeptide (residues 211–217) from within the region of sequence homology.

Immunologic Cross-reactivity Between the Ad12 E1b Protein, A-Gliadin, and A-Gliadin Peptides. Fig. 5 shows that rat antiserum to Ad12-transformed BRK cell extracts (i.e., E1b$^+$) cross-reacts by RIA with the synthetic A-gliadin heptapeptide (residues 211–217), whereas rat antiserum to extracts of control BRK$_{p17}$ cells (i.e., E1b$^-$) does not. Control antisera to adenovirus type 5 E1b protein, a protein that is highly homologous with Ad12 E1b (32, 33) but not with A-gliadin, also do not cross-react with the synthetic A-gliadin heptapeptide (Fig. 5).

Anti-A-gliadin heptapeptide (residues 211–217) antibody was affinity-purified from antiserum to Ad12-transformed BRK cell extracts on A-gliadin heptapep-

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              384       387 389                    395
Adl2,Elb     |L  R  R |G| M |F  R  P  S  Q| C |N|
A-Gliadin    |L  G  Q |G| S |F  R  P  S  Q| Q |N|
              206       209 211                    217
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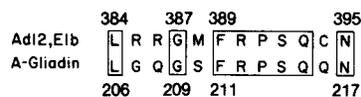
FIGURE 1.   Amino acid sequence of Ad12 E1b protein and A-gliadin beginning at residues 384 and 206, respectively. The single-letter code is that recommended by the IUPAC (43).
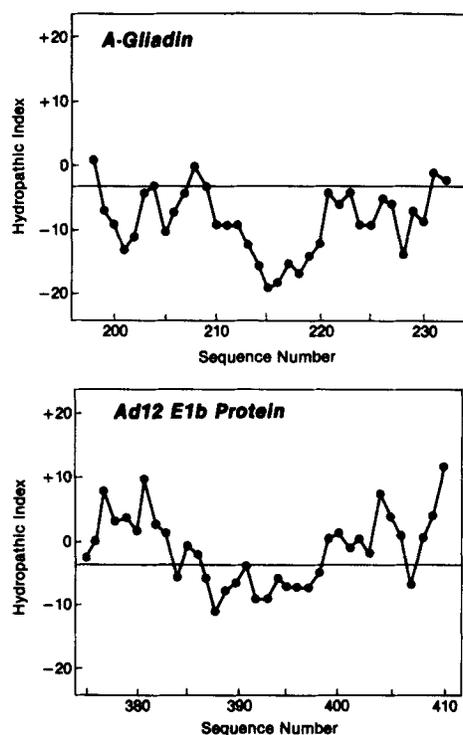
FIGURE 2. Hydropathic character of A-gliadin and Ad12 E1b protein. The figure depicts the hydrophilic and hydrophobic character of A-gliadin over the segment spanning residues 198–232 (*top*) and of Ad12 E1b protein over the segment spanning residues 375–410 (*bottom*). Regions below the solid line are likely to be on the exterior of the molecule.

tide (residues 211–217)/OVA/Sepharose 4B columns and tested by RIA for binding to A-gliadin and A-gliadin peptides. As shown in Fig. 6, affinity-purified antibody reacted with synthetic A-gliadin peptide (residues 211–217), intact A-gliadin, and the CNII peptide of A-gliadin (residues 128–246) containing the region of shared sequence between A-gliadin and the Ad12 E1b protein. The same affinity-purified antibody did not react with CNI peptide (A-gliadin residues 1–127), CNIII peptide (A-gliadin residues 247–268), or a control protein (BSA).

Further studies demonstrated that rat or hamster antiserum to Ad12-transformed BRK or BHK cell extracts cross-reacts with A-gliadin (Fig. 7). In contrast, control rat and hamster serum, antisera to control BRK$_{p17}$ cell extracts (E1b$^-$), and antisera to control BHK cell extracts (E1b$^-$) do not cross-react with A-gliadin (Fig. 7). Finally, as shown in Fig. 8, rabbit antisera to the synthetic A-gliadin peptide (residues 211–217) reacts with proteins immunoprecipitated from the Ad12-transformed BRK cell line and binding is specifically inhibited by free A-gliadin heptapeptide (Fig. 8).

Discussion

These studies demonstrate a region of amino acid sequence homology between the Ad12 E1b protein and A-gliadin, an alpha-gliadin component known to

A   B   C
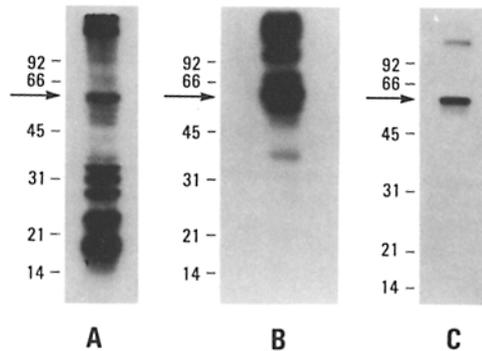
FIGURE 3. [35S]Methionine-labeled proteins were immunoprecipitated from Ad12-transformed BRK or BHK cells as described in Materials and Methods. Immunoprecipitates were run on 10% SDS-polyacrylamide gels. Autoradiograms were prepared as described in Materials and Methods. Arrow indicates the position of the 54-kD E1b protein. (A) Extracts of Ad12-transformed BRK cells immunoprecipitated with Ad12 antitumor sera from rats bearing a tumor of pAd12RIC-transformed BRK cells; (B) extracts of Ad12-transformed BRK cells immunoprecipitated with rat antiserum to BRK cell extracts; (C) extracts of Ad12-transformed BHK cells immunoprecipitated with rat antiserum to BRK cell extracts. Not shown: the 54-Kd E1b protein was not present in control BRK$_{p17}$ cells or nontransformed BHK cells and was not precipitated from Ad12-transformed BRK or BHK cells by normal rat or hamster serum or by serum from rats immunized with control BRK$_{p17}$ cell extracts.

BRK   BHK
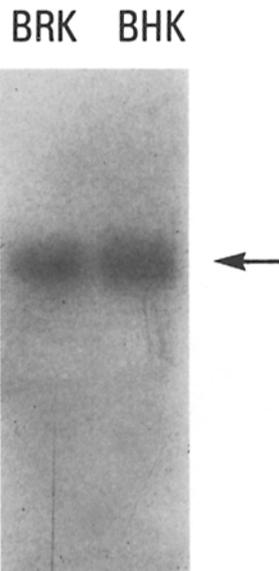
FIGURE 4. Western immunoblotting analysis of Ad12 E1b protein from Ad12-transformed BRK and BHK cells. Immunoprecipitates of Ad12-transformed BRK and BHK cells separated on SDS-polyacrylamide gels were electrophoretically transferred to nitrocellulose paper and then probed with ^{125}I-labeled affinity-purified rabbit antibody to synthetic A-gliadin peptide (residues 211–217). Antibody to the A-gliadin peptide reacted only with the 54-kD Ad12 E1b protein (arrow).

Serum Dilution (x 10⁻³)
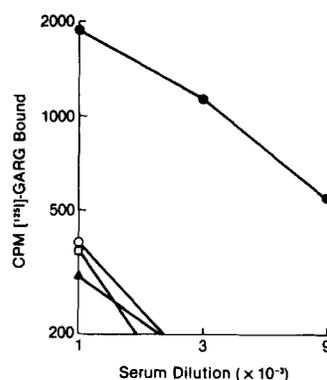
FIGURE 5. Antiserum to Ad12-transformed BRK cells cross-reacts with synthetic A-gliadin heptapeptide (residues 211–217). RIA in which wells of microtiter plates were sensitized with synthetic A-gliadin heptapeptide (residues 211–217) coupled to BSA as described in Materials and Methods. As shown, rat antiserum to Ad12-transformed BRK cells (E1b⁺) (●) reacts with synthetic A-gliadin heptapeptide (residues 211–217), whereas rat antiserum to control BRK$_{p17}$ cells (E1b⁻) (▲) does not. Two different rabbit antisera to adenovirus type 5 E1b protein, a protein that is highly homologous with Ad12 E1b but not with A-gliadin, do not react with the A-gliadin heptapeptide (○; □). Control prebleed rat serum, normal rat serum, and normal rabbit serum resulted in binding of 100–200 cpm at serum dilutions of 1:1000. Counts per minute obtained in control wells sensitized with BSA have been subtracted as background.

Antibody Concentration (ng/ml)
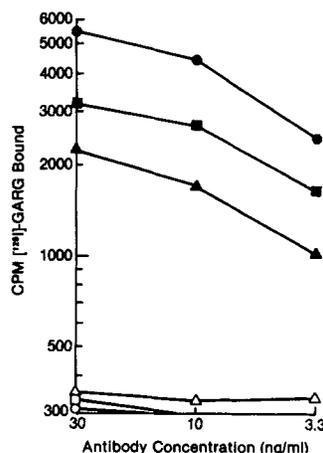
FIGURE 6. Anti-A-gliadin heptapeptide (residues 211–217) antibody affinity-purified from antiserum to Ad12-transformed BRK cell extracts cross-reacts with A-gliadin and A-gliadin peptides that share homology with the Ad12 E1b protein. Antibody to the synthetic A-gliadin peptide (residues 211–217) was affinity-purified from antiserum to Ad12-transformed BRK cells and tested by RIA for binding to A-gliadin and A-gliadin peptides. As shown, affinity-purified antibody reacts with A-gliadin heptapeptide (residues 211–217) (●), the CNII fragment of A-gliadin (residues 128–246) (■), and intact A-gliadin (residues 1–266) (▲), but not with the CNIII fragment (residues 247–266) (○) or the CNI fragment (residues 1–127) (□) of A-gliadin or with BSA (△).

activate human celiac disease (17). Ad12 is a human adenovirus that is usually isolated from the intestinal tract and can be detected as early as the first 1–2 yr of life (19, 20). Ad12 has not been implicated previously as a cause of human disease.
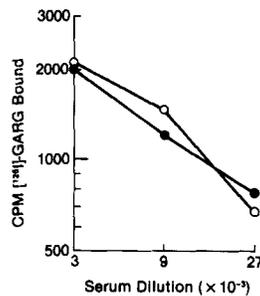
FIGURE 7. Antisera to Ad12-transformed BRK and BHK cell extracts cross-reacts with A-gliadin. RIA in which rat antiserum to Ad12-transformed BRK (E1b⁺) cell extracts (●) or hamster antiserum to Ad12-transformed BHK (E1b⁺) (○) cell extracts were added to wells of microtiter plates sensitized with A-gliadin. Binding with prebleed rat or hamster serum, antiserum to BRK$_{p17}$ cell extracts (E1b⁻), and antiserum to nontransformed BHK cell extracts (E1b⁻) was <500 cpm at serum dilutions of 1:1000.
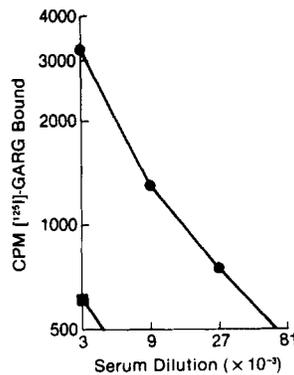
FIGURE 8. Rabbit antisera to synthetic A-gliadin peptide (residues 211–217) (●) binds to immunoprecipitates of Ad12-transformed BRK cells and binding is specifically inhibited by free A-gliadin heptapeptide. Wells of microtiter plates were sensitized with proteins immunoprecipitated from Ad12-transformed BHK cells as outlined in Methods and Materials. Binding of rabbit anti-A-gliadin heptapeptide antibody was specifically inhibited by free A-gliadin peptide (residues 211–217) (1 µM/ml) (■), but not by an unrelated peptide (cytochrome b sequence MTPMRKINPE) (not shown). Binding of hyperimmune rabbit serum to unrelated antigens (e.g., murine IgG2a, sonicates of the parasite Giardia lamblia, sheep erythrocytes) was <500 cpm at serum dilutions of 1:3000.

The region of homology between A-gliadin and the 54-kD Ad12 E1b protein spanned 12 amino acids and included 8 residue identities and an identical pentapeptide. A-gliadin has 32 glutamines and 15 prolines per 100 amino acid residues (18). However, the region of homology occurred in domain V of A-gliadin, a domain that lacks repeating sequences with a high glutamine and proline content (18). Further, the region included only a single glutamine and proline residue in the Ad12 E1b protein. Homology between the Ad12 E1b protein and A-gliadin is probably due to chance since these proteins are unrelated functionally and are not likely to share a common ancestry.

Enzyme digestion studies (3) have suggested that the essential peptide in gliadin that activates celiac disease may contain as few as 10 amino acids, although the frequency with which that sequence occurs in gliadin is not known. The high

glutamine and proline content of wheat storage proteins alone does not seem sufficient to activate celiac disease in that wheat glutenins are rich in proline and glutamine, but do not appear to activate disease (3). Omega-gliadins have a higher content of glutamine and proline than A-gliadin (3) (i.e., 45–56 glutamines and 20–30 prolines per 100 residues), but in a recent in vivo study (35), they did not appear to activate celiac disease. Among the 10 other proteins that had 8 or more amino acid identities with A-gliadin over a 20-residue segment, identities occurred mainly in regions rich in glutamine or proline. Further, identities between A-gliadin and those proteins included many gaps, and at most an identical tri- or tetrapeptide.

The region of homology was hydrophilic in both A-gliadin and the Ad12 E1b protein, and thus was more likely to be on the exterior of those proteins (31). Further, antisera reactive with the 54-kD Ad12 E1b protein specifically cross-reacted with A-gliadin, the 119 amino acid CNII A-gliadin peptide that contains the region of shared sequence, and the synthetic heptapeptide of A-gliadin from within the region of homology. The same antisera did not react with CNI or CNIII fragments of A-gliadin that are not homologous with the Ad12 E1b protein or with irrelevant control proteins. Serum from normal rats or hamsters and antiserum to extracts of control BRK_{p17} cells or normal hamster cells that lack the Ad12 E1b protein did not cross-react with A-gliadin or the synthetic A-gliadin heptapeptide. Although E1b from Ad5 is highly homologous with Ad12 E1b (32, 33), it does not share a region of homology with A-gliadin and, as predicted, antisera to Ad5 E1b do not cross-react with A-gliadin or its peptides. Taken together, these studies indicate that antibody to the native 54-kD Ad12 E1b protein specifically cross-reacts with A-gliadin in the region of shared sequence. Conversely, affinity-purified rabbit antibody to intact A-gliadin was shown to cross-react with CNII peptide, the synthetic A-gliadin heptapeptide, and E1b containing immunoprecipitates of Ad12 transformed BRK cells (data not shown).

Ad12 is a double-stranded DNA virus and is known to transform mammalian cell lines (19). Further, cells transformed by Ad12 induce tumors in rodents at a high frequency within a relatively short time period (36). The transforming activity of Ad12 has been assigned to the left-hand 11% of the viral genome (i.e., early region I) (37), which contains two transcriptional units, E1a and E1b in a 3.9-kb DNA segment (38, 39). Those units are the first to be expressed during lytic infection of cells. The E1b genomic region codes for the 54-kD protein described in this study, as well as a 19-kD polypeptide (32, 33). Recently, the oncogenic potential of Ad12 has been associated with a decreased expression of class 1 major histocompatibility complex antigens on the surface of infected cells, which may render those cells less susceptible to elimination by T cell killing (40, 41). In this regard, we note also that the risk of malignant neoplasms, particularly lymphoma and carcinoma of the small intestine, is significantly increased in patients with celiac disease (42).

Genetic, environmental, and immune factors all appear to have an important role in the pathogenesis of celiac disease. The basis for the association between celiac disease and specific HLA specificities is not known. Depending on geographic origin, HLA-B8, -DR3 and/or -DR7, and -DC3 occur in as many as 60–

100% of these patients (5–11). Although the same HLA specificities are present in 20–30% of the normal population, <0.2% of those individuals develop celiac disease, despite the ingestion of wheat and other grains known to activate disease. Moreover, disease is not always concordant among monozygotic twins (11–14).

The present studies suggest that the frequent occurrence of specific HLA haplotypes in celiac disease may reflect the presence of genes associated with those haplotypes that govern the host's immune response to specific viral infection. Encounter of the immune system with a protein produced during intestinal viral infection may be important in the pathogenesis of celiac disease, because of chance immunologic cross-reactivity between the viral protein and dietary gliadin. After the ingestion of gliadin, gliadin peptides associated with intestinal mucosal structures (4) might serve as the target of immune-mediated injury.

Summary

Celiac disease in humans is activated by the dietary ingestion of wheat, rye, triticale, barley, and possibly oats. Gliadins in wheat and similar proteins in the other grains are known to activate disease in susceptible individuals. There is a striking association between celiac disease and HLA-B8, -DR3 and/or -DR7, and -DC3. Nonetheless, <0.2% of individuals with those serologic HLA specificities develop celiac disease and disease is not always concordant among monozygotic twins. We propose that additional environmental factors may be important in the pathogenesis of celiac disease. To investigate that possibility, we examined a data bank of protein sequences for other proteins that might share amino acid sequence homologies with A-gliadin, an alpha-gliadin component known to activate celiac disease and whose complete primary amino acid sequence is known. These studies demonstrate that A-gliadin shares a region of amino acid sequence homology with the 54-kD E1b protein of human adenovirus type 12 (Ad12), an adenovirus usually isolated from the intestinal tract. The region spans 12 amino acid residues, includes 8 residue identities and an identical pentapeptide, and is hydrophilic in both proteins. Antibody reactive with the 54-kD Ad12 E1b protein cross-reacts with A-gliadin, a 119 amino acid cyanogen bromide peptide of A-gliadin that spans the region of homology and a synthetic heptapeptide of A-gliadin from within the region of homology. We suggest that an encounter of the immune system with antigenic determinants produced during intestinal viral infection may be important in the pathogenesis of celiac disease.

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