Studies in vivo of ω-gliadins in gluten sensitivity (coeliac sprue disease)

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ABSTRACT

1. Highly purified ω-gliadins from wheat were used to challenge gluten-sensitized individuals. Characteristic responses by mucosal CD3+ and γδ+ lymphocytes were demonstrated. Each lymphocyte subset showed an increase within 8–12 h post-challenge, indicating a specific response by the rectal mucosa to this gliadin species.

2. Available sequence data for the ω-gliadins and homologous proteins from barley and rye indicate a common repeating octapeptide motif (consensus PQQPFPQQ). The results indicate, therefore, that the octapeptide repeat, or a contained sequence such as PQQP, plays an important role in the mucosal immunopathology of gluten sensitivity.

INTRODUCTION

Gluten sensitivity (‘coeliac sprue disease’) is caused by the ingestion of gluten-containing foods in genetically predisposed (DQw2) individuals and mediated by activated T-lymphocytes (CD4+) within the lamina propria [1,2]. This sensitivity, induced by the consumption of wheat, barley and rye [1], is directed against certain prolamins, which are alcohol-soluble seed storage proteins. In wheat, the prolamins consist of monomeric gliadins and polymeric glutenins, of which only the gliadins have been clearly associated with coeliac disease [1]. There are α-, β-, γ- and ω-gliadins, but while the α-, β- and γ-gliadins are structurally related, consisting of repetitive N-terminal and non-repetitive C-terminal domains, the ω-gliadins are structurally distinct, comprising a single domain made up almost entirely of a single repeat motif [3].

Most studies of coeliac sprue have centred on the α-gliadins, either with the protein intact or enzymically cleaved or with synthetic peptides, but the immunopathogenicity of the ω-gliadins has hardly been explored [4]. Early studies implicated only the α-gliadins in disease activity [5], whereas later studies implied that β- and γ-, but not ω-, gliadins were also active [6,7]. Other studies have implicated all gliadin fractions, with decreasing activity from α- to ω-gliadins [8–11]. As reviewed by Cole and Kagnoff [12], discrepancies can arise due to a number of factors including the methodologies of gliadin fractionation and protein purity, extrapolation of data from in vitro systems to in vivo disease and different clinical and laboratory end points used to interpret results.

In this paper the in vivo activity of a highly purified ω-gliadin fraction, prepared by ion-exchange and covalent chromatography, was studied. The response, after local rectal infusion, was evaluated in terms of mucosal CD3+ and γδ+ lymphocytes. The results are discussed in relation to the present knowledge of ω-gliadin and prolamin structure.

MATERIALS AND METHODS

ω-Gliadin purification

Unbleached white wheat flour (cv. Mercia) was defatted with chloroform [10:1 (v/w), solvent/flour], filtered and air-dried. Non-prolamins were extracted by stirring with...
0.5 M NaCl [twice, 10:1 (v/w), solvent/flour] at room temperature for 2 h. After centrifugation the supernatant was discarded and the flour washed with distilled water to remove residual salt. The flour was extracted with 70% (v/v) aqueous ethanol [twice, 10:1 (v/w), solvent/flour] for 2 h at 4 °C and the supernatant dialysed against 1% (v/v) acetic acid and freeze-dried.

ω-Gliadins were separated by ion-exchange chromatography on carboxymethylcellulose in 3 M urea/10 mM glycine–acetate buffer, pH 4.6, and eluted with a linear salt gradient of 0 to 200 mM. Peaks were analysed by acid-PAGE [13] and ω-gliadin peaks bulked, dialysed and freeze-dried. The ion-exchange step was then repeated. Contaminating α-, β- and γ-gliadins (containing cysteine residues) were removed by passage over a column of activated thiol-Sepharose in 0.5 M NaCl/1 M urea/0.1 M Tris–HCl buffer, pH 8.0. The unbound fraction (ω-gliadin) was eluted with buffer, dialysed and freeze-dried. The bound gliadins (cysteine-containing) were removed with buffer containing 20 mM dithiothreitol.

Fractions were analysed by acid-PAGE and with previously characterized monoclonal antibodies specific for α-, β-, γ and ω-gliadins [14] and low-molecular-mass glutenin subunits [15].

**RESULTS**

**Characterization of ω-gliadins**

ω-Gliadins prepared by ion-exchange chromatography appeared to be essentially pure as judged by acid-PAGE (Figure 1, track 3). Thiol-Sepharose covalently binds cysteine-containing proteins, so ω-gliadins which lack cysteine residues are not bound by the column, whereas contaminating gliadins (α-, β- and γ-type) are covalently attached and unbound only by the addition of a reducing agent (dithiothreitol). The thiol-Sepharose column removed contaminating proteins from the ion-exchange purified ω-gliadin fraction, some with a mobility similar to that of the ω-gliadins, and others similar to the γ-gliadins (Figure 1, track 5). The proteins could not be sequenced as their N-termini were blocked. Dot blot assays using monoclonal antibodies specific for the ω-gliadins (and their homologues from barley and rye: C hordein and ω-secalins respectively), IFRN0061 and IFRN0614 [14], failed to react with the bound proteins. These proteins did react with two other antibodies, IFRN0065 and IFRN0610, with a broader reactivity to other gliadins and glutenins, but not ω-gliadins [15]. The contaminants constituted about 2–3% by weight of the ion-exchange purified ω-fraction. The presence of these contaminating gliadins, not readily detectable by acid-
In recent years the search for coeliac active peptides, by a variety of procedures, has indicated that certain tetrapeptide sequences (PSQQ, PQQP and QQQP) are common to all active peptides [1,24–26]. In a series of _in vivo_ challenges with a consecutive series of synthetic 12- and 13-mer oligopeptides derived from the N-terminus of α-gliadin (residues 3–96), we have shown that a mucosal effect only occurs in the presence of the motif PQQP. Peptides lacking this particular sequence appeared to lack any demonstrable effect on coeliac intestine [26]. This and other putative tetrapeptide motifs (e.g. PSQQ) are unlikely to constitute the active moiety, but may form part of larger excerpts, most of which are present in all prolamin species known to exacerbate the condition and cause mucosal damage [1,4].

Although there are no complete gene sequences for the α-gliadins, there are sequences for the homologous ω-secalins of rye [27], C hordeins of barley [28,29] and their N-termini determined directly from these proteins [30]. These sequences show that the various ω-species consist almost entirely of a repeating octapeptide motif of consensus sequence PQQPFPQQ [30]. The PSQQ and QQQP motifs are absent from the available N-terminal and gene sequences of α-gliadins, ω-secalins and C hordeins [30], while the PQPF motif is found in the predominant octapeptide repeat motif [30]. More recently, it has been suggested that the QQPY tetrapeptide motif may be associated with coeliac activity [31]. This motif is found in the N-terminal region of the α-gliadins of the relatives of wheat, that is, C hordein of barley and α-secalins of rye [30]. It is closely related to a tetrapeptide that constitutes part of a major repeated motif of α-gliadins, viz QQP. This again strongly suggests that the PQQPFPQQ motif is of major importance in disease activity.

The secondary structure of the C hordein of barley has been extensively studied and consists of an equilibrium between β-reverse turns and a poly-L-proline II-like structure, resulting in an extended conformation [32,33]. It has been postulated that immunogenicity is dependent on processing and presentation of gliadin peptides by antigen-presenting cells expressing the DQ2 or DQ8 class 2 MHC molecule [1,2]: also that peptide conformation, particularly β-reverse turn conformations, may be involved in recognition by such coeliac-associated class 2 alloantigens [34]. Furthermore, it should be noted that the β-reverse turns comprising α-gliadins certainly appear to be major immunogenic sites in the production of antibodies [14].

α-Type gliadins have been identified in the glutenin fraction of wheat gluten, bound covalently by cysteine residue(s) [35]. Low-molecular-mass subunits with α- and γ-type N-terminal sequences have been identified [35,36]. The few reported gene sequences for low-molecular-mass glutenin subunits contain coding regions with different degrees of sequence similarity to the...
octapeptide motif of the ω-gliadins and the motifs (e.g., SPQQ, PSQQ) associated with coeliac activity [37–39]. The activity of ω-gliadins reported in this study suggests that it would be difficult to breed wheat varieties with reduced effect on coeliac patients by reducing their gliadin content alone. In view of the activity of the ω-gliadins and the identification of cysteine-containing homologues in the glutenin fraction of wheat, the low- and high-molecular-mass subunits of glutenin also need to be examined for coeliac activity.

Concerning the immunogenic response in the rectal mucosa of the two patients studied, its validity is based on an earlier and extensive series of in vivo challenges. In our first study [40] with a lyophylate of digested gluten redissolved in saline, progressive time- and dose-related increments in epithelial lymphocytes were demonstrable by 12 h, being (+)10% (not significant), 25% (P > 0.05), 44% (P = 0.01) and 55% (P < 0.005) with doses of 100, 500, 1000 and 1500 mg of protein. Control experiments revealed that: (i) in six coeliac patients no lymphocyte response occurred after a 500-mg challenge with β-lactoglobulin; (ii) among 12 disease-control subjects, no lymphocyte infiltration nor mucosal changes were evoked with either 1000 mg (three challenges) or 1500 mg (four challenges) of gluten digest, nor with 500 mg of β-lactoglobulin (six challenges).

Subsequently [41], a dose of 1500 mg of gluten was shown to evoke a crypt epithelial lymphocyte infiltrate. With 3 g of gluten, crypt hypertrophy became an added feature in addition to increased epithelial infiltration, whereas with 6 g and 12 g of gluten, progressive villous flattening, increasing crypt hypertrophy with raised mitotic activity and lamina propria swelling was documented [41]. Epithelial cell height was significantly reduced (45%) with the 12-g challenge [41]. Thus with this dynamic approach, the entire spectrum of mucosal changes typical of, and specific to, gluten sensitivity [1] was evoked. No responses to gluten were obtained in the various groups of disease-control subjects, nor in either coeliac patients or controls with the milk-sensitizing protein β-lactoglobulin.

With this extensive background, the rectal response to gluten and β-lactoglobulin was similarly analysed in coeliac and a variety of disease-control subjects, thus reflecting (i) previous observations [42,43] and (ii) the physiological dogma that the homing of intestinal-sensitized T-lymphocytes proceeds via the microvasculature to all regions of the lamina propria, including colon and rectum [44–48].

As with jejunum, rectal challenge evoked specific, measurable and reproducible changes in coeliac patients to gluten, but not β-lactoglobulin. The temporal evolution of these responses was characterized by rises in CD3+ and γδ+ lymphocytes [49] accompanied by parallel up-regulation in microvascular VCAM-1 and ELAM-1 expression [50,51], while in a further series of double challenges with either gluten or β-lactoglobulin [52], there was a specific (i.e. gluten-induced) coeliac-associated up-regulation of mRNA for the pro-inflammatory cytokines interleukin-1β, interleukin-8 and monocyte chemotactic protein that preceded the lymphocyte infiltration occurring around 8–12 h post-challenge. These unique, coeliac-associated responses to rectal gluten (and not β-lactoglobulin) provided the basis on which two diagnostic indices of high specificity and sensitivity [53,54] were formulated.

The present study, therefore, extends the previous work on rectal challenge, indicating that highly purified ω-gliadins evoke identical immunohistopathological changes which characterize the coeliac-specific response to x-gluten-derived peptides, but not to other immunogens such as β-lactoglobulin. Although no controls were used here, it is reasonably safe to infer, given the extensive in vivo challenge background [40–42,49–54], that there would not have been a demonstrable response. Furthermore, in some of our most recent work on rectal challenge (A. Ensari, M. N. Marsh, S. Morgan, P. Crowe and K. Moriarty, unpublished work) with a similar quantity (1 g) of the x-gliadin sequences 44–55 (PQPQPFSPQQPYP) and 69–81 (SQPQPFSPQQPYP), identical CD3 and γδ lymphocyte responses were again demonstrated.

Thus, in conclusion, there can be no doubt that we have observed a specific immunopathological effect that was solely due to ω-gliadin, and not other preparative contaminants. Since ω-gliadins appear to be structured on a repeat octapeptide motif, the data suggest that PQPQPFPQQ, or a sequence or conformation within it, is immunopathogenic to gluten-sensitized individuals.

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REFERENCES


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