(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 218 751 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
01.03.2006 Bulletin 2006/09

(21) Application number: **00964460.0**

(22) Date of filing: **02.10.2000**

(51) Int Cl.:
G01N 33/68 *(2006.01)* ***C07K 14/415*** *(2006.01)*
A61P 37/08 *(2006.01)*

(86) International application number:
PCT/GB2000/003760

(87) International publication number:
WO 2001/025793 (12.04.2001 Gazette 2001/15)

(54) **DIAGNOSIS OF COELIAC DISEASE USING A GLIADIN EPITOPE**

DIAGNOSE VON EINHEIMISCHER SPRUE/ZÖLIAKIE MIT HILFE VON GLIADINEPITOPEN

DIAGNOSTIC DE LA MALADIE COELIAQUE A L'AIDE DE L'EPITOPE DE GLIADINE

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **01.10.1999 GB 9923306**

(43) Date of publication of application:
03.07.2002 Bulletin 2002/27

(60) Divisional application:
05027922.3

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Description

[0001] The invention relates to the diagnosis and therapy of coeliac disease, and to a gliadin protein which does not cause coeliac disease.

[0002] An immune reaction to gliadin (a component of gluten) in the diet causes coeliac disease. It is known that immune responses in the intestinal tissue preferentially respond to gliadin which has been modified by an intestinal transglutaminase. Coeliac disease is diagnosed by detection of anti-endomysial antibodies, but this requires confirmation by the finding of a lymphocytic inflammation in intestinal biopsies. The taking of such a biopsy is inconvenient for the patient.

[0003] Investigators have previously assumed that only intestinal T cell responses provide an accurate indication of the immune response against gliadins. Therefore they have concentrated on the investigation of T cell responses in intestinal tissue[1]. For example, EP 0905518 discloses T-cell epitopes from glutenin and gliadin. These are specifically recognised by intestinally derived gluten-specific or sensitive T-cells. The peptides have sequences SGQGSFQPSQQ (gliadin 206-216; SEQ ID NO:4) and GQQGYYPTSPQQSGQ (glutenin; SEQ ID NO:5). Gliadin epitopes which require transglutaminase modification (before they are recognised by the immune system) are known[2].

[0004] The inventors have found the immunodominant T cell epitope recognised by the immune system in coeliac disease, and have shown that this is recognised by T cells in the peripheral blood of individuals with coeliac disease. Such T cells were found to be present at high enough frequencies to be detectable without restimulation (i.e. a 'fresh response' detection system could be used). The epitope was identified using a non-T cell cloning based method which provided a more accurate reflection of the epitopes being recognised. The immunodominant epitope requires transglutaminase modification (causing substitution of a particular glutamine to glutamate) before immune system recognition.

[0005] Based on this work the inventors have developed a test which can be used to diagnose coeliac disease at an early stage. The test may be carried out on a sample from peripheral blood and therefore an intestinal biopsy is not required. The test is more sensitive than the antibody tests which are currently being used.

[0006] The invention thus provides a method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising:

(a) contacting a sample from the host with an agent selected from (i) the epitope comprising sequence which is: SEQ ID NO:1 or 2, or an equivalent sequence from a naturally occurring homologue of the gliadin represented by SEQ ID NO:3, (ii) an epitope comprising sequence comprising: SEQ ID NO:1, or an equivalent sequence from a naturally occurring homologue of the gliadin represented by SEQ ID NO:3, which epitope is an isolated oligopeptide derived from a gliadin protein, (iii) an analogue of (i) or (ii) which is capable of being recognised by a T cell receptor that recognises (i) or (ii), which in the case of a peptide analogue is not more than 50 amino acids in length, or (iv) a product comprising two or more agents as defined in (i), (ii) or (iii), and (b) determining *in vitro* whether T cells in the sample recognise the agent, recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

[0007] The invention also provides use of the agent for the preparation of a diagnostic means for use in a method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual, said method comprising determining whether T cells of the individual recognise the agent, recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

[0008] The finding of an immunodominant epitope which is modified by transglutaminase also allows diagnosis of coeliac disease based on determining whether other types of immune response to this epitope are present. Thus the invention also provides a method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising determining the presence of an antibody that binds to the epitope in a sample from the individual, the presence of the antibody indicating that the individual has, or is susceptible to, coeliac disease.

[0009] The invention additionally provides the agent, optionally in association with a carrier, for use in a method of treating or preventing coeliac disease by tolerising T cells which recognise the agent. Also provided is an antagonist of a T cell which has a T cell receptor that recognises (i) or (ii), optionally in association with a carrier, for use in a method of treating or preventing coeliac disease by antagonising such T cells. Additionally provided is the agent or an analogue that binds an antibody (that binds the agent) for use in a method of treating or preventing coeliac disease in an individual by tolerising the individual to prevent the production of such an antibody.

[0010] The invention provides a method of determining whether a composition is capable of causing coeliac disease comprising determining whether a protein capable of being modified by a trans glutaminase to an oligopeptide sequence as defined above is present in the composition, the presence of the protein indicating that the composition is capable of causing coeliac disease.

[0011] The invention also provides a mutant gliadin protein whose wild-type sequence can be modified by a transglutaminase to a sequence that comprises an epitope comprising sequence as defined above, but which mutant gliadin

protein has been modified in such a way that it does not contain sequence which can be modified by a transglutaminase to a sequence that comprises such an epitope comprising sequence; or a fragment of such a mutant gliadin protein which is at least 15 amino acids long and which comprises sequence which has been modified in said way.

[0012] The invention also provides a protein that comprises a sequence which is able to bind to a T cell receptor, which T cell receptor recognises the agent, and which sequence is able to cause antagonism of a T cell that carries such a T cell receptor.

[0013] Additionally the invention provides a food that comprises the proteins defined above.

[0014] The invention is illustrated by the accompanying drawings in which:

Figure 1 shows freshly isolated PBMC (peripheral blood mononuclear cell) IFNγ ELISPOT responses (vertical axis shows spot forming cells per 10^6 PBMC) to transglutaminase (tTG)-treated and untreated peptide pool 3 (each peptide 10 μg/ml) including five overlapping 15mers spanning A-gliadin 51-85 (see Table 1) and a-chymotrypsin-digested gliadin (40 μg/ml) in coeliac disease Subject 1, initially in remission following a gluten free diet then challenged with 200g bread daily for three days from day 1 **(a).** PBMC IFNγ ELISPOT responses by Subject 2 to tTG-treated A-gliadin peptide pools 1-10 spanning the complete A-gliadin protein during ten day bread challenge **(b)**. The horizontal axis shows days after commencing bread.

Figure 2 shows PBMC IFNγ ELISPOT responses to tTG-treated peptide pool 3 (spanning A-gliadin 51-85) in 7 individual coeliac disease subjects (vertical axis shows spot forming cells per 10^6 PBMC), initially in remission on gluten free diet, challenged with bread for three days (days 1 to 3). The horizontal axis shows days after commencing bread.**(a)**. PBMC IFNg Elispot responses to tTG-treated overlapping 15mer peptides included in pool 3; bars represent the mean (± SEM) response to individual peptides (10 μg/ml) in 6 Coeliac disease subjects on day 6 or 7 **(b)**. (In individual subjects, ELISPOT responses to peptides were calculated as a % of response elicited by peptide 12 - as shown by the vertical axis.)

Figure 3 shows PBMC IFNγ ELISPOT responses to tTG-treated truncations of A-gliadin 56-75 (0.1 μM). Bars represent the mean (± SEM) in 5 Coeliac disease subjects. (In individual subjects, responses were calculated as the % of the maximal response elicited by any of the peptides tested.)

Figure 4 shows how the minimal structure of the dominant A-gliadin epitope was mapped using tTG-treated 7-17mer A-gliadin peptides (0.1 μM) including the sequence, PQPQLPY (A-gliadin 62-68) **(a),** and the same peptides without tTG treatment but with the substitution Q-E65 **(b).** Each line represents PBMC IFNg ELISPOT responses in each of three Coeliac disease subjects on day 6 or 7 after bread was ingested on days 1-3. (In individual subjects, ELISPOT responses were calculated as a % of the response elicited by the 17mer, A-gliadin 57-73.)

Figure 5 shows the amino acids which were deamidated by tTG. A-gliadin 56-75 (LQLQPFPQPQLPYPQPQSFP) (0.1 μM) was incubated with tTG (50 μg/ml) at 37°C for 2 hours. A single product was identified and purified by reverse phase HPLC. Amino acid analysis allowed % deamidation (Q→E) of each Gln residue in A-gliadin 56-75 attributable to tTG to be calculated (vertical axis).

Figure 6 shows the effect of substituting Q→E in A-gliadin 57-73 at other positions in addition to Q65 using the 17mers: QLQPFPQPELPYPQPES (E57,65), QLQPFPQPELPYPQPES (E65,72), ELQPFPQPELPYPQPES (E57, 65, 72), and QLQPFPQPELPYPQPQS (E65) in three Coeliac disease subjects on day 6 or 7 after bread was ingested on days 1-3. Vertical axis shows % of the E65 response.

Figure 7 shows that tTG treated A-gliadin 56-75 (0.1 μM) elicited IFN-g ELISPOT responses in **(a)** CD4 and CD8 magnetic bead depleted PBMC. (Bars represent CD4 depleted PBMC responses as a % of CD8 depleted PBMC responses; spot forming cells per million CD8 depleted PBMC were: Subject 4: 29, and Subject 6: 535). **(b)** PBMC IFNγ ELISPOT responses (spot forming cells/million PBMC) after incubation with monoclonal antibodies to HLA-DR (L243), -DQ (L2) and -DP (B7.21) (10 μg/ml) 1h prior to tTG-treated 56-75 (0.1 μM) in two coeliac disease subjects homozygous for HLA-DQ a1*0501, b1*0201.

Figure 8 shows the effect of substituting Glu at position 65 for other amino acids in the immunodominant epitope. The vertical axis shows the % response in the 3 subjects in relation to the immunodominant epitope.

Figure 9 shows the immunoreactivity of naturally occurring gliadin peptides (measuring responses from 3 subjects) which contain the sequence PQLPY with (shaded) and without (clear) transglutaminase treatment.

Figure 10 shows CD8, CD4, β_7, and α^E -specific immunomagnetic bead depletion of peripheral blood mononuclear cells from two coeliac subjects 6 days after commencing gluten challenge followed by interferon gamma ELISpot. A-gliadin 57-73 QE65 (25mcg/ml), tTG-treated chymotrypsin-digested gliadin (100 mcg/ml) or PPD (10 mcg/ml) were used as antigen.

Figure 11 shows the optimal T cell epitope length.

Figure 12 shows a comparison of A-gliadin 57-73 QE65 with other peptides in a dose response study.

Figure 13 shows a comparison of gliadin and A-gliadin 57-73 QE65 specific responses.

Figure 14 shows the bioactivity of gliadin polymorphisms in coeliac subjects.

Figures 15 and 16 show the defining of the core epitope sequence.

Figures 17 to 27 show the agonist activity of A-gliadin 57-73 QE65 variants.
Figure 28 shows responses in different patient groups.

Detailed description of the invention

[0015] The term 'coeliac disease' encompasses a spectrum of conditions caused by varying degrees of gluten sensitivity, including a severe form characterised by a flat small intestinal mucosa (hyperplastic villous atrophy) and other forms characterised by milder symptoms.

[0016] The individual mentioned above (in the context of diagnosis or therapy) is human. They may have coeliac disease (symptomatic or asymptomatic) or be suspected of having it. They may be on a gluten free diet. They may be in an acute phase response (for example they may have coeliac disease, but have only ingested gluten in the last 24 hours before which they had been on a gluten free diet for 14 to 28 days).

[0017] The individual may be susceptible to coeliac disease, such as a genetic susceptibility (determined for example by the individual having relatives with coeliac disease or possessing genes which cause predisposition to coeliac disease).

The agent

[0018] The agent is typically a peptide, for example of length 7 to 50 amino acids, such as 10 to 40, or 15 to 30 amino acids in length.

[0019] SEQ ID NO: 1 is PQPELPY. SEQ ID NO:2 is QLQPFPQPELPYPQPQS. SEQ ID NO:3 is shown in Table 1 and is the sequence of a whole A-gliadin. The glutamate at position 4 of SEQ ID NO:1 (equivalent to position 9 of SEQ ID NO:2) is generated by transglutaminase treatment of A-gliadin.

[0020] The agent may be the peptide represented by SEQ ID NO:1 or 2 or an epitope comprising sequence that comprises SEQ ID NO:1 which is an isolated oligopeptide derived from a gliadin protein; or an equivalent of these sequences from a naturally occurring gliadin protein which is a homologue of SEQ ID NO:3. Thus the epitope may be a derivative of the protein represented by SEQ ID NO:3. Such a derivative is typically a fragment of the gliadin, or a mutated derivative of the whole protein or fragment. Therefore the epitope of the invention does not include this naturally occurring whole gliadin protein, and does not include other whole naturally occurring gliadins.

[0021] The epitope may thus be a fragment of A-gliadin (e.g. SEQ ID NO:3), which comprises the sequence of SEQ ID NO:1, obtainable by treating (fully or partially) with transglutaminase, i.e. with 1, 2, 3 or more glutamines substituted to glutamates (including the substitution within SEQ ID NO:1).

[0022] Such fragments may be or may include the sequences represented by positions 55 to 70, 58 to 73, 61 to 77 of SEQ ID NO:3 shown in Table 1. Typically such fragments will be recognised by T cells to at least the same extent that the peptides represented by SEQ ID NO:1 or 2 are recognised in any of the assays described herein using samples from coeliac disease patients.

[0023] In the case where the epitope comprises a sequence equivalent to the above epitopes (including fragments) from another gliadin protein (e.g. any of the gliadin proteins mentioned herein or any gliadins which cause coeliac disease), such equivalent sequences will correspond to a fragment of a gliadin protein typically treated (partially or fully) with transglutaminase. Such equivalent peptides can be determined by aligning the sequences of other gliadin proteins with SEQ ID NO:3 (for example using any of the programs mentioned herein). Transglutaminase is commercially available (e.g. Sigma T-5398). Table 4 provides examples of suitable equivalent sequences.

[0024] The agent which is an analogue is capable of being recognised by a TCR which recognises (i) or (ii). Therefore generally when the analogue is added to T cells in the presence of (i) or (ii), typically also in the presence of an antigen presenting cell (APC) (such as any of the APCs mentioned herein), the analogue inhibits the recognition of (i) or (ii), i.e. the analogue is able to compete with (i) or (ii) in such a system.

[0025] The analogue may be one which is capable of binding the TCR which recognises (i) or (ii). Such binding can be tested by standard techniques. Such TCRs can be isolated from T cells which have been shown to recognise (i) or (ii) (e.g. using the method of the invention). Demonstration of the binding of the analogue to the TCRs can then shown by determining whether the TCRs inhibit the binding of the analogue to a substance that binds the analogue, e.g. an antibody to the analogue. Typically the analogue is bound to a class II MHC molecule (e.g. HLA-DQ2) in such an inhibition of binding assay.

[0026] Typically the analogue inhibits the binding of (i) or (ii) to a TCR. In this case the amount of (i) or (ii) which can bind the TCR in the presence of the analogue is decreased. This is because the analogue is able to bind the TCR and therefore competes with (i) or (ii) for binding to the TCR.

[0027] T cells for use in the above binding experiments can be isolated from patients with coeliac disease, for example with the aid of the method of the invention. Other binding characteristics of the analogue may also be the same as (i) or (ii), and thus typically the analogue binds to the same MHC class II molecule to which the peptide binds (HLA-DQ2). The analogue typically binds to antibodies specific for (i) or (ii), and thus inhibits binding of (i) or (ii) to such antibodies.

[0028] The analogue is typically a peptide. It may have homology with (i) or (ii), typically at least 70% homology, preferably at least 80, 90%, 95%, 97% or 99% homology with (i) or (ii), for example over a region of at least 15 more (such as the entire length of the analogue and/or (i) or (ii), or across the region which contacts the TCR or binds the MHC molecule) contiguous amino acids. Methods of measuring protein homology are well known in the art and it will be understood by those of skill in the art that in the present context, homology is calculated on the basis of amino acid identity (sometimes referred to as "hard homology").

[0029] For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al* (1984) *Nucleic Acids Research* **12**, p387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul S. F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10.

[0030] Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci.* USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

[0031] The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad Sci.* USA 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0032] The homologous peptide analogues typically differ from (i) or (ii) by 1, 2, 3, 4, 5, 6, 7, 8 or more mutations (which may be substitutions, deletions or insertions). These mutation may be measured across any of the regions mentioned above in relation to calculating homology. The substitutions are preferably 'conservative'. These are defined according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

[0033] Typically the amino acids in the analogue at the equivalent positions to amino acids in (i) or (ii) which contribute to binding the MHC molecule or are responsible for the recognition by the TCR, are the same or are conserved.

[0034] Typically the analogue peptide comprises one or more modifications, which may be natural post-translation modifications or artificial modifications. The modification may provide a chemical moiety (typically by substitution of a hydrogen, e.g. of a C-H bond), such as an amino, acetyl, hydroxy or halogen (e.g. fluorine) group or carbohydrate group. Typically the modification is present on the N or C terminus.

[0035] The analogue may comprise one or more non-natural amino acids, for example amino acids with a side chain different from natural amino acids. Generally, the non-natural amino acid will have an N terminus and/or a C terminus. The non-natural amino acid may be an L- or a D- amino acid.

[0036] The analogue typically has a shape, size, flexibility or electronic configuration which is substantially similar to (i) or (ii). It is typically a derivative of (i) or (ii). In one embodiment the analogue is a fusion protein comprising the sequence

of SEQ ID NO:1 or 2, or any of the other peptides mentioned herein; and non-gliadin sequence.

[0037] In one embodiment the analogue is or mimics (i) or (ii) bound to a MHC class II molecule. 2, 3, 4 or more of such complexes may be associated or bound to each other, for example using a biotin/streptavidin based system, in which typically 2, 3 or 4 biotin labelled MHC molecules bind to a streptavidin moiety. This analogue typically inhibits the binding of the (i) or (ii)/MHC Class II complex to a TCR or antibody which is specific for the complex.

[0038] The analogue is typically an antibody or a fragment of an antibody, such as a Fab or $(Fab)_2$ fragment. The analogue may be immobilised on a solid support, particularly an analogue which mimics peptide bound to a MHC molecule.

[0039] The analogue is typically designed by computational means and then synthesised using methods known in the art. Alternatively the analogue can be selected from a library of compounds. The library may be a combinatorial library or a display library, such as a phage display library. The library of compounds may be expressed in the display library in the form of being bound to a MHC class II molecule, such as HLA-DQ2. Analogues are generally selected from the library based on their ability to mimic the binding characteristics (i) or (ii). Thus they may be selected based on ability to bind a TCR or antibody which recognises (i) or (ii).

[0040] Typically analogues will be recognised by T cells to at least the same extent as any of the agents (i) or (ii), for example at least to the same extent as the equivalent epitope and preferably to the same extent as the peptide represented by SEQ ID NO:2, is recognised in any of the assays described herein, typically using T cells from coeliac disease patients. Analogues may be recognised to these extents in *vivo* and thus may be able to induce coeliac disease symptoms to at least the same extent as any of the agents mentioned herein (e.g. in a human patient or animal model).

[0041] Analogues may be identified in a method comprising determining whether a candidate substance is recognised by a T cell receptor that recognises an epitope of the invention, recognition of the substance indicating that the substance is an analogue. Such TCRs may be any of the TCRs mentioned herein, and may be present on T cells. Any suitable assay mentioned herein can be used to identify the analogue. In one embodiment this method is carried out *in vivo.* As mentioned above preferred analogues are recognised to at least the same extent as the peptide SEQ ID NO:2, and so the method may be used to identify analogues which are recognised to this extent.

[0042] In one embodiment the method comprises determining whether a candidate substance is able to inhibit the recognition of an epitope of the invention, inhibition of recognition indicating that the substance is an analogue.

[0043] The agent may be a product comprising at least 2, 5, 10 or 20 agents as defined by (i), (ii) or (iii). Typically the composition comprises epitopes of the invention (or equivalent analogues) from different gliadins, such as any of the species or variety of or types of gliadin mentioned herein. Preferred compositions comprise at least one epitope of the invention, or equivalent analogue, from all of the gliadins present in any of the species or variety mentioned herein, or from 2, 3, 4 or more of the species mentioned herein (such as from the panel of species consisting of wheat, rye, barley, oats and triticale).

Diagnosis

[0044] As mentioned above the method of diagnosis of the invention may be based on the detection of T cells which bind the agent or on the detection of antibodies that recognise the agent.

[0045] The T cells which recognise the agent in the method (which includes the use mentioned above) are generally T cells which have been pre-sensitised *in vivo* to gliadin. As mentioned above such antigen-experienced T cells have been found to be present in the peripheral blood.

[0046] In the method the T cells can be contacted with the agent *in vitro* or *in vivo*, and determining whether the T cells recognise the agent can be performed *in vitro* or *in vivo.* Thus the invention provides the agent for use in a method of diagnosis practiced on the human body. Different agents are provided for simultaneous, separate or sequential use in such a method.

[0047] The *in vitro* method is typically carried out in aqueous solution into which the agent is added. The solution will also comprise the T cells (and in certain embodiments the APCs discussed below). The term 'contacting' as used herein includes adding the particular substance to the solution.

[0048] Determination of whether the T cells recognise the agent is generally done by detecting a change in the state of the T cells in the presence of the agent or determining whether the T cells bind the agent. The change in state is generally caused by antigen specific functional activity of the T cell after the TCR binds the agent. The change of state may be measured inside (e.g. change in intracellular expression of proteins) or outside (e.g. detection of secreted substances) the T cells.

[0049] The change in state of the T cell may be the start of or increase in secretion of a substance from the T cell, such as a cytokine, especially IFN-γ, IL-2 or TNF-α. Determination of IFN-γ secretion is particularly preferred. The substance can typically be detected by allowing it to bind to a specific binding agent and then measuring the presence of the specific binding agent/substance complex. The specific binding agent is typically an antibody, such as polyclonal or monoclonal antibodies. Antibodies to cytokines are commercially available, or can be made using standard techniques.

[0050] Typically the specific binding agent is immobilised on a solid support. After the substance is allowed to bind

the solid support can optionally be washed to remove material which is not specifically bound to the agent. The agent/substance complex may be detected by using a second binding agent which will bind the complex. Typically the second agent binds the substance at a site which is different from the site which binds the first agent. The second agent is preferably an antibody and is labelled directly or indirectly by a detectable label.

[0051] Thus the second agent may be detected by a third agent which is typically labelled directly or indirectly by a detectable label. For example the second agent may comprise a biotin moiety, allowing detection by a third agent which comprises a streptavidin moiety and typically alkaline phosphatase as a detectable label.

[0052] In one embodiment the detection system which is used is the *ex-vivo* ELISPOT assay described in WO 98/23960. In that assay IFN-γ secreted from the T cell is bound by a first IFN-γ specific antibody which is immobilised on a solid support. The bound IFN-γ is then detected using a second IFN-γ specific antibody which is labelled with a detectable label. Such a labelled antibody can be obtained from MABTECH (Stockholm, Sweden). Other detectable labels which can be used are discussed below.

[0053] The change in state of the T cell which can be measured may be the increase in the uptake of substances by the T cell, such as the uptake of thymidine. The change in state may be an increase in the size of the T cells, or proliferation of the T cells, or a change in cell surface markers on the T cell.

[0054] In one embodiment the change of state is detected by measuring the change in the intracellular expression of proteins, for example the increase in intracellular expression of any of the cytokines mentioned above. Such intracellular changes may be detected by contacting the inside of the T cell with a moiety that binds the expressed proteins in a specific manner and which allows sorting of the T cells by flow cytometry.

[0055] In one embodiment when binding the TCR the agent is bound to an MHC class II molecule (typically HLA-DQ2), which is typically present on the surface of an antigen presenting cell (APC). However as mentioned herein other agents can bind a TCR without the need to also bind an MHC molecule.

[0056] Generally the T cells which are contacted in the method are taken from the individual in a blood sample, although other types of samples which contain T cells can be used. The sample may be added directly to the assay or may be processed first. Typically the processing may comprise diluting of the sample, for example with water or buffer. Typically the sample is diluted from 1.5 to 100 fold, for example 2 to 50 or 5 to 10 fold.

[0057] The processing may comprise separation of components of the sample. Typically mononuclear cells (MCs)are separated from the samples. The MCs will comprise the T cells and APCs. Thus in the method the APCs present in the separated MCs can present the peptide to the T cells. In another embodiment only T cells, such as only CD4 T cells, can be purified from the sample. PBMCs, MCs and T cells can be separated from the sample using techniques known in the art, such as those described in Lalvani *et al* (1997) *J.Exp. Med*. **186,** p859-865.

[0058] In one embodiment the T cells used in the assay are in the form of unprocessed or diluted samples, or are freshly isolated T cells (such as in the form of freshly isolated MCs or PBMCs) which are used directly *ex vivo,* i.e. they are not cultured before being used in the method. Thus the T cells have not been restimulated in an antigen specific manner *in vitro*. However the T cells can be cultured before use, for example in the presence of one or more of the agents, and generally also exogenous growth promoting cytokines. During culturing the agent(s) are typically present on the surface of APCs, such as the APC used in the method. Pre-culturing of the T cells may lead to an increase in the sensitivity of the method. Thus the T cells can be converted into cell lines, such as short term cell lines (for example as described in Ota *et al* (1990) *Nature* **346**, p183-187).

[0059] The APC which is typically present in the method may be from the same individual as the T cell or from a different host. The APC may be a naturally occurring APC or an artificial APC. The APC is a cell which is capable of presenting the peptide to a T cell. It is typically a B cell, dendritic cell or macrophage. It is typically separated from the same sample as the T cell and is typically co-purified with the T cell. Thus the APC may be present in MCs or PBMCs. The APC is typically a freshly isolated *ex vivo* cell or a cultured cell. It may be in the form of a cell line, such as a short term or immortalised cell line. The APC may express empty MHC class II molecules on its surface.

[0060] In the method one or more (different) agents may be used. Typically the T cells derived from the sample can be placed into an assay with all the agents which it is intended to test or the T cells can be divided and placed into separate assays each of which contain one or more of the agents.

[0061] The invention also provides the agents such as two or more of any of the agents mentioned herein (e.g. the combinations of agents which are present in the composition agent discussed above) for simultaneous separate or sequential use (eg. for *in vivo* use).

[0062] In one embodiment agent *per se* is added directly to an assay comprising T cells and APCs. As discussed above the T cells and APCs in such an assay could be in the form of MCs. When agents which can be recognised by the T cell without the need for presentation by APCs are used then APCs are not required. Analogues which mimic the original (i) or (ii) bound to a MHC molecule are an example of such an agent.

[0063] In one embodiment the agent is provided to the APC in the absence of the T cell. The APC is then provided to the T cell, typically after being allowed to present the agent on its surface. The peptide may have been taken up inside the APC and presented, or simply be taken up onto the surface without entering inside the APC.

[0064] The duration for which the agent is contacted with the T cells will vary depending on the method used for determining recognition of the peptide. Typically 10^5 to 10^7, preferably $5x10^5$ to 10^6 PBMCs are added to each assay. In the case where agent is added directly to the assay its concentration is from 10^{-1} to 10^3μg/ml, preferably 0.5 to 50μg/ml or 1 to 10μg/ml.

[0065] Typically the length of time for which the T cells are incubated with the agent is from 4 to 24 hours, preferably 6 to 16 hours. When using *ex vivo* PBMCs it has been found that $0.3x10^6$ PBMCs can be incubated in 10μg/ml of peptide for 12 hours at 37°C.

[0066] The determination of the recognition of the agent by the T cells may be done by measuring the binding of the agent to the T cells (this can be carried out using any suitable binding assay format discussed herein). Typically T cells which bind the agent can be sorted based on this binding, for example using a FACS machine. The presence of T cells which recognise the agent will be deemed to occur if the frequency of cells sorted using the agent is above a 'control' value. The frequency of antigen-experienced T cells is generally 1 in 10^6 to 1 in 10^3, and therefore whether or not the sorted cells are antigen-experienced T cells can be determined.

[0067] The determination of the recognition of the agent by the T cells may be measured *in vivo.* Typically the agent is administered to the host and then a response which indicates recognition of the agent may be measured. The agent is typically administered intradermally or epidermally. The agent is typically administered by contacting with the outside of the skin, and may be retained at the site with the aid of a plaster or dressing. Alternatively the agent may be administered by needle, such as by injection, but can also be administered by other methods such as ballistics (e.g. the ballistics techniques which have been used to deliver nucleic acids). EP-A-0693119 describes techniques which can typically be used to administer the agent. Typically from 0.001 to 1000 μg, for example from 0.01 to 100 μg or 0.1 to 10 μg of agent is administered.

[0068] In one embodiment a product can be administered which is capable of providing the agent *in vivo*. Thus a polynucleotide capable of expressing the agent can be administered, typically in any of the ways described above for the administration of the agent. The polynucleotide typically has any of the characteristics of the polynucleotide provided by the invention which is discussed below. The agent is expressed from the polynucleotide *in vivo.* Typically from 0.001 to 1000 μg, for example from 0.01 to 100 μg or 0.1 to 10 μg of polynucleotide is administered.

[0069] Recognition of the agent administered to the skin is typically indicated by the occurrence of inflammation (e.g. induration, erythema or oedema) at the site of administration. This is generally measured by visual examination of the site.

[0070] The method of diagnosis based on the detection of an antibody that binds the agent is typically carried out by contacting a sample from the individual (such as any of the samples mentioned here, optionally processed in any manner mentioned herein) with the agent and determining whether an antibody in the sample binds the agent, such a binding indicating that the individual has, or is susceptible to coeliac disease. Any suitable format of binding assay may be used, such as any such format mentioned herein.

Therapy

[0071] The identification of the immunodominant epitope allows the therapeutic products to be made which target the T cells which recognise this epitope (such T cells being ones which participate in the immune response against gliadin). This finding also allows the prevention or treatment of coeliac disease by suppressing (by tolerisation) an antibody or T cell response to the epitope.

[0072] Certain agents of the invention bind the TCR which recognises the epitope of the invention (as measured using any of the binding assays discussed above) and cause tolerisation of the T cell that carries the TCR. Such agents, optionally in association with a carrier, can therefore be used to prevent or treat coeliac disease.

[0073] Generally tolerisation can be caused by the same peptides which can (after being recognised by the TCR) cause antigen specific functional activity of the T cell (such as any such activity mentioned herein, e.g. secretion of cytokines). Such agents cause tolerisation when they are presented to the immune system in a 'tolerising' context.

[0074] Tolerisation leads to a decrease in the recognition of a T cell or antibody epitope by the immune system. In the case of a T cell epitope this can be caused by the deletion or anergising of T cells which recognise the epitope. Thus T cell activity (for example as measured in suitable assays mentioned herein) in response to the epitope is decreased. Tolerisation of an antibody response means that a decreased amount of specific antibody to the epitope is produced when the epitope is administered.

[0075] Methods of presenting antigens to the immune system in such a context are known and are described for example in Yoshida et al. Clin. Immunol. Immunopathol. 82, 207-215 (1997), Thurau et al. Clin. Exp. Immunol. 109, 370-6 (1997), and Weiner et al. Res. Immunol. 148, 528-33 (1997). In particular certain routes of administration can cause tolerisation, such as oral, nasal or intraperitoneal. Particular products which cause tolerisation may be administered (e.g. in a composition which also comprises the agent) to the individual. Such products include cytokines, such as cytokines which favour a Th2 response (e.g. IL-4, TGF-β or IL-10). Products or agent may be administered at a dose which causes tolerisation.

[0076] The invention provides a protein which comprises a sequence able to act as an antagonist of the T cell (which T cell recognises the agent). Such proteins and such antagonists can also be used to prevent or treat coeliac disease. The antagonist will cause a decrease in the T cell response. In one embodiment the antagonist binds the TCR of the T cell (generally in the form of a complex with HLA-DQ2) but instead of causing normal functional activation causing an abnormal signal to be passed through the TCR intracellular signalling cascade which causes the T cell to have decreased function activity (e.g. in response to recognition of an epitope, typically as measured by any suitable assay mentioned herein).

[0077] In one embodiment the antagonist competes with epitope to bind a component of MHC processing and presentation pathway, such as an MHC molecule (typically HLA-DQ2). Thus the antagonist may bind HLA-DQ2 (and thus be a peptide presented by this MHC molecule), such as peptide TP (Table 10) or a homologue thereof.

[0078] Methods of causing antagonism are known in the art. In one embodiment the antagonist is a homologue of the epitopes mentioned above and may have any of the sequence, binding or other properties of the agent (particularly analogues). The antagonists typically differ from any of the above epitopes (which are capable of causing a normal antigen specific function in the T cell) by 1, 2, 3, 4 or more mutations (each of which may be a substitution, insertion or deletion). Such antagonists are termed "altered peptide ligands" or "APL" in the art. The mutations are typically at the amino acid positions which contact the TCR.

[0079] The antagonist may differ from the epitope by a substitution within the sequence which is equivalent to the sequence represented by amino acids 65 to 67 of A-gliadin (such antagonists are shown in Table 9). Thus preferably the antagonist has a substitution at the equivalent of position 64, 65 or 67. Preferably the substitution is 64W, 67W, 67M or 65T.

[0080] Since the T cell immune response to the epitope of the invention in an individual is polyclonal more than one antagonist may need to be administered to cause antagonism of T cells of the response which have different TCRs. Therefore the antagonists may be administered in a composition which comprises at least 2, 4, 6 or more different antagonists, which each antagonise different T cells.

[0081] The invention also provides a method of identifying an antagonist of a T cell (which recognises the agent) comprising contacting a candidate substance with the T cell and detecting whether the substance causes a decrease in the ability of the T cell to undergo an antigen specific response (e.g. using any suitable assay mentioned herein), the detecting of any such decrease in said ability indicating that the substance is an antagonist.

[0082] In one embodiment the antagonists (including combinations of antagonists to a particular epitope) or tolerising (T cell and antibody tolerising) agents are present in a composition comprising at least 2, 4, 6 or more antagonists or agents which antagonise or tolerise to different epitopes of the invention, for example to the combinations of epitopes discussed above in relation to the agents which are a product comprising more than one substance.

Testing whether a composition is capable of causing coeliac disease

[0083] As mentioned above the invention provides a method of determining whether a composition is capable of causing coeliac disease comprising detecting the presence of a protein sequence which is capable of being modified by a transglutaminase to as sequence comprising the agent or epitope of the invention (such transglutaminase activity may be a human intestinal transglutaminase activity). Typically this is performed by using a binding assay in which a moiety which binds to the sequence in a specific manner is contacted with the composition and the formation of sequence/moiety complex is detected and used to ascertain the presence of the agent. Such a moiety may be any suitable substance (or type of substance) mentioned herein, and is typically a specific antibody. Any suitable format of binding assay can be used (such as those mentioned herein).

[0084] In one embodiment the composition is contacted with at least 2, 5, 10 or more antibodies which are specific for epitopes of the invention from different gliadins, for example a panel of antibodies capable of recognising the combinations of epitopes discussed above in relation to agents of the invention which are a product comprising more than one substance.

[0085] The composition typically comprises material from a plant that expresses a gliadin which is capable of causing coeliac disease (for example any of the gliadins or plants mentioned herein). Such material may be a plant part, such as a harvested product (e.g. seed). The material may be processed products of the plant material (e.g. any such product mentioned herein), such as a flour or food that comprises the gliadin. The processing of food material and testing in suitable binding assays is routine, for example as mentioned in Kricka LJ, J. Biolumin. Chemilumin. 13, 189-93 (1998).

Binding assays

[0086] The determination of binding between any two substances mentioned herein may be done by measuring a characteristic of either or both substances that changes upon binding, such as a spectroscopic change.

[0087] The binding assay format may be a 'band shift' system. This involves determining whether the presence of one

substance (such as a candidate substance) advances or retards the progress of the other substance during gel electrophoresis.

[0088] The format may be a competitive binding method which determines whether the one substance is able to inhibit the binding of the other substance to an agent which is known to bind the other substance, such as a specific antibody.

Mutant gliadin proteins

[0089] The invention provides a gliadin protein in which an epitope sequence of the invention, or sequence which can be modified by a transglutaminase to provide such a sequence has been mutated so that it no longer causes, or is recognised by, a T cell response that recognises the epitope. In this context the term recognition refers to the TCR binding the epitope in such a way that normal (not antagonistic) antigen-specific functional activity of the T cell occurs.

[0090] Methods of identifying equivalent epitopes in other gliadins are discussed above. The wild type of the mutated gliadin is one which causes coeliac disease. Such a gliadin will have homology with SEQ ID NO:3, for example to the degree mentioned above (in relation to the analogue) across all of SEQ ID NO:3 or across 15, 30, 60, 100 or 200 contiguous amino acids of SEQ ID NO:3.

[0091] The mutated gliadin will not cause coeliac disease or will cause decreased symptoms of coeliac disease. Typically the mutation decreases the ability of the epitope to induce a T cell response. The mutated epitope may have a decreased binding to HLA-DQ2, a decreased ability to be presented by an APC or a decreased ability to bind to or to be recognised (i.e. cause antigen-specific functional activity) by T cells that recognise the agent. The mutated gliadin or epitope will therefore show no or reduced recognition in any of the assays mentioned herein in relation to the diagnostic aspects of the invention.

[0092] The mutation may be one or more deletions, additions or substitutions of length 1 to 3, 4 to 6, 6 to 10, 11 to 15 or more in the epitope, for example across the sequence SEQ ID NO:2 or its equivalent. Preferably the mutant gliadin has at least one mutation in the sequence SEQ ID NO:1. A preferred mutation is at position 65 in A-gliadin (or in an equivalent position in other gliadins). Typically the naturally occurring glutamine at this position is substituted to any of the amino acids shown in Table 3, preferably to histidine, tyrosine, tryptophan, lysine, proline, or arginine.

[0093] The invention thus also provides use of a mutation (such any of the mutations in any of the sequences discussed herein) in an epitope of a gliadin protein, which epitope is an epitope of the invention, to decrease the ability of the gliadin protein to cause coeliac disease.

[0094] In one embodiment the mutated sequence is able to act as an antagonist. Thus the invention provides a protein that comprises a sequence which is able to bind to a T cell receptor, which T cell receptor recognises an agent of the invention, and which sequence is able to cause antagonism of a T cell that carries such a T cell receptor.

[0095] The invention also provides proteins which are fragments of the above mutant gliadin proteins, which are at least 15 amino acids long (e.g. at least 30, 60, 100, 150, 200, or 250 amino acids long) and which comprise the mutations discussed above which decrease the ability of the gliadin to be recognised. Any of the mutant proteins (including fragments) mentioned herein may also be present in the form of fusion proteins, for example with other gliadins or with non-gliadin proteins.

[0096] The equivalent wild type protein to the mutated gliadin protein is typically from a graminaceous monocotyledon, such as a plant of genus Triticum, e.g. wheat, rye, barley, oats or triticale. The protein is typically an α, $\alpha\beta$, β, γ or ω gliadin. The gliadin may be an A-gliadin.

Kits

[0097] The invention also provides a kit for carrying out the method comprising one or more agents and optionally a means to detect the recognition of the agent by the T cell. Typically the different agents are provided for simultaneous, separate or sequential use. Typically the means to detect recognition allows or aids detection based on the techniques discussed above.

[0098] Thus the means may allow detection of a substance secreted by the T cells after recognition. The kit may thus additionally include a specific binding moiety for the substance, such as an antibody. The moiety is typically specific for IFN-γ. The moiety is typically immobilised on a solid support. This means that after binding the moiety the substance will remain in the vicinity of the T cell which secreted it. Thus 'spots' of substance/moiety complex are formed on the support, each spot representing a T cell which is secreting the substance. Quantifying the spots, and typically comparing against a control, allows determination of recognition of the agent.

[0099] The kit may also comprise a means to detect the substance/moiety complex. A detectable change may occur in the moiety itself after binding the substance, such as a colour change. Alternatively a second moiety directly or indirectly labelled for detection may be allowed to bind the substance/moiety complex to allow the determination of the spots. As discussed above the second moiety may be specific for the substance, but binds a different site on the substance than the first moiety.

[0100] The immobilised support may be a plate with wells, such as a microtitre plate. Each assay can therefore be carried out in a separate well in the plate.

[0101] The kit may additionally comprise medium for the T cells, detection moieties or washing buffers to be used in the detection steps. The kit may additionally comprise reagents suitable for the separation from the sample, such as the separation of PBMCs or T cells from the sample. The kit may be designed to allow detection of the T cells directly in the sample without requiring any separation of the components of the sample.

[0102] The kit may comprise an instrument which allows administration of the agent, such as intradermal or epidermal administration. Typically such an instrument comprises plaster, dressing or one or more needles. The instrument may allow ballistic delivery of the agent. The agent in the kit may be in the form of a pharmaceutical composition.

[0103] The kit may also comprise controls, such as positive or negative controls. The positive control may allow the detection system to be tested. Thus the positive control typically mimics recognition of the agent in any of the above methods. Typically in the kits designed to determine recognition *in vitro* the positive control is a cytokine. In the kit designed to detect *in vivo* recognition of the agent the positive control may be antigen to which most individuals should response.

[0104] The kit may also comprise a means to take a sample containing T cells from the host, such as a blood sample. The kit may comprise a means to separate mononuclear cells or T cells from a sample from the host.

Polynucleotides, cells, transgenic mammals and antibodies

[0105] The invention also provides a polynucleotide which is capable of expression to provide the agent or mutant gliadin proteins. Typically the polynucleotide is DNA or RNA, and is single or double stranded. The polynucleotide will preferably comprise at least 50 bases or base pairs, for example 50 to 100, 100 to 500, 500 to 1000 or 1000 to 2000 or more bases or base pairs. The polynucleotide therefore comprises sequence which encodes the sequence of SEQ ID NO: 1 or 2 or any of the agents mentioned herein. To the 5' and 3' of this coding sequence the polynucleotide of the invention has sequence or codons which are different from the sequence or codons 5' and 3' to these sequences in the corresponding gliadin gene.

[0106] 5' and/or 3' to the sequence encoding the peptide the polynucleotide has coding or non-coding sequence. Sequence 5' and/or 3' to the coding sequence may comprise sequences which aid expression, such as transcription and/or translation, of the sequence encoding the agent. The polynucleotide may be capable of expressing the agent prokaryotic or eukaryotic cell. In one embodiment the polynucleotide is capable of expressing the agent in a mammalian cell, such as a human, primate or rodent (e.g. mouse or rat) cell.

[0107] A polynucleotide of the invention may hybridise selectively to a polynucleotide that encodes SEQ ID NO:3 at a level significantly above background. Selective hybridisation is typically achieved using conditions of medium to high stringency (for example 0.03M sodium chloride and 0.03M sodium citrate at from about 50°C to about 60°C). However, such hybridisation may be carried out under any suitable conditions known in the art (see Sambrook *et al* (1989), Molecular Cloning: A Laboratory Manual). For example, if high stringency is required, suitable conditions include 0.2 x SSC at 60°C. If lower stringency is required, suitable conditions include 2 x SSC at 60°C.

[0108] Agents or proteins of the invention may be encoded by the polynucleotides described herein.

[0109] The polynucleotide may form or be incorporated into a replicable vector. Such a vector is able to replicate in a suitable cell. The vector may be an expression vector. In such a vector the polynucleotide of the invention is operably linked to a control sequence which is capable of providing for the expression of the polynucleotide. The vector may contain a selectable marker, such as the ampicillin resistance gene.

[0110] The polynucleotide or vector may be present in a cell. Such a cell may have been transformed by the polynucleotide or vector. The cell may express the agent. The cell will be chosen to be compatible with the said vector and may for example be a prokaryotic (bacterial), yeast, insect or mammalian cell. The polynucleotide or vector may be introduced into host cells using conventional techniques including calcium phosphate precipitation, DEAE-dextran transfection, or electroporation.

[0111] The invention provides processes for the production of the proteins of the invention by recombinant means. This may comprise (a) cultivating a transformed cell as defined above under conditions that allow the expression of the protein; and preferably (b)recovering the expressed polypeptide. Optionally, the polypeptide may be isolated and/or purified, by techniques known in the art.

[0112] The invention also provides TCRs which recognise (or bind) the agent, or fragments thereof which are capable of such recognition (or binding). These can be present in the any form mentioned herein (e.g. purity) discussed herein in relation to the protein of the invention. The invention also provides T cells which express such TCRs which can be present in any form (e.g. purity) discussed herein for the cells of the invention.

[0113] The invention also provides monoclonal or polyclonal antibodies which specifically recognise the agents (such as any of the epitopes of the invention) and which recognise the mutant gliadin proteins (and typically which do not recognise the equivalent wild-type gliadins) of the invention, and methods of making such antibodies. Antibodies of the

invention bind specifically to these substances of the invention.

[0114] For the purposes of this invention, the term "antibody" includes antibody fragments such as Fv, F(ab) and F $(ab)_2$ fragments, as well as single-chain antibodies.

[0115] A method for producing a polyclonal antibody comprises immunising a suitable host animal, for example an experimental animal, with the immunogen and isolating immunoglobulins from the serum. The animal may therefore be inoculated with the immunogen, blood subsequently removed from the animal and the IgG fraction purified. A method for producing a monoclonal antibody comprises immortalising cells which produce the desired antibody. Hybridoma cells may be produced by fusing spleen cells from an inoculated experimental animal with tumour cells (Kohler and Milstein (1975) *Nature* **256**, 495-497).

[0116] An immortalized cell producing the desired antibody may be selected by a conventional procedure. The hybridomas may be grown in culture or injected intraperitoneally for formation of ascites fluid or into the blood stream of an allogenic host or immunocompromised host. Human antibody may be prepared by *in vitro* immunisation of human lymphocytes, followed by transformation of the lymphocytes with Epstein-Barr virus.

[0117] For the production of both monoclonal and polyclonal antibodies, the experimental animal is suitably a goat, rabbit, rat or mouse. If desired, the immunogen may be administered as a conjugate in which the immunogen is coupled, for example via a side chain of one of the amino acid residues, to a suitable carrier. The carrier molecule is typically a physiologically acceptable carrier. The antibody obtained may be isolated and, if desired, purified.

[0118] The polynucleotide, agent, protein or antibody of the invention, may carry a detectable label. Detectable labels which allow detection of the secreted substance by visual inspection, optionally with the aid of an optical magnifying means, are preferred. Such a system is typically based on an enzyme label which causes colour change in a substrate, for example alkaline phosphatase causing a colour change in a substrate. Such substrates are commercially available, e.g. from BioRad. Other suitable labels include other enzymes such as peroxidase, or protein labels, such as biotin; or radioisotopes, such as ^{32}P or ^{35}S. The above labels may be detected using known techniques.

[0119] Polynucleotides, agents, proteins, antibodies or cells of the invention may be in substantially purified form. They may be in substantially isolated form, in which case they will generally comprise at least 80% e.g. at least 90, 95, 97 or 99% of the polynucleotide, peptide, antibody, cells or dry mass in the preparation. The polynucleotide, agent, protein or antibody is typically substantially free of other cellular components. The polynucleotide, agent, protein or antibody may be used in such a substantially isolated, purified or free form in the method or be present in such forms in the kit.

[0120] The invention also provides a transgenic mammal which expresses a TCR of the invention. This may be any of the mammals discussed herein (e.g. in relation to the production of the antibody). Preferably the mammal has, or is susceptible, to coeliac disease. The mammal may also express HLA-DQ2 and/or may be given a diet comprising a gliadin which cause coeliac disease (e.g. any of the gliadin proteins mentioned herein). Thus the mammal may act as an animal model for coeliac disease.

[0121] The invention also provides a method of identifying a product which is therapeutic for coeliac disease comprising administering a candidate substance to a mammal of the invention which has, or which is susceptible to, coeliac disease and determining whether substance prevents or treats coeliac disease in the mammal, the prevention or treatment of coeliac disease indicating that the substance is a therapeutic product. Such a product may be used to treat or prevent coeliac disease.

[0122] The invention provides therapeutic (including prophylactic) agents or diagnostic substances (the agents, proteins and polynucleotides of the invention). These substances are formulated for clinical administration by mixing them with a pharmaceutically acceptable carrier or diluent. For example they can be formulated for topical, parenteral, intravenous, intramuscular, subcutaneous, intraocular, intradermal, epidermal or transdermal administration. The substances may be mixed with any vehicle which is pharmaceutically acceptable and appropriate for the desired route of administration. The pharmaceutically carrier or diluent for injection may be, for example, a sterile or isotonic solution such as Water for Injection or physiological saline, or a carrier particle for ballistic delivery.

[0123] The dose of the substances may be adjusted according to various parameters, especially according to the agent used; the age, weight and condition of the patient to be treated; the mode of administration used; the severity of the condition to be treated; and the required clinical regimen. As a guide, the amount of substance administered by injection is suitably from 0.01 mg/kg to 30 mg/kg, preferably from 0.1 mg/kg to 10 mg/kg.

[0124] The routes of administration and dosages described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and dosage for any particular patient and condition.

[0125] The substances of the invention may thus be used in a method of treatment of the human or animal body, or in a diagnostic method practised on the human body. In particular they may be used in a method of treating or preventing coeliac disease. The invention also provide the agents for use in a method of manufacture of a medicament for treating or preventing coeliac disease.

[0126] The agent of the invention can be made using standard synthetic chemistry techniques, such as by use of an automated synthesizer. The agent may be made from a longer polypeptide e.g. a fusion protein, which polypeptide

typically comprises the sequence of the peptide. The peptide may be derived from the polypeptide by for example hydrolysing the polypeptide, such as using a protease; or by physically breaking the polypeptide. The polynucleotide of the invention can be made using standard techniques, such as by using a synthesiser.

Plant cells and plants that express mutant gliadin proteins or express proteins comprising sequences which can act as antagonists

[0127] The cell of the invention may be a plant cell, such as a cell of a graminaceous monocotyledonous species. The species may be one whose wild-type form expresses gliadins, such as any of the gliadin proteins mentioned herein (including gliadins with any degree of homology to SEQ ID NO:3 mentioned herein). Such a gliadin may cause coeliac disease in humans. The cell may be of wheat, maize, oats, rye, rice, barley, triticale, sorghum, or sugar cane. Typically the cell is of the Triticum genus, such as aestivum, spelta, polonicum or monococcum.

[0128] The plant cell of the invention is typically one which does not express a wild-type gliadin (such as any of the gliadins mentioned herein which may cause coeliac disease), or one which does not express a gliadin comprising a sequence that can be recognised by a T cell that recognises the agent. Thus if the wild-type plant cell did express such a gliadin then it may be engineered to prevent or reduce the expression of such a gliadin or to change the amino acid sequence of the gliadin so that it no longer causes coeliac disease (typically by no longer expressing the epitope of the invention).

[0129] This can be done for example by introducing mutations into 1, 2, 3 or more or all of such gliadin genes in the cell, for example into coding or non-coding (e.g. promoter regions). Such mutations can be any of the type or length of mutations discussed herein (e.g in relation to homologous proteins). The mutations can be introduced in a directed manner (e.g using site directed mutagenesis or homologous recombination techniques) or in a random manner (e.g. using a mutagen, and then typically selecting for mutagenised cells which no longer express the gliadin (or a gliadin sequence which causes coeliac disease)).

[0130] In the case of plants or plant cells that express a protein that comprises a sequence able to act as an antagonist such a plant or plant cell may express a wild-type gliadin protein (e.g. one which causes coeliac disease). Preferably though the presence of the antagonist sequence will cause reduced coeliac disease symptoms (such as no symptoms) in an individual who ingests a food comprising protein from the plant or plant cell.

[0131] The polynucleotide which is present in (or which was transformed into) the plant cell will generally comprise promoter capable of expressing the mutant gliadin protein the plant cell. Depending on the pattern of expression desired, the promoter may be constitutive, tissue- or stage-specific; and/or inducible. For example, strong constitutive expression in plants can be obtained with the CAMV 35S, Rubisco ssu, or histone promoters. Also, tissue-specific or stage-specific promoters may be used to target expression of protein of the invention to particular tissues in a transgenic plant or to particular stages in its development. Thus, for example seed-specific, root-specific, leaf-specific, flower-specific etc promoters may be used. Seed-specific promoters include those described by Dalta *et al* (Biotechnology Ann. Rev. (1997), 3, pp.269-296). Particular examples of seed-specific promoters are napin promoters (EP-A-0 255, 378), phaseolin promoters, glutenine promoters, helianthenine promoters (WO92/17580), albumin promoters (WO98/45460), oleosin promoters (WO98/45461) and ATS1 and ATS3 promoters (PCT/US98/06798).

[0132] The cell may be in any form. For example, it may be an isolated cell, e.g. a protoplast, or it may be part of a plant tissue, e.g. a callus, or a tissue excised from a plant, or it may be part of a whole plant. The cell may be of any type (e.g of any type of plant part). For example, an undifferentiated cell, such as a callus cell; or a differentiated cell, such as a cell of a type found in embryos, pollen, roots, shoots or leaves. Plant parts include roots; shoots; leaves; and parts involved in reproduction, such as pollen, ova, stamens, anthers, petals, sepals and other flower parts.

[0133] The invention provides a method of obtaining a transgenic plant cell comprising transforming a plant cell with a polynucleotide or vector of the invention to give a transgenic plant cell. Any suitable transformation method may be used (in the case of wheat the techniques disclosed in Vasil V et al, Biotechnology 10, 667-674 (1992) may be used). Preferred transformation techniques include electroporation of plant protoplasts and particle bombardment. Transformation may thus give rise to a chimeric tissue or plant in which some cells are transgenic and some are not.

[0134] The cell of the invention or thus obtained cell may be regenerated into a transgenic plant by techniques known in the art. These may involve the use of plant growth substances such as auxins, giberellins and/or cytokinins to stimulate the growth and/or division of the transgenic cell. Similarly, techniques such as somatic embryogenesis and meristem culture may be used. Regeneration techniques are well known in the art and examples can be found in, e.g. US 4,459,355, US 4,536,475, US 5,464,763, US 5, 177,010, US 5, 187,073, EP 267,159, EP 604, 662, EP 672, 752, US 4,945,050, US 5,036,006, US 5,100,792, US 5,371,014, US 5,478,744, US 5,179,022, US 5,565,346, US 5,484,956, US 5,508,468, US 5,538,877, US 5,554,798, US 5,489,520, US 5,510,318, US 5,204,253, US 5,405,765, EP 442,174, EP 486,233, EP 486,234, EP 539,563, EP 674,725, WO91/02071 and WO 95/06128.

[0135] In many such techniques, one step is the formation of a callus, i.e. a plant tissue comprising expanding and/or dividing cells. Such calli are a further aspect of the invention as are other types of plant cell cultures and plant parts.

Thus, for example, the invention provides transgenic plant tissues and parts, including embryos, meristems, seeds, shoots, roots, stems, leaves and flower parts. These may be chimeric in the sense that some of their cells are cells of the invention and some are not. Transgenic plant parts and tissues, plants and seeds of the invention may be of any of the plant species mentioned herein.

[0136] Regeneration procedures will typically involve the selection of transformed cells by means of marker genes.

[0137] The regeneration step gives rise to a first generation transgenic plant. The invention also provides methods of obtaining transgenic plants of further generations from this first generation plant. These are known as progeny transgenic plants. Progeny plants of second, third, fourth, fifth, sixth and further generations may be obtained from the first generation transgenic plant by any means known in the art.

[0138] Thus, the invention provides a method of obtaining a transgenic progeny plant comprising obtaining a second-generation transgenic progeny plant from a first-generation transgenic plant of the invention, and optionally obtaining transgenic plants of one or more further generations from the second-generation progeny plant thus obtained.

[0139] Progeny plants may be produced from their predecessors of earlier generations by any known technique. In particular, progeny plants may be produced by:

obtaining a transgenic seed from a transgenic plant of the invention belonging to a previous generation, then obtaining a transgenic progeny plant of the invention belonging to a new generation by growing up the transgenic seed; and/or

propagating clonally a transgenic plant of the invention belonging to a previous generation to give a transgenic progeny plant of the invention belonging to a new generation; and/or

crossing a first-generation transgenic plant of the invention belonging to a previous generation with another compatible plant to give a transgenic progeny plant of the invention belonging to a new generation; and optionally

obtaining transgenic progeny plants of one or more further generations from the progeny plant thus obtained.

[0140] These techniques may be used in any combination. For example, clonal propagation and sexual propagation may be used at different points in a process that gives rise to a transgenic plant suitable for cultivation. In particular, repetitive backcrossing with a plant taxon with agronomically desirable characteristics may be undertaken. Further steps of removing cells from a plant and regenerating new plants therefrom may also be carried out.

[0141] Also, further desirable characteristics may be introduced by transforming the cells, plant tissues, plants or seeds, at any suitable stage in the above process, to introduce desirable coding sequences other than the polynucleotides of the invention. This may be carried out by the techniques described herein for the introduction of polynucleotides of the invention.

[0142] For example, further transgenes may be selected from those coding for other herbicide resistance traits, e.g. tolerance to: Glyphosate (e.g. using an EPSP synthase gene (e.g. EP-A-0 293,358) or a glyphosate oxidoreductase (WO 92/000377) gene); or tolerance to fosametin; a dihalobenzonitrile; glufosinate, e.g. using a phosphinothrycin acetyl transferase (PAT) or glutamine synthase gene (cf. EP-A-0 242,236); asulam, e.g. using a dihydropteroate synthase gene (EP-A-0 369,367); or a sulphonylurea, e.g. using an ALS gene); diphenyl ethers such as acifluorfen or oxyfluorfen, e.g. using a protoporphyrogen oxidase gene); an oxadiazole such as oxadiazon; a cyclic imide such as chlorophthalim; a phenyl pyrazole such as TNP, or a phenopylate or carbamate analogue thereof.

[0143] Similarly, genes for beneficial properties other than herbicide tolerance may be introduced. For example, genes for insect resistance may be introduced, notably genes encoding *Bacillus thuringiensis* (*Bt*) toxins. Likewise, genes for disease resistance may be introduced, e.g. as in WO91/02701 or WO95/06128.

[0144] Typically, a protein of the invention is expressed in a plant of the invention. Depending on the promoter used, this expression may be constitutive or inducible. Similarly, it may be tissue- or stage-specific, i.e. directed towards a particular plant tissue (such as any of the tissues mentioned herein) or stage in plant development.

[0145] The invention also provides methods of obtaining crop products by harvesting, and optionally processing further, transgenic plants of the invention. By crop product is meant any useful product obtainable from a crop plant.

Products that contain mutant gliadin proteins or proteins that comprise sequence capable of acting as an antagonist

[0146] The invention provides a product that comprises the mutant gliadin proteins or protein that comprises sequence capable of acting as an antagonist. This is typically derived from or comprise plant parts from plants mentioned herein which express such proteins. Such a product may be obtainable directly by harvesting or indirectly, by harvesting and further processing the plant of the invention. Directly obtainable products include grains. Alternatively, such a product may be obtainable indirectly, by harvesting and further processing. Examples of products obtainable by further processing are flour or distilled alcoholic beverages; food products made from directly obtained or further processed material, e.g.

baked products (e.g. bread) made from flour. Typically such food products, which are ingestible and digestible (i.e. non-toxic and of nutrient value) by human individuals.

[0147] In the case of food products that comprise the protein which comprises an antagonist sequence the food product may also comprise wild-type gliadin, but preferably the antagonist is able to cause a reduction (e.g. completely) in the coeliac disease symptoms after such food is ingested.

[0148] The invention is illustrated by the following Examples:

Example 1

[0149] We carried out epitope mapping in Coeliac disease by using a set of 51 synthetic 15-mer peptides that span the complete sequence of a fully characterized a-gliadin, "A-gliadin" (see Table 1). A-Gliadin peptides were also individually treated with tTG to generate products that might mimic those produced in vivo[3]. We also sought to study Coeliac disease patients at the point of initiation of disease relapse to avoid the possibility that epitope "spreading" or "exhaustion" may have occurred, as described in experimental infectious and autoimmune diseases.

Clinical and A-gliadin specific T cell responses with 3 and 10 day bread challenge

[0150] In a pilot study, two subjects with Coeliac disease in remission, defined by absence of serum anti-endomysial antibody (EMA), on a gluten free diet were fed four slices of standard gluten-containing white bread daily in addition to their usual gluten free diet. Subject 1 ceased bread because of abdominal pain, mouth ulcers and mild diarrhoea after three days, but Subject 2 continued for 10 days with only mild nausea at one week. The EMA became positive in Subject 2 one week after the bread challenge, indicating the bread used had caused a relapse of Coeliac disease. But in Subject 1, EMA remained negative up to two months after bread challenge. In both subjects, symptoms that appeared with bread challenge resolved within two days after returning to gluten free diet.

[0151] PBMC responses in IFNγ ELISPOT assays to A-gliadin peptides were not found before or during bread challenge. But from the day after bread withdrawal (Day 4) in Subject 1 a single pool of 5 overlapping peptides spanning A-gliadin 51-85 (Pool 3) treated with tTG showed potent IFNg responses (see Figure 1a). In Subject 1, the PBMC IFNg response to A-gliadin peptide remained targeted to Pool 3 alone and was maximal on Day 8. The dynamics and magnitude of the response to Pool 3 was similar to that elicited by α-chymotrypsin digested gliadin. PBMC IFNγ responses to tTG-treated Pool 3 were consistently 5 to 12-fold greater than Pool 3 not treated with tTG, and responses to α-chymotrypsin digested gliadin were 3 to 10-fold greater if treated with tTG. In Subject 2, Pool 3 treated with tTG was also the only immunogenic set of A-gliadin peptides on Day 8, but this response was weaker than Subject 1, was not seen on Day 4 and by Day 11 the response to Pool 3 had diminished and other tTG-treated pools of A-gliadin peptides elicited stronger IFNα responses (see Figure 1b).

[0152] The pilot study indicated that the initial T cell response in these Coeliac disease subjects was against a single tTG-treated A-gliadin pool of five peptides and was readily measured in peripheral blood. But if antigen exposure is continued for ten days instead of three, T cell responses to other A-gliadin peptides appear, consistent with epitope spreading.

Coeliac disease-specific IFN-g induction by tTG-treated A-gliadin peptides

[0153] In five out of six further Coeliac disease subjects on gluten free diet (see Table 1), bread challenge for three days identified tTG-treated peptides in Pool 3, and in particular, peptides corresponding to 56-70 (12) and 60-75 (13) as the sole A-gliadin components eliciting IFNγ from PBMC (see Figure 2). IL-10 ELISPOT assays run in parallel to IFNγ ELISPOT showed no IL-10 response to tTG-treated peptides 12 or 13. In one subject, there were no IFNγ responses to any A-gliadin peptide or α-chymotrypsin digested gliadin before, during or up to four days after bread challenge. In none of these Coeliac disease subjects did EMA status change from baseline when measured for up to two months after bread challenge.

[0154] PBMC from four healthy, EMA-negative subjects with the HLA-DQ alleles α1*0501, β1*0201 (ages 28-52, 2 females) who had been challenged for three days with bread after following a gluten free diet for one month, showed no IFNγ responses above the negative control to any of the A-gliadin peptides with or without tTG treatment. Thus, induction of IFNγ in PBMC to tTG-treated Pool 3 and A-gliadin peptides 56-70 (12) and 60-75 (13) were Coeliac disease specific (7/8 vs 0/4, $p<0.01$ by Chi-squared analysis).

Fine mapping of the minimal A-gliadin T cell epitope

[0155] tTG-treated peptides representing truncations of A-gliadin 56-75 revealed that the same core peptide sequence (QPQLP) was essential for antigenicity in all of the five Coeliac disease subjects assessed (see Figure 3). PBMC IFNγ

responses to tTG-treated peptides spanning this core sequence beginning with the 7-mer PQPQLPY and increasing in length, indicated that the tTG-treated 17-mer QLQPFPQPQLPYPQPQS (A-gliadin 57-73) possessed optimal activity in the IFNγ ELISPOT (see Figure 4).

Deamidation of Q65 by tTG generates the immunodominant T cell epitope in A-gliadin

[0156] HPLC analysis demonstrated that tTG treatment of A-gliadin 56-75 generated a single product that eluted marginally later than the parent peptide. Amino acid sequencing indicated that out of the six glutamine (Q) residues contained in A-gliadin 56-75, Q65 was preferentially deamidated by tTG (see Figure 5). Bioactivity of peptides corresponding to serial expansions from the core A-gliadin 62-68 sequence in which glutamate (E) replaced Q65, was equivalent to the same peptides with Q65 after tTG-treatment (see Figure 4a). Replacement of Q57 and Q72 by E together or alone, with E65 did not enhance antigenicity of the 17-mer in the three Coeliac disease subjects studied (see Figure 6). Q57 and Q72 were investigated because glutamine residues followed by proline in gliadin peptides are not deamidated by tTG in vitro (W. Vader et al, Proceedings 8th International Symposium Coeliac Disease). Therefore, the immunodominant T cell epitope was defined as QLQPFPQPELPYPQPQS.

Immunodominant T cell epitope response is DQ2-restricted and CD4 dependent

[0157] In two Coeliac disease subjects homozygous for HLA-DQ α1*0501, β1*0201, anti-DQ monoclonal antibody blocked the ELISPOT IFNγ response to tTG-treated A-gliadin 56-75, but anti-DP and -DR antibody did not (see Figure 7). Anti-CD4 and anti-CD8 magnetic bead depletion of PBMC from two Coeliac disease subjects indicated the IFNγ response to tTG-treated A-gliadin 56-75 is CD4 T cell-mediated.

Discussion

[0158] In this study we describe a rather simple dietary antigen challenge using standard white bread to elicit a transient population of CD4 T cells in peripheral blood of Coeliac disease subjects responsive to a tTG-treated A-gliadin 17-mer with the sequence: QLQPFPQPELPYPQPQS (residues 57-73). The immune response to A-gliadin 56-75 (Q-E65) is restricted to the Coeliac disease-associated HLA allele, DQ α1*0501, β1*0201. Tissue transglutaminase action in vitro selectively deamidates Q65. Elicited peripheral blood IFNg responses to synthetic A-gliadin peptides with the substitution Q→E65 is equivalent to tTG-treated Q65 A-gliadin peptides; both stimulate up to 10-fold more T cells in the IFNg ELISPOT than unmodified Q65 A-gliadin peptides.

[0159] We have deliberately defined this Coeliac disease-specific T cell epitope using in vivo antigen challenge and short-term ex vivo immune assays to avoid the possibility of methodological artifacts that may occur with the use of T cell clones in epitope mapping. Our findings indicate that peripheral blood T cell responses to ingestion of gluten are rapid but short-lived and can be utilized for epitope mapping. In vivo antigen challenge has also shown there is a temporal hierarchy of immune responses to A-gliadin peptides; A-gliadin 57-73 modified by tTG not only elicits the strongest IFNg response in PBMC but it is also the first IFNg response to appear.

[0160] Because we have assessed only peptides spanning A-gliadin, there may be other epitopes in other gliadins of equal or greater importance in the pathogenesis of Coeliac disease. Indeed, the peptide sequence at the core of the epitope in A-gliadin that we have identified (PQPQLPY) is shared by several other gliadins (SwissProt and Trembl accession numbers: P02863, Q41528, Q41531, Q41533, Q9ZP09, P04722, P04724, P18573). However, A-gliadin peptides that have previously been shown to possess bioactivity in biopsy challenge and in vivo studies (for example: 31-43, 44-55, and 206-217)[4,5] did not elicit IFNg responses in PBMC following three day bread challenge in Coeliac disease subjects. These peptides may be "secondary" T cell epitopes that arise with spreading of the immune response.

Example 2

The effect on T cell recognition of substitutions in the immunodominant epitope

[0161] The effect of substituting the glutamate at position 65 in the 57-73 A-gliadin epitope was determined by measuring peripheral blood responses against the substituted epitopes in an IFNγ ELISPOT assay using synthetic peptides (at 50 μg/ml). The responses were measured in 3 Coeliac disease subjects 6 days after commencing gluten challenge (4 slices bread daily for 3 days). Results are shown in table 3 and Figure 8. As can be seen substitution of the glutamate to histidine, tyrosine, tryptophan, lysine, proline or arginine stimulated a response whose magnitude was less than 10% of the magnitude of the response to the immunodominant epitope. Thus mutation of A-gliadin at this position could be used to produce a mutant gliadin with reduce or absent immunoreactivity.

Example 3

Testing the immunoreactivity of equivalent peptides from other naturally occurring gliadins

[0162] The immunoreactivity of equivalent peptides form other naturally occurring wheat gliadins was assessed using synthetic peptides corresponding to the naturally occurring sequences which were then treated with transglutaminase. These peptides were tested in an ELISPOT in the same manner and with PBMCs from the same subjects as described in Example 2. At least five of the peptides show immunoreactivity comparable to the A-gliadin 57-73 E65 peptide (after transglutaminase treatment) indicating that other gliadin proteins in wheat are also likely to induce this Coeliac disease-specific immune response (Table 4 and Figure 9).

Methods

[0163]

Subjects: Patients used in the study attended a Coeliac Clinic in Oxford, United Kingdom. Coeliac disease was diagnosed on the basis of typical small intestinal histology, and normalization of symptoms and small intestinal histology with gluten free diet.

Tissue typing: Tissue typing was performed using DNA extracted from EDTA-anticoagulated peripheral blood. HLA-DQA and DQB genotyping was performed by PCR using sequence-specific primer mixes[6-8].

Anti-endomysial antibody assay: EMA were detected by indirect immunofluorescence using patient serum diluted 1:5 with monkey oesophagus, followed by FITC-conjugated goat anti-human IgA. IgA was quantitated prior to EMA, none of the subjects were IgA deficient.

Antigen Challenge: Coeliac disease subjects following a gluten free diet, consumed 4 slices of gluten-containing bread (50g/slice, Sainsbury's "standard white sandwich bread") daily for 3 or 10 days. EMA was assessed the week before and up to two months after commencing the bread challenge. Healthy subjects who had followed a gluten free diet for four weeks, consumed their usual diet including four slices of gluten-containing bread for three days, then returned to gluten free diet for a further six days.

IFNγ and IL-10 ELISPOT: PBMC were prepared from 50-100 ml of venous blood by Ficoll-Hypaque density centrifugation. After three washes, PBMC were resuspended in complete RPMI containing 10% heat inactivated human AB serum. ELISPOT assays for single cell secretion of IFNγ and IL-10 were performed using commercial kits (Mabtech; Stockholm, Sweden) with 96-well plates (MAIP-S-45; Millipore, Bedford, MA) according to the manufacturers instructions (as described elsewhere[9]) with 2-5x10^5 (IFNγ) or 0.4-1x10^5 (IL-10) PBMC in each well. Peptides were assessed in duplicate wells, and Mycobacterium tuberculosis purified protein derivative (PPD RT49) (Serum Institute; Copenhagen, Denmark) (20 μg/ml) was included as a positive control in all assays.

Peptides: Synthetic peptides were purchased from Research Genetics (Huntsville, Alabama) Mass-spectroscopy and HPLC verified peptides' authenticity and >70% purity. Digestion of gliadin (Sigma; G-3375) (100 mg/ml) with α-chymotrypsin (Sigma; C-3142) 200:1 (w/w)was performed at room temperature in 0.1 M NH_4HCO_3 with 2M urea and was halted after 24 h by heating to 98°C for 10 minutes. After centrifugation (13 000g, 10 minutes), the gliadin digest supernatant was filter-sterilized (0.2 mm). Digestion of gliadin was verified by SDS-PAGE and protein concentration assessed. α-Chymotrypsin-digested gliadin (640 μg/ml) and synthetic gliadin peptides (15-mers: 160 μg/ml, other peptides: 0.1 mM) were individually treated with tTG (Sigma; T-5398) (50 μg/ml) in PBS + $CaCl_2$ 1 mM for 2 h at 37°C. Peptides and peptide pools were aliquotted into sterile 96-well plates and stored frozen at -20°C until use.

Amino acid sequencing of peptides: Reverse phase HPLC was used to purify the peptide resulting from tTG treatment of A-gliadin 56-75. A single product was identified and subjected to amino acid sequencing (automated sequencer Model 494A, Applied Biosystems, Foster City, California). The sequence of unmodified G56-75 was confirmed as: LQLQPFPQPQLPYPQPQSFP, and tTG treated G56-75 was identified as: LQLQPFPQPELPYPQPQSFP . Deamidation of glutamyl residues was defined as the amount (pmol) of glutamate recovered expressed as a percent of the combined amount of glutamine and glutamate recovered in cycles 2, 4, 8, 10, 15 and 17 of the amino acid sequencing. Deamidation attributable to tTG was defined as (% deamidation of glutamine in the tTG treated peptide - % deamidation in the untreated peptide) / (100 - % deamidation in the untreated

peptide).

CD4/CD8 and HLA Class II Restriction: Anti-CD4 or anti-CD8 coated magnetic beads (Dynal, Oslo, Norway) were washed four times with RPMI then incubated with PBMC in complete RPMI containing 10% heat inactivated human AB serum ($5x10^6$ cells/ml) for 30 minutes on ice. Beads were removed using a magnet and cells remaining counted. In vivo HLA-class II restriction of the immune response to tTG-treated A-gliadin 56-75 was established by incubating PBMC ($5x10^6$ cells/ml) with anti-HLA-DR (L243), -DQ (L2), and -DP (B7.21) monoclonal antibodies (10 μg/ml) at room temperature for one hour prior to the addition of peptide.

Example 4

Mucosal integrin expression by gliadin -specific peripheral blood lymphocytes

[0164] Interaction between endothelial and lymphocyte adressins facilitates homing of organ-specific lymphocytes. Many adressins are known. The herterodimer $\alpha_4\beta_7$ is specific for lamina propria gut and other mucosal lymphocytes, and $\alpha^E\beta_7$ is specific and intra-epithelial lymphocytes in the gut and skin. Approximately 30% of perpheral blood CD4 T cells express $\alpha_4\beta_7$ and are presumed to be in transit to a mucosal site, while 5% of perpheral blood T cells express $\alpha^E\beta_7$. Immunomagnetic beads coated with antibody specifc for α^E or β_7 deplete PBMC of cells expressing $\alpha^E\beta_7$ or $\alpha^E\beta_7$ and $\alpha_4\beta_7$, respectively. In combination with ELISpot assay, immunomagnetic bead depletion allows determination of gliadin-specific T cell addressin expression that may identify these cells as homing to a mucosal surface. Interestingly, gluten challenge in vivo is associated with rapid influx of CD4 T cells to the small intestinal lamina propria (not intra-epithelial sites), where over 90% lymphocytes express $\alpha_4\beta_7$.

[0165] Immunomagnetic beads were prepared and used to deplete PBMC from coeliac subjects on day 6 or 7 after commencing 3 day gluten challenge. FACS analysis demonstrated α^E beads depleted approximately 50% of positive CD4 T cells, while β_7 beads depleted all β_7 positive CD4 T cells. Depletion of PBMC using CD4- or β_7-beads, but not CD8- or α^E -beads, abolished responses in the interferon gamma ELISpot. tTG gliadin and PPD responses were abolished by CD4 depletion, but consistently affected by integrin-specific bead depletion.

[0166] Thus A-gliadin 57-73 QE65-specific T cells induced after gluten challenge in coeliac disease express the integrin, $\alpha_4\beta_7$, present on lamina propria CD4 T cells in the small intestine.

Example 5

Optimal T cell Epitope Length

[0167] Previous data testing peptides from 7 to 17 aminoacids in length spanning the core of the dominant T cell epitope in A-gliadin indicated that the 17mer, A-gliadin 57-73 QE65 induced maximal responses in the interferon gamma Elispot using peripheral blood mononuclear cells (PBMC) from coeliac volunteers 6 days after commencing a 3-day gluten challenge.

[0168] Peptides representing expansions form the core sequence of the dominant T cell epitope in A-gliadin were assessed in the IFN gamma ELISPOT using peripheral blood mononuclear cells (PBMC) from coeliac volunteers in 6 days after commencing a 3-day gluten challenge (n=4). Peptide 13: A-gliadin 59-71 QE65 (13mer), peptide 15: 58-72 QE65 (15mer), ..., peptide 27: 52-78 QE65 (27mer).

[0169] As shown in Figure 11 expansion of the A-gliadin 57-73 QE65 sequence does not substantially enhance response in the IFNgamma Elispot. Subsequent Examples characterise the agonist and antagonist activity of A-gliadin 57-73 QE65 using 17mer peptides.

Example 6

Comparison of A-gliadin 57-73 QE65 with other DQ2-restricted T cell epitopes in coeliac disease

[0170] Dose response studies were performed using peptides corresponding to unmodified and transglutaminase-treated peptides corresponding to T cell epitopes of gluten-specific T cell clones and lines from intestinal biopsies of coeliac subjects. Responses to peptides were expressed as percent of response to A-gliadin 57-73 QE65. All subjects were HLA-DQ2+ (none were DQ8+).

[0171] The studies indicate that A-gliadin 57-73 QE65 is the most potent gliadin peptide for induction of interferon gamma in the ELISpot assay using coeliac PBMC after gluten challenge (see Figure 12a-h, and Tables 5 and 6). The second and third epitopes are suboptimal fragments of larger peptides i.e. A-gliadin 57-73 QE65 and GDA4_WHEAT P04724-84-100 QE92. The epitope is only modestly bioactive (approximately $1/20^{th}$ as active as A-gliadin 57-73 QE65

after blank is substracted).

[0172] A-gliadin 57-73 QE65 is more potent than other known T cell epitopes in coeliac disease. There are 16 polymorphisms of A-gliadin 57-73 (including the sequence PQLPY) amongst sequenced gliadin genes, their bioactivity is assessed next.

Example 7

Comparison of gliadin- and A-gliadin 57-73 QE65-specific responses in peripheral blood

[0173] The relative contribution of the dominant epitope, A-gliadin 57-73 QE65, to the total T cell response to gliadin in coeliac disease is a critical issue. Pepsin-trypsin and chymotrypsin-digested gliadin have been traditionally used as antigen for development of T cell lines and clones in coeliac disease. However, it is possible that these proteases may cleave through certain peptide epitopes. Indeed, chymotrypsin digestion of recombinant α9-gliadin generates the peptide QLQPFPQPELPY, that is a truncation of the optimal epitope sequence QLQPFPQPELPYPQPQS (see above). Transglutaminase-treatment substantially increases the potency of chymotrypsin-digested gliadin in poliferation assays of gliadin-specific T cell clones and lines. Hence, transglutaminase-treated chymotrypsin-digested gliadin (tTG gliadin) may not be an ideal antigen, but responses against this mixture may approximate the "total" number of peripheral blood lymphocyte specific for gliadin. Comparison of responses against A-gliadin 57-73 QE65 and tTG gliadin in the ELISpot assay gives an indication of the contribution of this dominant epitope to the overall immune response to gliadin in coeliac disease, and also be a measure of epitope spreading.

[0174] PBMC collected on day 6 or 7 after commencing gluten challenge in 4 coeliac subjects were assessed in dose response studies using chymotrypsin-digested gliadin +/- tTG treatment and compared with ELISpot responses to an optimal concentration of A-gliadin 57-73 QE65 (25mcg/ml). TTG treatment of gliadin enhanced PBMC responses in the ELISpot approximately 10-fold (tTG was comparable to blank when assessed alone) (see Figure 13a-c). In the four coeliac subjects studied, A-gliadin 57-73 QE65 (25 mcg/ml) elicited responses between 14 and 115% those of tTG gliadin (500 mcg/ml), and the greater the response to A-gliadin 57-73 QE65 the greater proportion it represented of the tTG gliadin response.

[0175] Relatively limited data suggest that A-gliadin 57-73 QE65 responses are comparable to tTG gliadin in some subjects. Epitope spreading associated with more evolved anti-gliadin T cell responses may account for the smaller contribution of A-gliadin 57-73 QE65 to "total" gliadin responses in peripheral blood in some individuals. Epitope spreading may be maintained in individuals with less strictly gluten free diets.

Example 8

Definition of gliadin peptides bioactive in coeliac disease: polymorphisms of A-gliadin 57-73

[0176] Overlapping 15mer peptides spanning the complete sequence of A-gliadin were assessed in order to identify the immunodominant sequence in coeliac disease. A-gliadin was the first fully sequenced alpha gliadin protein and gene, but is one of approximately 30-50 related alpha gliadin proteins in wheat. Twenty five distinct alpha-gliadin genes have been identified by searching protein data bases, Swiss-Prot and TREMBL describing a further 8 alpha-gliadins. Contained within these 25 alpha-gliadins, there are 16 distinct polymorphisms of the sequence corresponding to A-gliadin 57-73 (see Table 7).

[0177] Synthetic peptides corresponding to these 16 polymorphisms, in an unmodified form, after treatment with transglutaminase in vitro, as well as with glutamate substituted at position 10 (equivalent to QE65 in A-gliadin 57-73) were assessed using PBMC from coeliac subjects, normally following a gluten free diet, day 6 or 7 after gluten challenge in interferon gamma ELISpot assays. Glutamate-substituted peptides were compared at three concentrations (2.5, 25 and 250 mcg/ml), unmodified peptide and transglutaminase-treated peptides were assessed at 25 mcg/ml only. Bioactivity was expressed as % of response associated with A-gliadin 57-73 QE65 25 mcg/ml in individual subjects (n=4). (See Fig. 14).

[0178] Bioactivity of "wild-type" peptides was substantially increased (>5-fold) by treatment with transglutaminase. Transglutaminase treatment of wild-type peptides resulted in bioactivity similar to that of the same peptides substituted with glutamate at position 10. Bioactivities of five glutamate-substituted peptides (B, C, K, L, M), were >70% that of A-gliadin 57-73 QE65 (A), but none was significantly more bioactive than A-gliadin 57-73 QE65. PBMC responses to glutamate-substituted peptides at concentrations of 2.5 and 250 mcg/ml were comparable to those at 25 mcg/ml. Six glutamate-substituted gliadin peptides (H, I, J, N, O, P) were <15% as bioactive as A-gliadin 57-73 QE65. Other peptides were intermediate in bioactivity.

[0179] At least six gliadin-derived peptides are equivalent in potency to A-gliadin 57-73 QE65 after modfication by transglutaminase. Relatively non-bioactive polymorphisms of A-gliadin 57-73 also exist. These data indicate that trans-

glutaminase modification of peptides from several gliadins of Tricetum aestivum, T. uartu and T. spelta may be capable of generating the immunodominant T cell epitope in coeliac disease.

[0180] Genetic modification of wheat to generate non-coeliac-toxic wheat is likely require removal or modification of multiple gliadin genes. Generation of wheat containing gliadins or other proteins or peptides incorporating sequences defming altered peptide ligand antagonists of A-gliadin 57-73 is an alternative strategy to generate genetically modified wheat that is therapeutic rather than "non-toxic" in coeliac disease.

Example 9

Definition of Core Epitope Sequence:

[0181] Comparison of peptides corresponding to truncations of A-gliadin 56-75 from the N- and C-terminal indicated that the core sequence of the T cell epitope is PELPY (A-gliadin 64-68). Attempts to define non-agonists and antagonists will focus on variants of A-gliadin that are substituted at residues that substantially contribute to its bioactivity.

[0182] Peptides corresponding to A-gliadin 57-73 QE65 with alanine (Figure 15) or lysine (Figure 16) substituted for residues 57 to 73 were compared in the IFN gamma ELISPOT usng peripheral blood mononuclear cells (PBMC) from coeliac volunteers 6 days after commencing a 3-day gluten challenge (n=8). [BL is blank, E is A-gliadin 57-73 QE65: QLQPFPQPELPYPQPQS].

[0183] It was found that residues corresponding to A-gliadin 60-70 QE65 (PFPQPELPYPQ) contribute substantially to the bioactivity in A-gliadin 57-73 QE65. Variants of A-gliadin 57-73 QE65 substituted at positions 60-70 are assessed in a 2-step procedure. Initially, A-gliadin 57-73 QE65 substituted at positions 60-70 using 10 different aminoacids with contrasting properties are assessed. A second group of A-gliadin 57-73 QE65 variants (substituted with all other naturally occurring aminoacids except cysteine at positions that prove are sensitive to modification) are assessed in a second round.

Example 10

Agonist activity of substituted variants of A-gliadin 57-73 QE65

[0184] A-gliadin 60-70 QE65 is the core sequence of the dominant T cell epitope in A-gliadin. Antagonist and non-agonist peptide variants of this epitope are most likely generated by modification of this core sequence. Initially, A-gliadin 57-73 QE65 substituted at positions 60-70 using 10 different aminoacids with contrasting properties will be assessed in the IFNgamma ELISPOT using PBMC from coeliac subjects 6 days after starting 3 day gluten challenge. A second group of A-gliadin 57-73 QE65 variants (substituted with all other naturally occurring aminoacids except cysteine) at positions 61-70 were also assessed. Both groups of peptides (all at 50 mcg/ml, in duplicate) were assessed using PBMC from 8 subjects and compared to the unmodified peptide (20 replicates per assay). Previous studies indicate that the optimal concentration for A-gliadin 57-73 QE65 in this assay is between 10 and 100 mcg/ml.

[0185] Results are expressed as mean response in spot forming cells (95% confidence interval) as % A-G 57-73 QE65 mean response in each individual. Unpaired t-tests will be used to compare ELISPOT responses of modified peptides with A-G 57-73 QE65. Super-agonists were defined as having a greater response than A-G 57-73 QE65 at a level of significance of $p<0.01$; partial agonists as having a response less than A-G 57-73 QE65 at a level of significance of $p<0.01$, and non-agonists as being not significantly different ($p>0.01$) from blank (buffer without peptide). Peptides with agonist activity 30% or less that of A-gliadin 57-73 QE65 were considered "suitable" partial or non-agonists to assess for antagonistic activity (see Table 8 and Figures 17-27).

[0186] The IFNgamma ELISPOT response of PBMC to A-gliadin 57-73 QE65 is highly specific at a molecular level. Proline at position 64 (P64), glutamate at 65 (E65) and leucine at position 66 (L66), and to a lesser extent Q63, P67, Y68 and P69 are particularly sensitive to modification. The substitutions Y61 and Y70 both generate super-agonists with 30% greater bioactivity than the parent peptide, probably by enhancing binding to HLA-DQ2 since the motif for this HLA molecule indicates a preference for bulky hydrophobic resides at positions 1 and 9. Eighteen non-agonist peptides were identified. Bioactivities of the variants (50 mcg/ml): P65, K64, K65 and Y65 (bioactivity 7-8%) were comparable to blank (7%). In total, 57 mutated variants of A-gliadin 57-73 QE65 were 30% or less bioactive than A-gliadin 57-73 QE65.

[0187] The molecular specificity of the peripheral blood lymphocyte (PBL) T cell response to the dominant epitope, A-gliadin 57-73 QE65, is consistently reproducible amongst HLA-DQ2+ coeliac subjects, and is highly specific to a restricted number of aminoacids in the core 7 aminoacids. Certain single-aminoacid variants of A-gliadin 57-73 QE65 are consistently non-agonists in all HLA-DQ2+ coeliac subjects.

Example 11

Antagonist activity of substituted variants

[0188] The homogeneity of the PBL T cell response to A-gliadin 57-73 QE65 in HLA-DQ2+ coeliac disease suggests that altered peptide ligands (APL) capable of antagonism in PBMC ex vivo may exist, even though the PBL T cell response is likely to be poly- or oligo-clonal. APL antagonists are generally weak agonists. Fifty-seven single aminoacid-substituted variants of A-gliadin 57-73 QE65 with agonist activity 30% or less have been identified and are suitable candidates as APL antagonists. In addition, certain weakly bioactive naturally occurring polymorphisms of A-gliadin 57-73 QE65 have also been identified (see below) and may be "naturally occurring" APL antagonists. It has also been suggested that competition for binding MHC may also antagonise antigen-specific T cell immune. Hence, non-gliadin peptides that do not induce IFNgamma responses in coeliac PBMC after gluten challenge but are known to bind to HLA-DQ2 may be capable of reducing T cell responses elicited by A-gliadin 57-73 QE65. Two peptides that bind avidly to HLA-DQ2 are HLA class 1α 46-60 (HLA 1a) (PRAPWIEQEGPEYW) and thyroid peroxidase (tp) 632-645Y (IDVWLGGLLAENFLPY).

[0189] Simultaneous addition of peptide (50μg/ml) or buffer and A-gliadin 57-73 QE65 (10μg/ml) in IFNgamma ELISPOT using PBMC from coeliac volunteers 6 days after commencing 3 day gluten challenge (n=5). Results were expressed as response with peptide plus A-G 57-73 QE65 (mean of duplicates) as % response with buffer plus A-G 57-73 QE65 (mean of 20 replicates). (See Table 9).

[0190] Four single aminoacid-substituted variants of A-gliadin 57-73 QE65 reduce the interferon gamma PBMC ELISPOT response to A-gliadin 57-73 QE65 ($p<0.01$) by between 25% and 28%, 13 other peptide variants reduce the ELISPOT response by between 18% and 24% ($p<0.06$). The HLA-DQ2 binder, thyroid peroxidase (tp) 632-645Y reduces PBMC interferon gamma responses to A-gliadin 57-73 QE65 by 31% ($p<0.0001$) but the other HLA-DQ2 binder, HLA class 1α 46-60, does not alter responses (see Table 9). The peptide corresponding to a transglutaminase-modified polymorphism of A-glaidin 57-73, SwissProt accession no.: P04725 82-98 QE90 (PQPQPFPPELPYPQPQS) reduces responses to A-gliadin 57-73 QE65 by 19% ($p<0.009$) (see Table 11).

[0191] Interferon gamma responses of PBMC to A-gliadin 57-73 QE65 in ELISPOT assays are reduced by co-administration of certain single-aminoacid A-gliadin 57-73 QE65 variants, a polymorphism of A-gliadin 57-73 QE65, and an unrelated peptide known to bind HLA-DQ2 in five-fold excess. These finding suggest that altered peptide ligand antagonists of A-gliadin 57-73 QE65 exist. Not only putative APL antagonists but also certain peptides that bind HLA-DQ2 effectively reduce PBL T cell responses to A-gliadin 57-73 QE65.

[0192] These findings support two strategies to interrupt the T cell response to the dominant A-gliadin epitope in HLA-DQ2+ coeliac disease.

1. Optimisation of APL antagonists by substituting aminoacids at more than one position (64-67) for use as "traditional" peptide pharmaceuticals or for specific genetic modification of gliadin genes in wheat.
2. Use of high affinity HLA-DQ2 binding peptides to competitively inhibit presentation of A-gliadin 57-73 QE65 in association with HLA-DQ2.

[0193] These two approaches may be mutually compatible. Super-agonists were generated by replacing F61 and Q70 with tyrosine residues. It is likely these super-agonists resulted from improved binding to HLA-DQ2 rather than enhanced contact with the T cell receptor. By combining these modifications with other substitutions that generate modestly effective APL antagonists might substantially enhance the inhibitory effect of substituted A-gliadin 57-73 QE65 variants.

Example 12

Development of interferon gamma ELISpot using PBMC and A-gliadin 57-73 OE65 and P04724 84-100 QE92 as a diagnostic for coeliac disease: Definition of immune-responsiveness in newly diagnosed coeliac disease

[0194] Induction of responsiveness to the dominant A-gliadin T cell epitope in PBMC measured in the interferon gamma ELISpot follows gluten challenge in almost all DQ2+ coeliac subjects following a long term strict gluten free diet (GFD) but not in healthy DQ2+ subjects after 4 weeks following a strict GFD. A-gliadin 57-73 QE65 responses are not measurable in PBMC of coeliac subjects before gluten challenge and pilot data have suggested these responses could not be measured in PBMC of untreated coeliacs. These data suggest that in coeliac disease immune-responsiveness to A-gliadin 57-73 QE65 is restored following antigen exclusion (GFD). If a diagnostic test is to be developed using the ELISpot assay and PBMC, it is desireable to define the duration of GFD required before gluten challenge is capable of inducing responses to A-gliadin 57-73 QE65 and other immunoreactive gliadin peptides in blood.

[0195] Newly diagnosed DQ2+ coeliac subjects were recruited from the gastroenterology outpatient service. PBMC were prepared and tested in interferon gamma ELISpot assays before subjects commenced GFD, and at one or two weeks after commencing GFD. In addition, gluten challenge (3 days consuming 4 slices standard white bread, 204g/day) was performed at one or two weeks after starting GFD. PBMC were prepared and assayed on day six are after commencing gluten challenge. A-gliadin 57-73 QE65 (A), P04724 84-100 QE92 (B) (alone and combined) and A-gliadin 57-73 QP65 (P65) (non-bioactive variant, see above) (all 25 mcg/ml) were assessed.

[0196] All but one newly diagnosed coeliac patient was DQ2+ (one was DQ8+) (n=11). PBMC from newly diagnosed coeliacs that were untreated, or after 1 or 2 weeks following GFD did not show responses to A-gliadin 57-73 QE65 and P04724 84-100 QE92 (alone or combined) that were not significantly different from blank or A-gliadin 57-73 QP65 (n=9) (see Figure 28). Gluten challenge in coeliacs who had followed GFD for only one week did not substantially enhance responses to A-gliadin 57-73 QE65 or P04724 84-100 QE92 (alone or combined). But gluten challenge 2 weeks after commencing GFD did induce responses to A-gliadin 57-73 QE65 and P04724 84-100 QE92 (alone or combined) that were significantly greater than the non-bioactive variant A-gliadin 57-73 QP65 and blank. Although these responses after gluten challenge at 2 weeks were substantial they appear to be less than in subjects >2 months after commencing GFD. Responses to A-gliadin 57-73 QE65 alone were equivalent or greater than responses to P04724 84-100 QE92 alone or when mixed with A-gliadin 57-73 QE65. None of the subjects experienced troubling symptoms with gluten challenge.

[0197] Immune responsiveness (as measured in PBMC after gluten challenge) to A-gliadin is partially restored 2 weeks after commencing GFD, implying that "immune unresponsiveness" to this dominant T cell epitope prevails in untreated coeliac disease and for at least one week after starting GFD. The optimal timing of a diagnostic test for coeliac disease using gluten challenge and measurement of responses to A-gliadin 57-73 QE65 in the ELISpot assay is at least 2 weeks after commencing a GFD.

[0198] Interferon gamma-secreting T cells specific to A-gliadin 57-73 QE65 cannot be measured in the peripheral blood in untreated coeliacs, and can only be induced by gluten challenge after at least 2 weeks GFD (antigen exclusion). Therefore, timing of a diagnostic test using this methodology is crucial and further studies are needed for its optimization. These finding are consistent with functional anergy of T cells specific for the dominant epitope, A-gliadin 57-73 QE65, reversed by antigen exclusion (GFD). This phenomenon has not been previously demonstrated in a human disease, and supports the possibility that T cell anergy may be inducible with peptide therapy in coeliac disease.

References

[0199]

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Table 1. A-Gliadin protein sequence (based on amino acid sequencing)

```
VRVPVPQLQP QNPSQQQPQE QVPLVQQQQF PGQQQQFPPQ QPYPQPQPFP SQQPYLQLQP FPQPQLPYPQ
1          11         21         31         41         51         61
PQSFPPQQPY PQPQPQYSQP QQPISQQQAQ QQQQQQQQQQ QQQILQQILQ QQLIPCMDVV LQQHNIAHAR

71         81         91         101        111        121        131
SQVLQQSTYQ LLQELCCQHL WQIPEQSQCQ AIHNVVHAII LHQQQKQQQQ PSSQVSFQQP LQQYP
LGQGS
141        151        161        171        181        191        201
FRPSQQNPQA QGSVQPQQLP QFEEIRNLAL QTLPAMCNVY IAPYCTIAPF GIFGTN
211        221        231        241        251        261
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Table 2. Coeliac disease subjects studied

	Age Sex	Gluten free diet	HLA-DQ2	Bread challenge	Symptoms with bread
1	64 f	14 yr	Homozygote	3 days	Abdominal pain, lethargy, mouth ulcers, diarrhoea
2	57 m	1 yr	Heterozygote	10 days	Lethargy, nausea
3	35 f	7 yr	Heterozygote	3 days	Nausea
4	36 m	6 wk	Homozygote	3 days	Abdominal pain, mouth ulcers, diarrhoea
5	26 m	19 yr	Heterozygote	3 days	None
6	58 m	35 yr	Heterozygote	3 days	None
7	55 m	1 yr	Heterozygote	3 days	Diarrhoea
8	48 f	15 yr	Homozygote	3 days	Abdominal pain, diarrhoea

Table 3

Aminoacid at position 65	Range	Mean
Glutamate	(100)	100%
Asparagine	(50-84)	70%
Aspartate	(50-94)	65%
Alanine	(44-76)	64%
Cysteine	(45-83)	62%
Serine	(45-75)	62%
Valine	(24-79)	56%
Threonine	(46-66)	55%
Glycine	(34-47)	40%
Leucine	(8-46)	33%
Glutamine	(16-21)	19%
Isoleucine	(3-25)	14%
Methionine	(3-32)	14%
Phenylalanine	(0-33)	12%
Histidine	(0-13)	8%
Tyrosine	(0-17)	8%
Tryptophan	(0-17)	8%
Lysine	(0-11)	4%
Proline	(0-4)	2%
Arginine	(0-2)	1%

Elisopt response		Peptide sequence	Corresponding residues in gliadin protein sequences (Accession no.)	
No TG	TG			
8 (1-13)		QLQPFPQPQLPYPQPQS	57-73	α-Gliadin (T. aestivum) Q41545
	100 (100)	QLQPFPQPELPYPQPQS	57-73	α-Gliadin (T. aestivum) Q41545
5 (1-7)	53 (44-67)	QLQPFPQPQLPYSQPQP	77-93	α/β-Gliadin precursor (Triticum. aestivum) P02863
			76-92	α-Gliadin (T. aestivum) Q41528
			77-93	α-Gliadin storage protein (T. aestivum) Q41531
			57-73	α-Gliadin mature peptide (T. aestivum) Q41533
			77-93	α-Gliadin precursor (T. spelta) Q9ZP09
12 (0-20)	83 (61-113)	QLQPFPQPQLPYPQPQP	77-93	α/β-Gliadin A-II precursor (T. aestivum) P0472
19 (0-33)	83 (74-97)	QLQPFPQPQLPYPQPQL	77-93	α/β-Gliadin A-IV precursor (T. aestivum) P04724
			77-93	α/β-Gliadin MM1 precursor (T. aestivum) P18573
3 (0-7)	109 (41-152)	PQLPYPQPQLPYPQPQP	84-100	α/β-Gliadin A-IV precursor (T. aestivum) P04724
ND		PQLPYPQPQLPYPQPQL	84-100	α/β-Gliadin MM1 precursor (T. aestivum) P18573
0 (0-1)	3 (0-7)	QLQPFLQPQLPYSQPQP	77-93	α/β-Gliadin A-I precursor (T. aestivum) P04721
			77-93	α-Gliadin (T. aestivum) Q41509
0 (0-0)	2 (0-7)	QLQPFSQPQLPYSQPQP	77-93	α-Gliadin storage protein (T. aestivum) Q41530
ND		PQPQPFPPQLPYPQTQP	77-93	α/β-Gliadin A-III precursor (T. aestivum) P04723
17 (0-40)	24 (11-43)	PQPQPFPPQLPYPQPQS	82-98	α/β-Gliadin A-V precursor (T. aestivum) P04725
10 (0-30)	19 (11-33)	PQPQPFPPQLPYPQPPP	82-98	α/β-Gliadin clone PW1215 precursor (T. aestivum) P04726
			82-98	α/β-Gliadin (T. urartu) Q41632
10 (0-30)	21 (11-33)	PQPQPFLPQLPYPQPQS	79-95	α/β-Gliadin clone PW8142 precursor (T. aestivum) P04726
			79-95	α-Gliadin (T. aestivum) Q41529
			79-95	α/β-Gliadin precursor (T. aestivum) Q41546

Table 4

Table 5. T cell epitopes described in coeliac disease

Source	Restriction	Frequency	Sequence*
Gamma -gliadin	DQ2	3/NS (iTCC)	QQLPQPEQPQQSFPEQERPF
Alpha-gliadin	DQ2	12/17 (iTCL)	QLQPFPQPELPY
Alpha-gliadin	DQ2	11/17 (iTCL)	PQPELPYPQPELPY
Alpha-gliadin	DQ2	1/23 (bTCC)	LGQQQPFPPQQPYPQPQPF
Alpha-gliadin	DQ8	3/NS (iTCC)	QQYPSGEGSFQPSQENPQ
Glutenin	DQ8	1/1 (iTCC)	GQQGYYPTSPQQSGQ
Alpha-gliadin	DQ2	11/12 in vivo	QLQPFPQPELPYPQPQS

NS not stated in original publication, iTCC intestinal T cell clone, iTCL intestinal polyclonal T cell line, bTCC peripheral blood T cell clone

*All peptides are the products of transglutaminase modifying wild type gluten peptides except the fourth and sixth peptides

Table 6. Relative bioactivity of gliadin T cell epitopes in coeliac PBMC after gluten challenge

Sequence*	ELISpot response as % A-gliadin 57-73 QE65 (all 25mcg/ml)		
	Wild type	Wildtype+tTG	E-substituted
QQLPQPEQPQQSFPEQERPF	9 (3)	18 (7)	10 (5)
QLQPFPQPELPY	6 (2)	19 (1)	8 (3)
PQPELPYPQPELPY	13 (6)	53 (8)	48 (9)
QQYPSGEGSFQPSQENPQ	10 (3)	9 (3)	14 (8)
QLQPFPQPELPYPQPQS	18 (7)	87 (7)	100
PQLPYPQPELPYPQPQP	14 (4)	80 (17)	69 (20)

* sequence refers that of transglutaminase (tTG) modified peptide and the T cell epitope. Wild type is the unmodified gliadin peptide. Data from 4 subjects. Blank was 5 (1) %.

Table 7. Polymorphisms of A-gliadin 57-73

A. Sequences derived from Nordic autumn wheat strain Mjoelner

Alpha-gliadin protein (single letter code refers to Fig. 14 peptides)	Polymorphism
Q41545 A-gliadin (from sequenced protein) 57-73 (A)	QLQPFPQPQLPYPQPQS
Gli alpha 1,6: (EMBL: AJ133605 & AJ133602 58-74) (J)	QPQPFPPPQLPYPQTQP
Gli alpha 3,4,5: (EMBL: AJ133606, AJ133607, AJ133608 57-73) (I)	QLQPFPQPQLSYSQPQP
Gli alpha 7: (EMBL: AJ133604 57-73) (E)	QLQPFPRPQLPYPQPQP
Gli alpha 8, 9, 11: (EMBL:) (F)	QLQPFPQPQLPYSQPQP
Gli alpha 10: (EMBL: AJ133610 57-73) (D)	QLQPFPQPQLPYLQPQS

B. SWISSPROT and TREMBL scan (10.12.99) for gliadins containing the sequence: XXXXXXXPQLPYXXXXX

Wheat (Triticum aestivum unless stated) gliadin accession number	Polymorphism
Q41545 A-gliadin (from sequenced protein) 57-73 (A)	QLQPFPQPQLPYPQPQS
SWISSPROT:	
GDA0_WHEAT P02863 77-93 (F)	QLQPFPQPQLPYSQPQP
GDA1_WHEAT P04721 77-93 (G)	QLQPFLQPQLPYSQPQP
GDA2_WHEAT P04722 77-93 (B)	QLQPFPQPQLPYPQPQP
GDA3_WHEAT P04723 77-93 (O)	PQPQPFPPQLPYPQTQP
GDA4_WHEAT P04724 77-93 (C)	QLQPFPQPQLPYPQPQL
GDA4_WHEAT P04724 84-100 (K)	PQLPYPQPQLPYPQPQP
GDA5_WHEAT P04725 82-98 (N)	PQPQPFPPQLPYPQPQS
GDA6_WHEAT P04726 82-98 (P)	PQPQPFPPQLPYPQPPP
GDA7_WHEAT P04727 79-95 (M)	PQPQPFLPQLPYPQPQS
GDA9_WHEAT P18573 77-93 (C)	QLQPFPQPQLPYPQPQL
GDA9_WHEAT P18573 84-100 (L)	PQLPYPQPQLPYPQPQL
GDA9_WHEAT P18573 91-107 (K)	PQLPYPQPQLPYPQPQP
TREMBL	
Q41509 ALPHA-GLIADIN 77-93 (G)	QLQPFLQPQLPYSQPQP
Q41528 ALPHA-GLIADIN 76-92 (F)	QLQPFPQPQLPYSQPQP
Q41529 ALPHA-GLIADIN 79-95 (M)	PQPQPFLPQLPYPQPQS
Q41530 ALPHA-GLIADIN 77-93 (H)	QLQPFSQPQLPYSQPQP
Q41531 ALPHA-GLIADIN 77-93 (F)	QLQPFPQPQLPYSQPQP
Q41533 ALPHA-GLIADIN 57-73 (F)	QLQPFPQPQLPYSQPQP
Q41546 ALPHA/BETA-GLIADIN 79-95 (M)	PQPQPFLPQLPYPQPQS
Q41632 ALPHA/BETA-TYPE GLIADIN. Triticum urartu 82-98 (P)	PQPQPFPPQLPYPQPPP
Q9ZP09 ALPHA-GLIADIN Triticum spelta 77-93 (F)	QLQPFPQPQLPYSQPQP

Table 8. Bioactivity of substituted variants of A-gliadin 57-73 QE65 (Subst) compared to unmodified A-gliadin 57-73 QE65 (G) (mean 100%, 95% CI 97-104) and blank (no peptide, bl) (mean 7.1%, 95% CI: 5.7-8.5)

Subst	%	P vs G	Subst	%	P vs G	Subst	%	P vs G	Subst	%	P vs G	P vs bl
Super-agonists			**F62**	71	0.001	**H62**	47	<0.0001	**N66**	24	<0.0001	
Y61	129	<0.00 01	**V63**	70	<0.0001	**G69**	47	<0.0001	**R64**	24	<0.0001	
Y70	129	0.0006	**S69**	70	<0.0001	**N63**	47	<0.0001	**K63**	23	<0.0001	
Agonists			**H63**	70	<0.0001	**H68**	47	<0.0001	**V65**	23	<0.0001	
W70	119	0.017	**F63**	70	0.008	**M68**	46	<0.0001	**H66**	23	<0.0001	
K57	118	0.02	**P70**	69	<0.0001	**D68**	46	<0.0001	**H67**	22	<0.0001	
Y59	117	0.04	**T62**	69	<0.0001	**V69**	46	<0.0001	**L64**	22	<0.0001	
A57	116	0.046	**L61**	69	<0.0001	**G63**	45	<0.0001	**S66**	22	<0.0001	
S70	116	0.045	**S61**	69	<0.0001	**V64**	45	<0.0001	**F67**	21	<0.0001	
K58	114	0.08	**T61**	69	<0.0001	**E61**	45	<0.0001	**W66**	21	<0.0001	
W59	110	0.21	**T63**	69	<0.0001	**A69**	43	<0.0001	**G64**	21	<0.0001	
A73	109	0.24	**M66**	68	<0.0001	**R62**	42	<0.0001	**G65**	21	<0.0001	
I59	108	0.37	**T69**	67	<0.0001	**G68**	42	<0.0001	**D64**	21	<0.0001	
G59	108	0.34	**K60**	66	<0.0001	**A64**	42	<0.0001	**I65**	21	<0.0001	
A58	108	0.35	**S62**	66	<0.0001	**C65**	42	<0.0001	**M64**	20	<0.0001	<0.0001
W60	105	0.62	**M61**	66	<0.0001	**N67**	41	<0.0001	**G67**	19	<0.0001	<0.0001
A59	104	0.61	**P61**	65	<0.0001	**W63**	41	<0.0001	**T65**	19	<0.0001	0.003
K72	104	0.65	**M62**	64	<0.0001	**F69**	41	<0.0001	**A66**	19	<0:0001	<0.0001
S59	103	0.76	**Q61**	64	<0.0001	**N68**	40	<0.0001	**I64**	19	<0.0001	0.0003
K73	102	0.8	**G61**	64	<0.0001	**V66**	40	<0.0001	**R63**	19	<0.0001	<0.0001
A70	102	0.81	**A63**	64	<0.0001	**H69**	40	<0.0001	**W67**	19	<0.0001	<0.0001
Y60	101	0.96	**L62**	60	<0.0001	**M69**	40	<0.0001	**K68**	18	<0.0001	<0.0001
A72	100	0.94	**I68**	60	<0.0001	**R69**	40	<0.0001	**H64**	18	<0.0001	<0.0001
S63	98	0.67	**S67**	59	<0.0001	**W69**	40	<0.0001	**W64**	18	<0.0001	0.0001
K59	96	0.46	**N61**	59	<0.0001	**Q69**	39	<0.0001	**Q65**	18	<0.0001	0.0002
I60	96	0.5	**I69**	59	<0.0001	**L67**	38	<0.0001	**F64**	16	<0.0001	0.0008
G70	95	0.41	**V61**	58	<0.0001	**K69**	38	<0.0001	**L65**	16	<0.0001	0.0022
D65	95	0.44	**D61**	58	<0.0001	**K62**	38	<0.0001	**N64**	16	<0.0001	<0.0001
E70	93	0.27	**E60**	57	<0.0001	**E67**	37	<0.0001	**F65**	16	<0.0001	0.12
163	92	0.19	**A61**	57	<0.0001	**L69**	37	<0.0001	**Q67**	15	<0.0001	0.0012
S60	92	0.23	**Q62**	56	<0.0001	**S64**	36	<0.0001	**M65**	14	<0.0001	0.015
P59	88	0.08	**F68**	56	<0.0001	**G62**	36	<0.0001	**D66**	14	<0.0001	0.013

Table continued

Subst		%	P vs G	Subst	%	P vs G	Subst	%	P vs G	Subst	%	P vs G	P vs bl
	M63	87	0.03	**N65**	56	<0.0001	**E69**	36	<0.0001	**R67**	14	<0.0001	0.002
	K71	85	0.047	**A62**	56	<0.0001	**E68**	36	<0.0001			**Non-agonists**	
	V62	84	0.04	**A68**	53	<0.0001	**V67**	35	<0.0001	**P63**	13	<0.0001	0.012
	I70	84	0.04	**P66**	53	<0.0001	**D62**	35	<0.0001	**E64**	12	<0.0001	0.053
	I61	83	0.01	**R61**	53	<0.0001	**R68**	34	<0.0001	**W65**	11	<0.0001	0.24
	V68	82	0.0045	**S68**	53	<0.0001	**Q66**	34	<0.0001	**Q64**	11	<0.0001	0.15
	E59	81	0.01	**Y63**	52	<0.0001	**A67**	33	<0.0001	**G66**	11	<0.0001	0.07
	Partial agonists			**N69**	51	<0.0001	**N62**	32	<0.0001	**R65**	11	<0.0001	0.26
	W61	79	0.002	**E63**	51	<0.0001	**F66**	31	<0.0001	**Y67**	10	<0.0001	0.13
	A60	78	0.002	**T64**	51	<0.0001	**E62**	31	<0.0001	**E66**	10	<0.0001	0.17
	Y62	78	0.006	**T67**	51	<0.0001	**D69**	31	<0.0001	**K66**	10	<0.0001	0.21
	G60	77	0.003	**Y69**	50	<0.0001	**D67**	30	<0.0001	**R66**	10	<0.0001	0.23
	A71	77	0.003	**D63**	50	<0.0001	**M67**	29	<0.0001	**K67**	10	<0.0001	0.11
	W62	76	0.0009	**A65**	49	<0.0001	**Y66**	28	<0.0001	**P65**	8	<0.0001	0.57
	Q60	76	0.001	**K61**	49	<0.0001	**I67**	28	<0.0001	**K64**	8	<0.0001	0.82
	L63	74	0.0002	**I66**	49	<0.0001	**H65**	26	<0.0001	**K65**	8	<0.0001	0.63
	I62	74	0.0005	**T68**	48	<0.0001	P68	26	<0.0001	**Y65**	7	<0.0001	0.9
	K70	74	0.001	**S65**	48	<0.0001	Y64	25	<0.0001				
	H61	72	<0.0001	**L68**	48	<0.0001	**EK6** 5	25	<0.0001				
	W68	72	<0.0001	**Q68**	48	<0.0001	**T66**	25	<0.0001				

Table 9. Antagonism of A-gliadin 57-73 QE65 interferon gamma ELISPOT response by substituted variants of A-gliadin 57-73 QE65 (Subst) (P is significance level in unpaired t-test). Agonist activity (% agonist) of peptides compared to A-gliadin 57-73 QE65 is also shown.

Subst	% Inhibit.	P	% agonist.	Subst	% Inhibit.	P	% agonist.
Antagonists				**65R**	13	0.18	11
65T	28	0.004	19	**65M**	13	0.16	14
67M	27	0.005 22	29	**68P**	13	0.16	26
64W	26	0.007	18	**63R**	13	0.19	19
67W	25	0.008 8	19	**66G**	12	0.19	11
Potential antagonists				**65Q**	12	0.2	18
67I	24	0.013	10	**65Y**	12	0.22	7
67Y	24	0.013	21	**66S**	12	0.22	22
64G	21	0.03	21	**67F**	11	0.25	21
64D	21	0.029	16	66R	10	0.29	10
65L	20	0.046	26	**67K**	10	0.29	10
66N	20	0.037	24	**64F**	10	0.29	16
65H	20	0.038	16	**65F**	9	0.41	16
64N	19	0.05	16	**63P**	8	0.42	13
64Y	19	0.06	25	**65EK**	8	0.39	25
66Y	19	0.048	28	**64Q**	7	0.49	11
64E	19	0.049	12	**64I**	5	0.6	21
67A	18	0.058	30	**68K**	5	0.56	19
67H	18	0.052	22	**67Q**	5	0.61	18
Non-antagonists				**65G**	5	0.62	15
65V	17	0.07	23	**64M**	4	0.7	20
65I	17	0.086	21	**66H**	4	0.66	23
66T	17	0.069	25	**66 E**	3	0.76	10
65W	15	0.11	11	**66D**	1	0.9	14
67R	15	0.13	14	**63K**	1	0.88	23
65P	15	0.13	8	**64H**	1	0.93	18
65K	15	0.11	8	**66K**	0	0.98	10
66W	15	0.12	21	**64K**	-2	0.88	8
67G	14	0.14	19	**64L**	-11	0.26	22
66A	14	0.14	19				

Table 10. Inhibition of A-gliadin 57-73 QE65 interferon gamma ELISPOT response by peptides known to bind HLA-DQ2 (**P** is significance level in unpaired t-test).

Peptide	% Inhibit.	P
TP	**31**	**<0.0001**
HLA1a	**0**	**0.95**

Table 11. Antagonism of A-gliadin 57-73 QE65 interferon gamma ELISpot response by naturally occurring polymorphisms of A-gliadin 57-73 QE65 (P is significance level in unpaired t-test).

A-gliadin 57-73 QE65 polymorphism		% Inhibit.	P
P04725 82-98 QE90	PQPQPFPPELPYPQPQS	19	0.009
Q41509 77-93 QE85	QLQPFLQPELPYSQPQP	11	0.15
Gliα 1,6 58-74 QE66	QPQPFPPPELPYPQTQP	11	0.11
P04723 77-93 QE85	PQPQPFPPELPYPQTQP	10	0.14
Gliα 3-5 57-73 QE65	QLQPFPQPELSYSQPQP	7	0.34
P02863 77-93 QE85	QLQPFPQPELPYSQPQP	6	0.35
Q41509 77-93 QE85	QLQPFLQPELPYSQPQP	6	0.41
P04727 79-95 QE65	PQPQPFLPELPYPQPQS	6	0.39
P04726 82-98 QE90	PQPQPFPPELPYPQPPP	5	0.43

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            20                  25                  30

Gln Gln Gln Gln Phe Pro Pro Gln Gln Pro Tyr Pro Gln Pro Gln Pro
        35                  40                  45

Phe Pro Ser Gln Gln Pro Tyr Leu Gln Leu Gln Pro Phe Pro Gln Pro
    50                  55                  60
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65                  70                  75                  80

Pro Gln Pro Gln Pro Gln Tyr Ser Gln Pro Gln Gln Pro Ile Ser Gln
                85                  90                  95

Gln Gln Ala Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
            100                 105                 110

Gln Ile Leu Gln Gln Ile Leu Gln Gln Gln Leu Ile Pro Cys Met Asp
        115                 120                 125

Val Val Leu Gln Gln His Asn Ile Ala His Ala Arg Ser Gln Val Leu
    130                 135                 140

Gln Gln Ser Thr Tyr Gln Leu Leu Gln Glu Leu Cys Cys Gln His Leu
145                 150                 155                 160

Trp Gln Ile Pro Glu Gln Ser Gln Cys Gln Ala Ile His Asn Val Val
                165                 170                 175

His Ala Ile Ile Leu His Gln Gln Gln Lys Gln Gln Gln Gln Pro Ser
            180                 185                 190

Ser Gln Val Ser Phe Gln Gln Pro Leu Gln Gln Tyr Pro Leu Gly Gln
        195                 200                 205

Gly Ser Phe Arg Pro Ser Gln Gln Asn Pro Gln Ala Gln Gly Ser Val
    210                 215                 220

Gln Pro Gln Gln Leu Pro Gln Phe Glu Glu Ile Arg Asn Leu Ala Leu
225                 230                 235                 240

Gln Thr Leu Pro Ala Met Cys Asn Val Tyr Ile Ala Pro Tyr Cys Thr
                245                 250                 255

Ile Ala Pro Phe Gly Ile Phe Gly Thr Asn
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Gln Ser Phe Pro
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<400> 64

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Ser

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Pro

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Pro

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<400> 67

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Pro

<210> 68
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Gln Leu Gln Pro Phe Pro Gln Pro Glu Leu Ser Tyr Ser Gln Pro Gln
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Pro

<210> 69
<211> 17
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<400> 69

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1 5 10 15

Pro

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Ser

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<400> 71

Gln Leu Gln Pro Phe Leu Gln Pro Glu Leu Pro Tyr Ser Gln Pro Gln
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Pro

<210> 72
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<400> 72

Gln Pro Gln Pro Phe Pro Pro Pro Glu Leu Pro Tyr Pro Gln Thr Gln
1 5 10 15

Pro

<210> 73
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Pro Gln Pro Gln Pro Phe Pro Pro Glu Leu Pro Tyr Pro Gln Thr Gln
1 5 10 15

Pro

<210> 74
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<400> 74

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1 5 10 15

Pro

<210> 75
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<213> Homo sapiens;

<400> 75

Gln Leu Gln Pro Phe Pro Gln Pro Glu Leu Pro Tyr Ser Gln Pro Gln
1 5 10 15

Pro

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<213> Homo sapiens;

<400> 76

Gln Leu Gln Pro Phe Leu Gln Pro Glu Leu Pro Tyr Ser Gln Pro Gln
1 5 10 15

Pro

<210> 77
<211> 17
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<400> 77

Pro Gln Pro Gln Pro Phe Leu Pro Glu Leu Pro Tyr Pro Gln Pro Gln
1 5 10 15

Ser

<210> 78
<211> 17
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<400> 78

Pro Gln Pro Gln Pro Phe Pro Pro Glu Leu Pro Tyr Pro Gln Pro Pro
1 5 10 15

Pro

Claims

1. A peptide comprising the amino acid sequence PQPELPY (SEQ ID NO:1).

2. A fusion protein comprising (a) the amino acid sequence PQPELPY (SEQ ID NO:1) and (b) other gliadin or non-gliadin sequence.

3. A peptide according to claim 1, or a fusion protein according to claim 2, which is not more than 50 amino acids in length.

4. A peptide according to claim 3 which is 10 to 40 amino acids in length.

5. A peptide or fusion protein according to any preceding claim, comprising the amino acid sequence QLQPF-PQPELPYPQPQS (SEQ ID NO:2).

6. A fusion protein according to claim 2, wherein the gliadin sequence is from wheat, rye, barley, oats or triticale.

7. A fusion protein according to claim 2, wherein (b) is non-gliadin sequence.

8. A composition comprising two or more of the peptides or fusion proteins according to any of claims 1 to 7.

9. A pharmaceutical composition comprising a peptide or fusion protein according to any of claims 1 to 7 and a pharmaceutically acceptable carrier or diluent.

10. A pharmaceutical composition according to claim 9 comprising (a) at least one peptide according to claim 1 and (b) a gliadin epitope from at least one of wheat, rye, barley, oats and triticale.

11. A peptide or fusion protein as defined in any of claims 1 to 7, or a pharmaceutical composition as defined claim 9 or 10 for use in a method of treating or preventing coeliac disease.

12. A peptide, fusion protein or pharmaceutical composition as defined in claim 11, wherein said treatment or prevention of coeliac disease is by tolerisation.

13. A method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising:

    (a) contacting a sample from the host with a peptide or fusion protein of any of claims 1 to 7, and
    (b) determining in vitro whether T cells in the sample recognise the peptide or fusion protein; recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

14. Use of a peptide or fusion protein of any of claims 1 to 7 for the preparation of a diagnostic means for use in a method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual, said method comprising determining whether T cells of the individual recognise the peptide, recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

15. Use according to claim 14 wherein the method comprises administering the peptide or fusion protein to the skin of an individual and detecting the presence of inflammation at the site of administration, the detection of inflammation indicating that the T cells of the individual recognise the peptide or fusion protein.

16. A method according to claim 13 or a use according to claim 14, wherein the sample is a blood sample.

17. A method according to claim 13 or 16 or use according to claim 14 wherein the T cells are not re-stimulated in

antigen specific manner *in vitro* before the said determining.

18. A method or use according to any of claims 13 to 17 in which the recognition of the peptide or fusion protein by the T cells is determined by detecting the secretion of a cytokine from the T cells.

19. A method or use according to claim 18 in which the cytokine is IFN-γ.

20. A method or use according to claim 18 or 19 in which the cytokine is detected by allowing the cytokine to bind to an immobilised antibody specific to the cytokine and then detecting the presence of the antibody/cytokine complex.

21. A method or use according to any one of claims 13 to 17 wherein said determining is done by measuring whether the peptide or fusion protein binds the T cell receptor.

22. A method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising determining the presence of an antibody that binds to the amino acid sequence PQPELPY (SEQ ID NO:1) in a sample from the individual, the presence of the antibody indicating that the individual has, or is susceptible to, coeliac disease.

23. A method of determining whether a composition is capable of causing coeliac disease comprising determining whether a sequence capable of being modified by a transglutaminase to a peptide as defined in claim 1 is present in the composition, the presence of the sequence indicating that the composition is capable of causing coeliac disease.

24. A method according to claim 23 wherein the said determining is done by contacting the composition with an antibody specific for the sequence which is capable of being modified to the peptide sequence, binding of the antibody to a protein in the composition indicating the composition is capable of causing coeliac disease.

25. A kit for carrying out a method or use according to any one of claims 13 to 21 comprising a peptide or fusion protein of any of claims 1 to 7 and a means to detect the recognition of the peptide or fusion protein by the T cell.

26. A kit according to claim 25 wherein the means to detect recognition comprises an antibody to IFN-γ.

27. A kit according to claim 26 wherein the antibody is immobilised on a solid support and optionally the kit also comprises a means to detect the antibody/IFN-γ complex.

28. Use of a peptide or fusion protein as defined in any of claims 1 to 7 to produce an antibody specific to the peptide.

29. A polynucleotide that comprises a coding sequence that encodes a peptide or fusion protein as defined in any of claims 1 to 7.

30. A polynucleotide according to claim 29, wherein sequence 5' and/or 3' to the coding sequence comprises sequences which aid expression of the sequence encoding the peptide or fusion protein.

31. A polynucleotide according to claim 30, wherein the polynucleotide is capable of expressing the peptide or fusion protein in a prokaryotic or eukaryotic cell.

32. A polynucleotide according to claim 31, wherein the eukaryotic cell is a mammalian cell.

33. An expression vector comprising a polynucleotide according to any one of claims 29 to 32, wherein the polynucleotide is operably linked to a control sequence which is capable of providing for the expression of the polynucleotide.

34. A cell comprising a polynucleotide or expression vector as defined in any one of claims 29 to 33 or which has been transformed with such a polynucleotide or expression vector.

35. A cell according to claim 34 which is a prokaryotic cell or a mammalian cell.

36. A protein comprising: (a) a mutant gliadin protein with at least one mutation in the epitope ^{62}PQPQLPY^{68}, wherein the mutation decreases the ability of the epitope to induce a T cell response; or (b) a fragment of the mutant protein of (a), which fragment is at least 15 amino acids long and comprises the mutated PQPQLPY sequence.

37. A protein according to claim 36, wherein the mutation is at position 65 in A-gliadin, or in an equivalent positions in other gliadins.

38. A protein according to claim 37, wherein the glutamine residue at position 65 is substituted to a histidine, tyrosine, tryptophan, lysine, proline or arginine residue.

39. A polynucleotide that comprises a coding sequence that encodes a protein as defined in any of claims 36 to 38.

40. A polynucleotide according to claim 39 that additionally comprises one or more regulatory sequences operably linked to the coding sequence, which regulatory sequences are capable of securing the expression of the coding sequence in a cell.

41. A polynucleotide according to claims 39 or 40 which is a vector or which is in the form of a vector.

42. A cell comprising a polynucleotide as defined in any one of claims 39 to 41 or which has been transformed with such a polynucleotide.

43. A cell according to claim 42 which is a cell of a graminaceous monocotyledonous species.

44. A cell according to claim 43 which is a cell of wheat, maize, oats, rye, rice, barley, triticale, sorghum, or sugar cane.

45. A process for the production of a protein encoded by a coding sequence as defined in claim 39 which process comprises:

(a) cultivating a cell according to any one of claims 42 to 44 under conditions that allow the expression of the protein; and optionally
(b) recovering the expressed protein.

46. A method of obtaining a transgenic plant cell comprising: (a) transforming a plant cell with a vector according to claim 41 to give a transgenic plant cell.

47. A transgenic plant cell obtainable by a method according to claim 46.

48. A transgenic plant or plant seed comprising plant cells according to any one of claims 42 to 44.

49. A transgenic plant cell callus comprising plant cells according to claim 43 or 44 obtainable from a transgenic plant cell as defined in claim 43, 44 or 47.

50. A plant or callus according to claim 48 or 49 which is of a species as defined in claim 43 or 44.

51. A method of obtaining a crop product comprising harvesting a crop product from a plant according to claim 48 or 50 and optionally further processing the harvested product.

52. A method according to claim 51 wherein the plant is a wheat plant and the harvested crop product is grain; optionally further processed into flour or another grain product.

53. A crop product obtainable by a method according to claim 51 or 52.

54. A food that comprises a protein as defined in any of claims 36 to 38.

55. A food according to claim 54, in which a protein as defined in any of claims 36 to 38 is used instead of wild-type gliadin.

Patentansprüche

1. Peptid, umfassend die Aminosäuresequenz PQPELPY (SEQ ID NO: 1).

2. Protein, umfassend (a) die Aminosäuresequenz PQPELPY (SEQ ID NO: 1) und (b) andere Gliadin oder nicht-Gliad-

insequenz.

3. Peptid nach Anspruch 1, oder ein Fusionsprotein nach Anspruch 2, das nicht länger als 50 Aminosäuren ist.

4. Peptid nach Anspruch 3, das 10-40 Aminosäuren lang ist.

5. Peptid oder Fusionsprotein nach einem der vorstehenden Ansprüche, umfassend die Aminosäuresequenz QLQPF-PQPELPYPQPQS (SEQ ID NO: 2).

6. Fusionsprotein nach Anspruch 2, wobei die Gliadinsequenz von Weizen, Roggen, Gerste, Hafer oder Tritikale ist.

7. Fusionsprotein nach Anspruch 2, wobei (b) eine nicht-Gliadinsequenz ist.

8. Zusammensetzung, umfassend zwei oder mehr der Peptide oder Fusionsproteine nach einem der Ansprüche 1 bis 7.

9. Pharmazeutische Zusammensetzung, umfassend ein Peptid oder Fusionsprotein nach einem der Ansprüche 1 bis 7 und einen pharmazeutisch akzeptablen Träger oder Verdünnungsmittel.

10. Pharmazeutische Zusammensetzung nach Anspruch 9, umfassend (a) mindestens ein Peptid nach Anspruch 1, und (b) ein Gliadin-Epitop von mindestens einem von Weizen, Roggen, Gerste, Hafer und Tritikale.

11. Peptid oder Fusionsprotein wie in einem der Ansprüche 1 bis 7 definiert oder eine pharmazeutische Zusammensetzung wie in Anspruch 9 oder 10 definiert, zur Verwendung in einem Verfahren zur Behandlung oder Vorbeugung von Erkrankungen der Bauchhöhle.

12. Peptid, Fusionsprotein oder pharmazeutische Zusammensetzung wie in Anspruch 11 definiert, wobei die Behandlung oder Vorbeugung von Erkrankungen der Bauchhöhle durch Tolerierung erfolgt.

13. Verfahren zur Diagnose von Bauchhöhlenerkrankungen oder der Empfindlichkeit gegenüber Bauchhöhlenerkrankungen in einem Individuum, umfassend:

(a) in Kontakt bringen einer Probe von dem Wirt mit einem Peptid oder Fusionsprotein nach einem der Ansprüche 1 bis 7, und
(b) Bestimmen *in vitro,* ob T-Zellen in der Probe das Peptid oder Fusionsprotein erkennen; wobei die Erkennung durch die T-Zellen anzeigt, daß das Individuum eine Bauchhöhlenerkrankung aufweist oder dieser gegenüber empfindlich ist.

14. Verwendung eines Peptids oder Fusionsproteins nach einem der Ansprüche 1 bis 7 zur Herstellung eines diagnostischen Mittels zur Verwendung in einem Verfahren der Diagnose von Bauchhöhlenerkrankungen oder Empfindlichkeit gegenüber Bauchhöhlenerkrankungen in einem Individuum, wobei das Verfahren ein Bestimmen umfaßt, ob T-Zellen des Individuums das Peptid erkennen, wobei die Erkennung durch die T-Zellen anzeigt, daß das Individuum eine Bauchhöhlenerkrankung aufweist oder ihr gegenüber empfindlich ist.

15. Verwendung nach Anspruch 14, wobei das Verfahren eine Verabreichung des Peptids oder Fusionsproteins auf die Haut eines Individuums und Nachweisen der Anwesenheit von Entzündung an der Stelle der Verabreichung umfaßt, wobei der Nachweis von Entzündung anzeigt, daß die T-Zellen des Individuums das Peptid oder Fusionsprotein erkennen.

16. Verfahren nach Anspruch 13 oder eine Verwendung nach Anspruch 14, wobei die Probe eine Blutprobe ist.

17. Verfahren nach Anspruch 13 oder 16 oder die Verwendung nach Anspruch 14, wobei die T-Zellen vor der Bestimmung nicht *in vitro* auf Antigen spezifische Beweise re-stimuliert werden.

18. Verfahren oder Verwendung nach einem der Ansprüche 13-17, wobei die Erkennung des Peptids oder Fusionsproteins durch die T-Zellen durch die Sekretion eines Zytokins aus den T-Zellen nachgewiesen wird.

19. Verwendung nach Anspruch 18, wobei das Zytokin IFN-γ ist.

20. Verfahren oder Verwendung nach Anspruch 18 oder 19, wobei das Zytokin durch Ermöglichen des Zytokins, an einen immobilisierten Antikörper zu binden, der für das Zytokin spezifisch ist, und dann Nachweisen der Anwesenheit des Antikörper-/Zytokinkomplexes nachgewiesen wird.

21. Verfahren oder Verwendung nach einem der Ansprüche 13-17, wobei die Bestimmung durch Messen durchgeführt wird, ob das Peptid oder Fusionsprotein den T-Zell Rezeptor bindet.

22. Verfahren zur Diagnose von Bauchhöhlenerkrankung oder einer Empfindlichkeit gegenüber dieser Bauchhöhlenerkrankung in einem Individuum, umfassend Bestimmen der Anwesenheit des Antikörpers, der an die Aminosäuresequenz PQPELPY (SEQ ID NO: 1) in der Probe eines Individuums bindet, wobei die Anwesenheit des Antikörpers anzeigt, daß das Individuum eine Bauchhöhlenerkrankung aufweist oder dieser gegenüber empfindlich ist.

23. Verfahren zur Bestimmung, ob eine Zusammensetzung in der Lage ist, eine Bauchhöhlenerkrankung zu verursachen, umfassend Bestimmen, ob eine Sequenz in der Zusammensetzung vorhanden ist, die in der Lage ist, durch eine Transglutaminase in ein Peptid wie in Anspruch 1 definiert modifiziert zu werden, wobei die Anwesenheit der Sequenz anzeigt, daß die Zusammensetzung in der Lage ist, eine Bauchhöhlenerkrankung zu verursachen.

24. Verfahren nach Anspruch 23, wobei das Bestimmen durch in Kontakt bringen der Zusammensetzung mit einem Antikörper durchgeführt wird, der für Sequenz spezifisch ist, die in der Lage ist, zu der Peptidsequenz modifiziert zu werden, wobei eine Bindung des Antikörpers an ein Protein in der Zusammensetzung anzeigt, daß die Zusammensetzung in der Lage ist, eine Bauchhöhlenerkrankung zu verursachen.

25. Kit zur Durchführung eines Verfahrens oder der Verwendung nach einem der Ansprüche 13-21, umfassend ein Peptid oder Fusionsprotein nach einem der Ansprüche 1-25 und ein Mittel zum Nachweis der Erkennung des Peptids oder Fusionsproteins durch die T-Zelle.

26. Kit nach Anspruch 25, wobei das Mittel zum Nachweisen der Erkennung einen Antikörper gegen IFN-γ umfaßt.

27. Kit nach Anspruch 26, wobei der Antikörper auf einem festen Träger immobilisiert ist und gegebenenfalls das Kit auch ein Mittel umfaßt, um den Antikörper-/IFN-γ- Komplex nachzuweisen.

28. Verwendung eines Peptids oder Fusionsproteins wie in einem der Ansprüche 1 bis 7 definiert, um einen gegen das Peptid spezifischen Antikörper herzustellen.

29. Polynukleotid, das eine kodierende Sequenz umfaßt, die ein Peptid oder Fusionsprotein wie in einem der Ansprüche 1 bis 7 definiert kodiert.

30. Polynukleotid nach Anspruch 29. wobei die Sequenz 5' und/oder 3' zu der kodierenden Sequenz Sequenzen umfaßt, die bei der Expression der Sequenz helfen, die für das Peptid oder Fusionsprotein kodieren.

31. Polynukleotid nach Anspruch 30, wobei das Polynukleotid in der Lage ist, das Peptid oder Fusionsprotein in einer prokaryontischen oder eukaryontischen Zelle zu exprimieren.

32. Polynukleotid nach Anspruch 31, wobei die eukaryontische Zelle eine Säugerzelle ist.

33. Expressionsvektor, umfassend ein Polynukleotid nach einem der Ansprüche 29 bis 32, wobei das Polyonukleotid operativ mit einer Kontrollsequenz verbunden ist, die in der Lage ist, die Expression des Polynukleotids zur Verfügung zu stellen.

34. Zelle, umfassend ein Polynukleotid oder Expressionsvektor, wie in einem der Ansprüche 29 bis 33 definiert oder die mit solch einem Polynukleotid oder Expressionsvektor transformiert wurde.

35. Zelle nach Anspruch 34, die eine prokaryontische Zelle oder eine Säugerzelle ist.

36. Protein, umfassend: (a) ein Mutanten-Gliadinprotein mit mindestens einer Mutation in dem Epitop ^{62}PQPQLPY^{68}, wobei die Mutation die Fähigkeit des Epitops verringert, eine T-Zell Antwort zu induzieren; oder (b) ein Fragment des Mutantenproteins von (a), wobei das Fragment mindestens 15 Aminosäuren lang ist und die mutierte PQPQLPY Sequenz umfaßt.

37. Protein nach Anspruch 36, wobei die Mutation an Position 65 in A-Gliadin vorliegt oder in einer äquivalenten Position in anderen Gliadinen.

38. Protein nach Anspruch 37, wobei der Glutaminrest an Position 65 durch einen Histidin-, Tyrosin-, Tryptophan-, Lysin-, Prolin- oder Argininrest substituiert ist.

39. Polynukleotid, das eine kodierende Sequenz umfaßt, die für ein Protein wie in einem der Ansprüche 36 bis 38 definiert kodiert.

40. Polynukleotid nach Anspruch 39, das zusätzlich eine oder mehrere regulatorische Sequenzen umfaßt, die operativ mit der kodierenden Sequenz verbunden sind, wobei die regulatorischen Sequenzen in der Lage sind, die Expression der kodierenden Sequenz in einer Zelle sicher zu stellen.

41. Polynukleotid nach Anspruch 39 oder 40, das ein Vektor ist oder das in Form eines Vektors vorliegt.

42. Zelle, umfassend ein Polynukleotid wie definiert in einem der Ansprüche 39 bis 41 oder die mit solch einem Polynukleotid transformiert wurde.

43. Zelle nach Anspruch 42, die eine Zelle einer Graminaceen-monokotyledonen Spezies ist.

44. Zelle nach Anspruch 43, die eine Zelle von Weizen, Mais, Hafer, Roggen, Reis, Gerste, Tritikale, Sorghum oder Zuckerrohr ist.

45. Verfahren zur Herstellung eines Proteins, das durch eine kodierende Sequenz wie in Anspruch 39 definiert kodiert wird, wobei das Verfahren umfaßt:

(a) Kultivieren einer Zelle nach einem der Ansprüche 42 bis 44 unter Bedingungen, die die Expression des Proteins erlauben; und gegebenenfalls
(b) Aufreinigen des exprimierten Proteins.

46. Verfahren zum Erhalt einer transgenen Pflanzenzelle, umfassend: (a) Transformieren einer Pflanzenzelle mit einem Vektor nach Anspruch 41, um eine transgene Pflanzenzelle zu ergeben.

47. Transgene Pflanzenzelle, erhältlich durch ein Verfahren nach Anspruch 46.

48. Transgene Pflanze oder Pflanzensamen, umfassend Pflanzenzellen nach einem der Ansprüche 42 bis 44.

49. Transgener Pflanzenzellkallus, umfassend Pflanzenzellen nach Anspruch 43 oder 44, erhältlich von einer transgenen Pflanzenzelle, wie in Anspruch 43, 44 und 47 definiert.

50. Pflanze oder Kallus nach Anspruch 48 oder 49, die eine Spezies wie in Anspruch 43 oder 44 definiert ist.

51. Verfahren zum Inhalt eines Getreideprodukts, umfassend Ernten eines Getreideprodukts von einer Pflanze nach Anspruch 48 oder 50, und gegebenenfalls weitere Verarbeitung des geernteten Produkts.

52. Verfahren nach Anspruch 51, wobei die Pflanze eine Weizenpflanze ist und das geerntete Getreideprodukt Körner sind, gegebenenfalls weiterverarbeitet in Mehl oder ein anderes Körnerprodukt.

53. Getreideprodukt, erhältlich durch ein Verfahren nach einem der Ansprüche 51 oder 52.

54. Nahrungsmittel, das ein Protein wie in einem der Ansprüche 36 bis 38 definiert umfaßt.

55. Nahrungsmittel nach Anspruch 54, wobei ein Protein wie in einem der Ansprüche 36 bis 38 an Stelle eines Wildtyp Gliadins verwendet wird.

Revendications

1. Peptide comprenant la séquence d'acides aminés PQPELPY (SEQ ID NO:1).

2. Protéine de fusion comprenant (a) la séquence d'acides aminés PQPELPY (SEQ ID NO:1) et (b) une autre séquence de gliadine ou qui n'est pas de gliadine.

3. Peptide selon la revendication 1, ou protéine de fusion selon la revendication 2, qui ne dépasse pas 50 acides aminés de long.

4. Peptide selon la revendication 3, qui a une longueur de 10 à 40 acides aminés.

5. Peptide ou protéine de fusion selon l'une quelconque des revendications précédentes, comprenant la séquence d'acides aminés QLQPFPQPELPYPQPQS (SEQ ID NO:2).

6. Protéine de fusion selon la revendication 2, dans laquelle la séquence de gliadine provient de blé, de seigle, d'orge, d'avoine ou de triticale.

7. Protéine de fusion selon la revendication 2, dans laquelle (b) est une séquence qui n'est pas de gliadine.

8. Composition comprenant au moins deux des peptides ou protéines de fusion selon l'une quelconque des revendications 1 à 7.

9. Composition pharmaceutique comprenant un peptide ou une protéine de fusion selon l'une quelconque des revendications 1 à 7 et un véhicule ou diluant pharmaceutiquement acceptable.

10. Composition pharmaceutique selon la revendication 9, comprenant (a) au moins un peptide selon la revendication 1, et (b) un épitope de gliadine provenant de l'un au moins parmi le blé, le seigle, l'orge, l'avoine et le triticale.

11. Peptide ou protéine de fusion tel que défini dans l'une quelconque des revendications 1 à 7, ou composition pharmaceutique telle que définie dans la revendication 9 ou 10, à utiliser dans un procédé de traitement ou de prévention de la maladie coeliaque.

12. Peptide, protéine de fusion ou composition pharmaceutique tel que défini dans la revendication 11, où ledit traitement ou ladite prévention de la maladie coeliaque se fait par tolérisation.

13. Procédé de diagnostic de la maladie coeliaque ou d'une prédisposition à la maladie coeliaque chez un individu, comprenant:

    (a) la mise en contact d'un échantillon provenant de l'hôte avec un peptide ou une protéine de fusion selon l'une quelconque des revendications 1 à 7, et
    (b) la détermination in vitro pour savoir si les cellules T dans l'échantillon reconnaissent le peptide ou la protéine de fusion; la reconnaissance par les cellules T indiquant que l'individu souffre de la maladie coeliaque ou a une prédisposition à cette maladie.

14. Utilisation d'un peptide ou d'une protéine de fusion selon l'une quelconque des revendications 1 à 7 pour la préparation d'un moyen de diagnostic à utiliser dans un procédé de diagnostic de la maladie coeliaque ou d'une prédisposition à la maladie coeliaque chez un individu, ledit procédé comprenant l'étape qui consiste à déterminer si les cellules T de l'individu reconnaissent le peptide, la reconnaissance par les cellules T indiquant que l'individu souffre de la maladie coeliaque ou a une prédisposition à cette maladie.

15. Utilisation selon la revendication 14, dans laquelle le procédé comprend l'administration du peptide ou de la protéine de fusion à la peau d'un individu et la détection de la présence d'inflammation au niveau du site d'administration, la détection d'une inflammation indiquant que les cellules T de l'individu reconnaissent le peptide ou la protéine de fusion.

16. Procédé selon la revendication 13 ou utilisation selon la revendication 14, dans lequel l'échantillon est un échantillon de sang.

17. Procédé selon la revendication 13 ou 16 ou utilisation selon la revendication 14, dans lequel les cellules T ne sont pas restimulées de manière spécifique d'antigène in vitro avant ladite détermination.

18. Procédé ou utilisation selon l'une quelconque des revendications 13 à 17, dans lequel la reconnaissance du peptide ou de la protéine de fusion par les cellules T est déterminée par la détection de la sécrétion d'une cytokine par les cellules T.

19. Procédé ou utilisation selon la revendication 18, dans lequel la cytokine est IFN-γ.

20. Procédé ou utilisation selon la revendication 18 ou 19, dans lequel la cytokine est détectée en la laissant se lier à un anticorps immobilisé, spécifique de la cytokine puis en détectant la présence du complexe anticorps/cytokine.

21. Procédé ou utilisation selon l'une quelconque des revendications 13 à 17, dans lequel ladite détermination s'effectue en mesurant pour savoir si le peptide ou la protéine de fusion se lie au récepteur des cellules T.

22. Procédé de diagnostic de la maladie coeliaque ou d'une prédisposition à la maladie coeliaque chez un individu, comprenant la détermination de la présence d'un anticorps qui se lie à la séquence d'acides aminés PQPELPY (SEQ ID NO:1) dans un échantillon provenant de l'individu, la présence de l'anticorps indiquant que l'individu souffre de la maladie coeliaque ou a une prédisposition à cette maladie.

23. Procédé pour déterminer si une composition est capable de causer une maladie coeliaque, comprenant la détermination pour savoir si une séquence susceptible d'être modifiée par une transglutaminase en un peptide tel que défini dans la revendication 1 est présente dans la composition, la présence de la séquence indiquant que la composition est capable de causer la maladie coeliaque.

24. Procédé selon la revendication 23, dans lequel ladite détermination s'effectue par mise en contact de la composition avec un anticorps spécifique de la séquence qui est susceptible d'être modifiée pour donner la séquence peptidique, la liaison de l'anticorps à une protéine dans la composition indiquant que la composition est susceptible de provoquer la maladie coeliaque.

25. Trousse pour mettre en oeuvre un procédé ou une utilisation selon l'une quelconque des revendications 13 à 21, comprenant un peptide ou une protéine de fusion selon l'une quelconque des revendications 1 à 7 et un moyen pour détecter la reconnaissance du peptide ou de la protéine de fusion par la cellule T.

26. Trousse selon la revendication 25, dans laquelle le moyen pour détecter la reconnaissance comprend un anticorps dirigé contre IFN-γ.

27. Trousse selon la revendication 26, dans laquelle l'anticorps est immobilisé sur un support solide et éventuellement, la trousse comprend aussi un moyen pour détecter le complexe anticorps/IFN-γ.

28. Utilisation d'un peptide ou d'une protéine de fusion tel que défini dans l'une quelconque des revendications 1 à 7 pour produire un anticorps spécifique du peptide.

29. Polynucléotide qui comprend une séquence codante qui code pour un peptide ou une protéine de fusion tel que défini dans l'une quelconque des revendications 1 à 7.

30. Polynucléotide selon la revendication 29, dans lequel la séquence en 5' et/ou en 3' de la séquence codante comprend des séquences qui aident à l'expression de la séquence codant pour le peptide ou la protéine de fusion.

31. Polynucléotide selon la revendication 30, ledit polynucléotide étant capable d'exprimer le peptide ou la protéine de fusion dans une cellule procaryote ou eucaryote.

32. Polynucléotide selon la revendication 31, où la cellule eucaryote est une cellule de mammifère.

33. Vecteur d'expression comprenant un polynucléotide selon l'une quelconque des revendications 29 à 32, dans lequel le polynucléotide est lié de manière opérationnelle à une séquence de contrôle qui est capable d'assurer l'expression du polynucléotide.

34. Cellule comprenant un polynucléotide ou un vecteur d'expression tel que défini dans l'une quelconque des revendications 29 à 33 ou qui a été transformée avec un tel polynucléotide ou vecteur d'expression.

35. Cellule selon la revendication 34 qui est une cellule procaryote ou une cellule de mammifères.

36. Protéine comprenant: (a) une protéine gliadine mutante ayant au moins une mutation dans l'épitope ^{62}PQPQLPY^{68}, dans laquelle la mutation réduit la capacité de l'épitope à induire une réponse des cellules T; ou (b) un fragment de la protéine mutante en (a), ledit fragment ayant au moins une longueur de 15 acides aminés et comprend la séquence PQPQLPY mutée.

37. Protéine selon la revendication 36, dans laquelle la mutation se trouve en position 65 dans la gliadine A, ou en une position équivalente dans d'autres gliadines.

38. Protéine selon la revendication 37, dans laquelle le résidu glutamine en position 65 est substitué à un résidu histidine, tyrosine, tryptophane, lysine, proline ou arginine.

39. Polynucléotide qui comprend une séquence codante qui code pour une protéine telle que définie dans l'une quelconque des revendications 36 à 38.

40. Polynucléotide selon la revendication 39, qui comprend en outre une ou plusieurs séquences régulatrices liées de manière opérationnelle à la séquence codante, lesdites séquences régulatrices étant capables d'assurer l'expression de la séquence codante dans une cellule.

41. Polynucléotide selon la revendication 39 ou 40, qui est un vecteur ou qui est sous forme d'un vecteur.

42. Cellule comprenant un polynucléotide tel que défini dans l'une quelconque des revendication 39 à 41 ou qui a été transformée avec un tel polynucléotide.

43. Cellule selon la revendication 42 qui est une cellule d'une espèce monocotylédone graminée.

44. Cellule selon la revendication 43 qui est une cellule de blé, de maïs, d'avoine, de seigle, de riz, d'orge, de triticale, de sorgho ou de canne à sucre.

45. Procédé pour la production d'une protéine codée par une séquence codante telle que définie dans la revendication 39, ledit procédé comprenant:

(a) la culture d'une cellule selon l'une quelconque des revendications 42 à 44 dans des conditions qui permettent l'expression de la protéine; et éventuellement
(b) la récupération de la protéine exprimée.

46. Procédé d'obtention d'une cellule de plantes transgéniques, comprenant: (a) la transformation d'une cellule végétale avec un vecteur selon la revendication 41 pour donner une cellule de plantes transgéniques.

47. Cellule de plantes transgéniques que l'on peut obtenir par un procédé selon la revendication 46.

48. Plante transgénique ou graine de plante transgénique comprenant les cellules de plantes selon l'une quelconque des revendications 42 à 44.

49. Cal cellulaire de plantes transgéniques comprenant les cellules de plantes selon la revendication 43 ou 44, que l'on peut obtenir à partir d'une cellule de plantes transgéniques telle que définie dans la revendication 43, 44 ou 47.

50. Plante ou cal selon la revendication 48 ou 49, qui est d'une espèce telle que définie dans la revendication 43 ou 44.

51. Procédé d'obtention d'un produit de culture, comprenant la récolte d'un produit de culture provenant d'une plante selon la revendication 48 ou 50 et éventuellement, la transformation ultérieure du produit récolté.

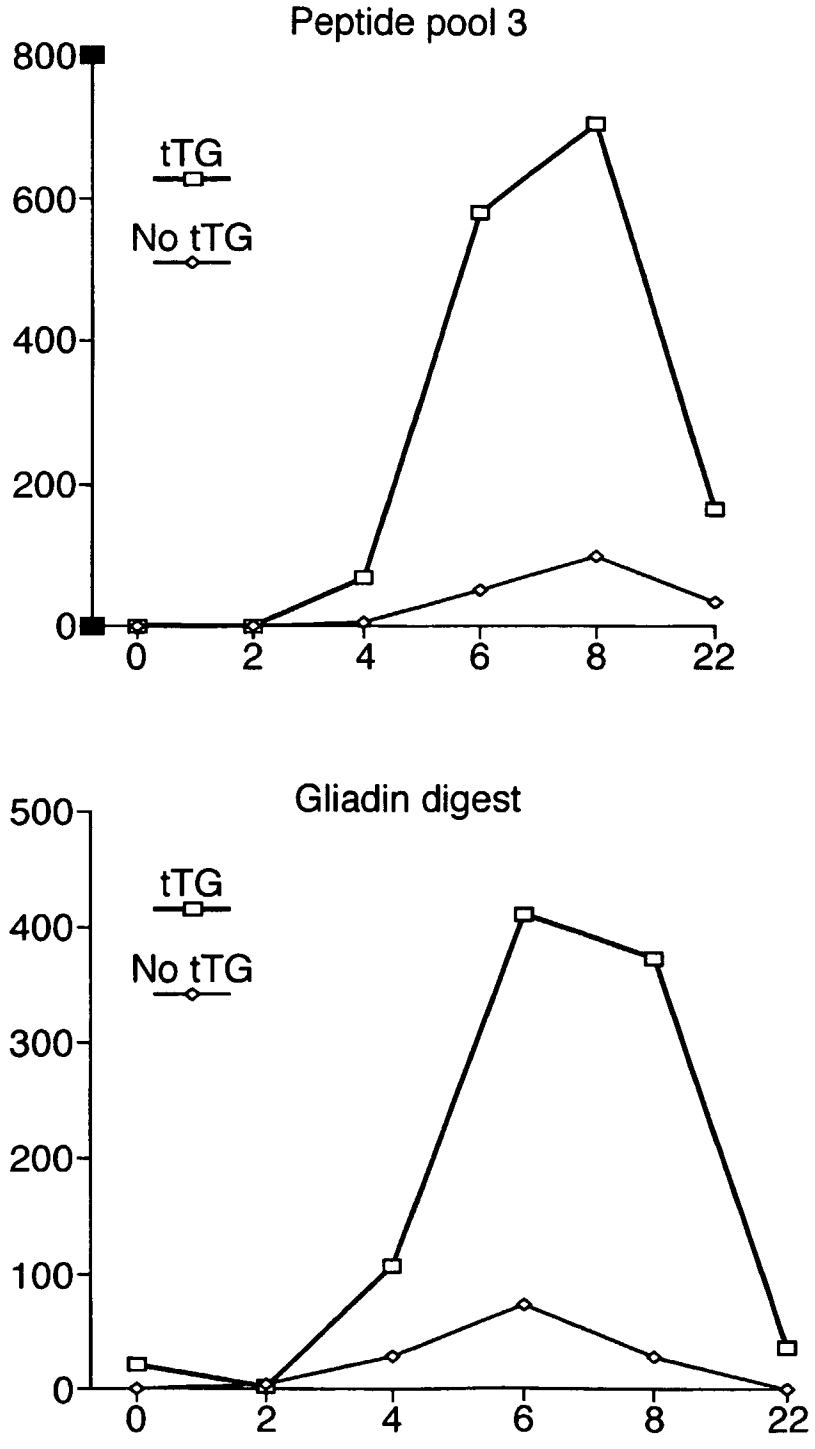
52. Procédé selon la revendication 51, dans lequel la plante est une plante de blé et le produit de culture récolté est un grain; éventuellement, transformé ultérieurement en farine ou un autre produit de grain.

53. Produit de culture que l'on peut obtenir par un procédé selon la revendication 51 ou 52.

54. Aliment qui comprend une protéine telle que définie dans l'une quelconque des revendications 36 à 38.

55. Aliment selon la revendication 54, dans lequel une protéine telle que définie dans l'une quelconque des revendications 36 à 38 est utilisée à la place de la gliadine de type sauvage.

Fig.1a.


Peptide pool 3
800
600
400
200
0
tTG
No tTG
0
2
4
6
8
22
Gliadin digest
500
400
300
200
100
0
tTG
No tTG
0
2
4
6
8
22

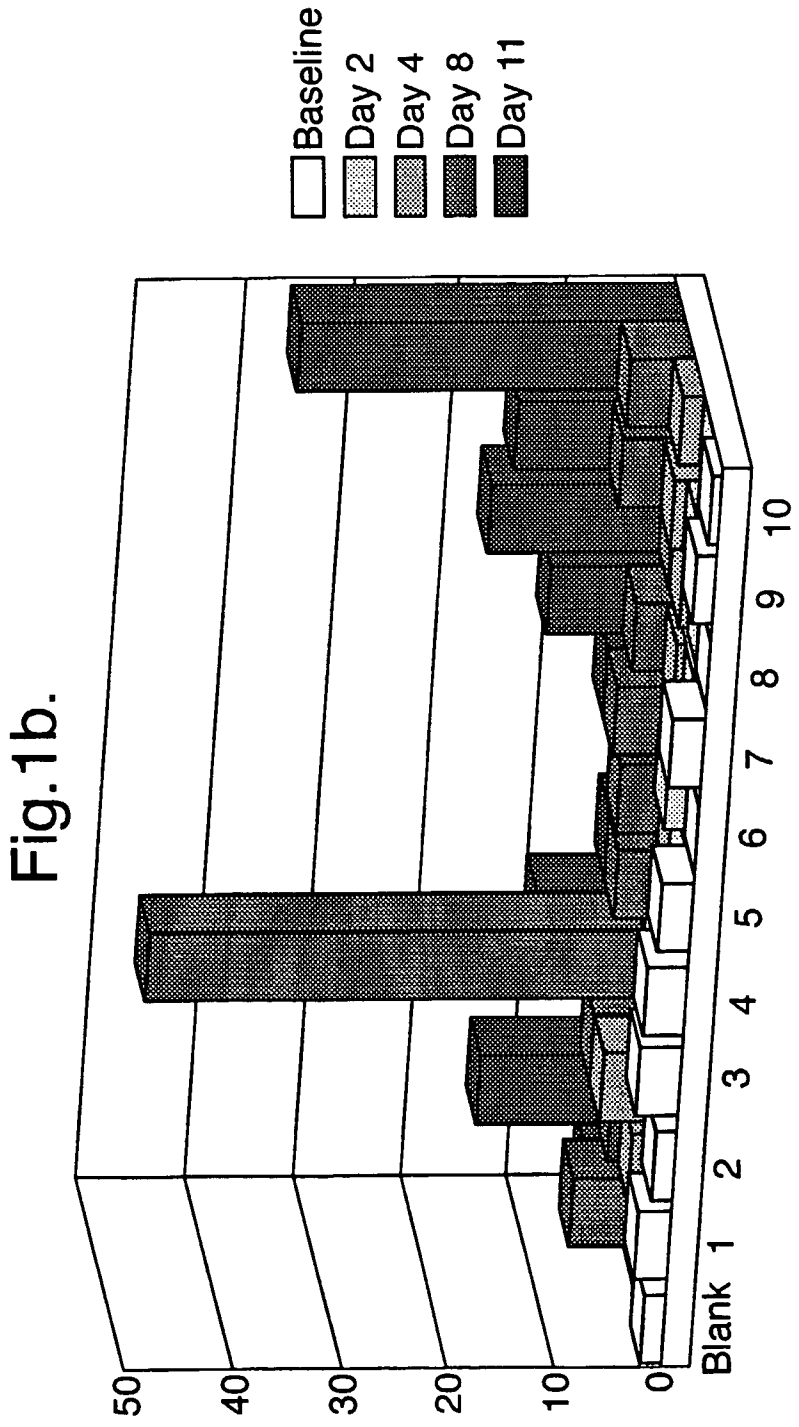

Baseline
Day 2
Day 4
Day 8
Day 11
50
40
30
20
10
0
Blank 1
2
3
4
5
6
7
8
9
10

Fig.1b.

Fig.2a.

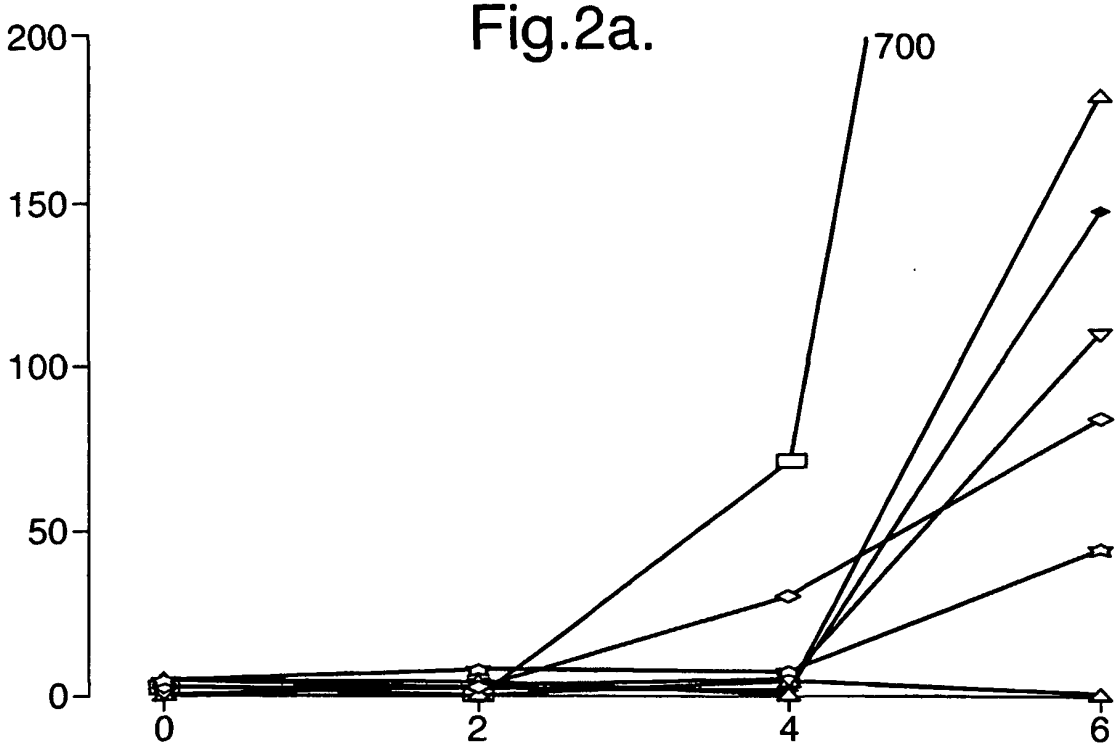


Fig.2b.

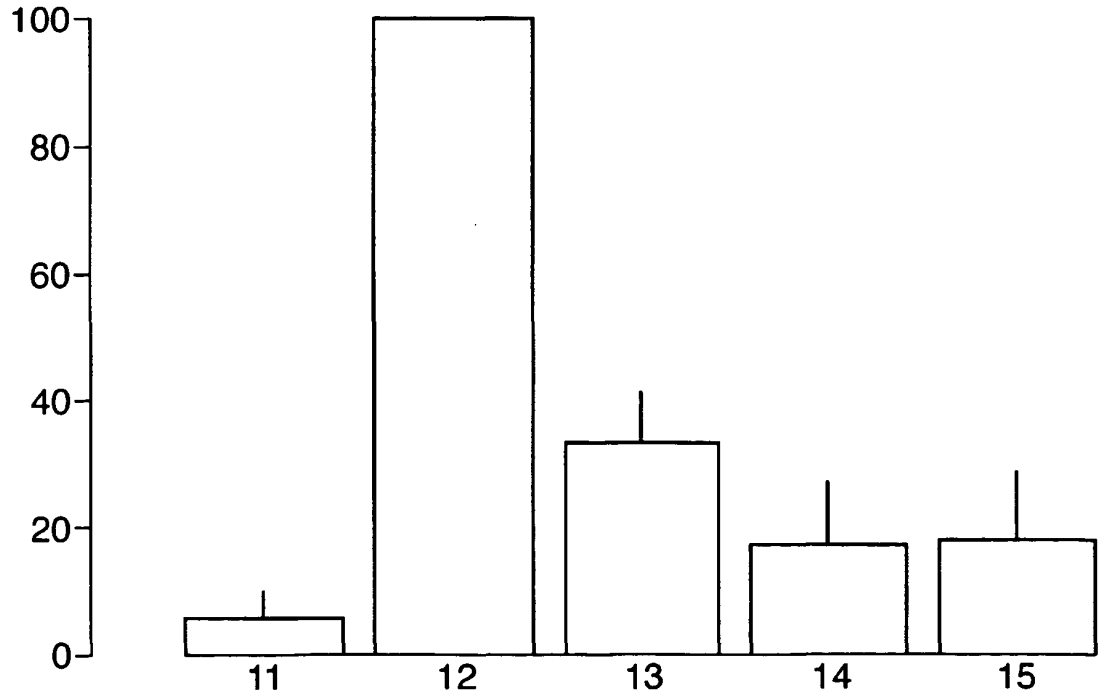


Fig.3.

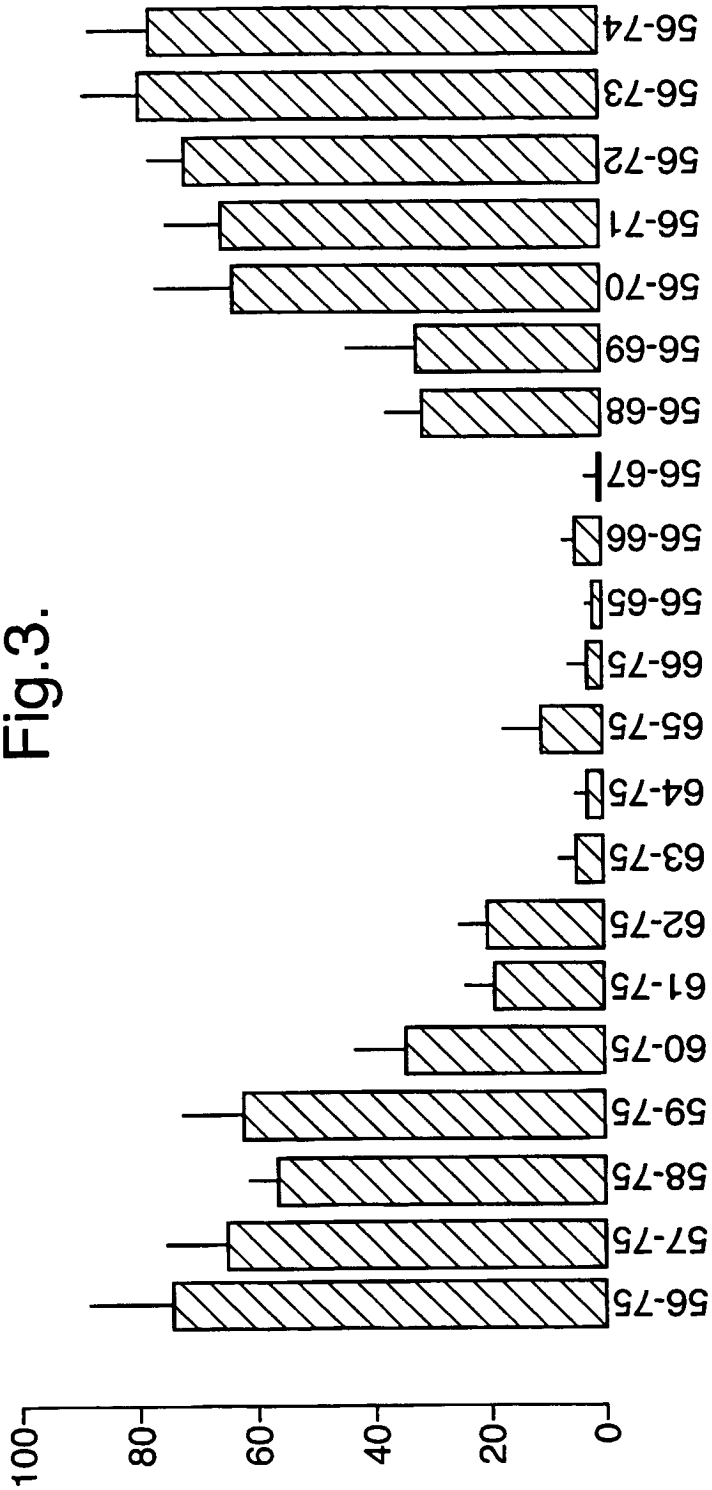

56-74
56-73
56-72
56-71
56-70
56-69
56-68
56-67
56-66
56-65
66-75
65-75
64-75
63-75
62-75
61-75
60-75
59-75
58-75
57-75
56-75
100
80
60
40
20
0

Fig.4a.

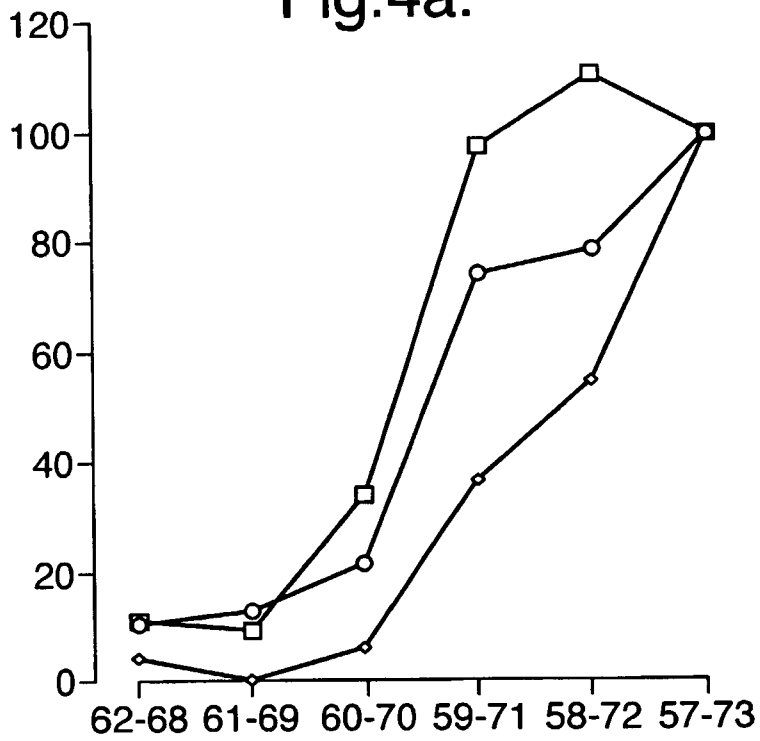

120
100
80
60
40
20
0
62-68 61-69 60-70 59-71 58-72 57-73

Fig.4b.

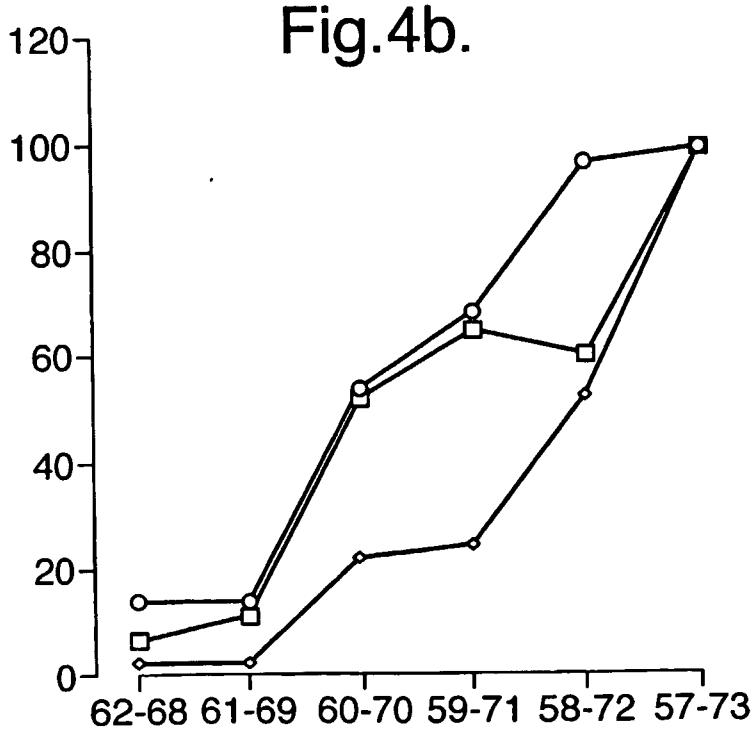

120
100
80
60
40
20
0
62-68 61-69 60-70 59-71 58-72 57-73

Fig.5.

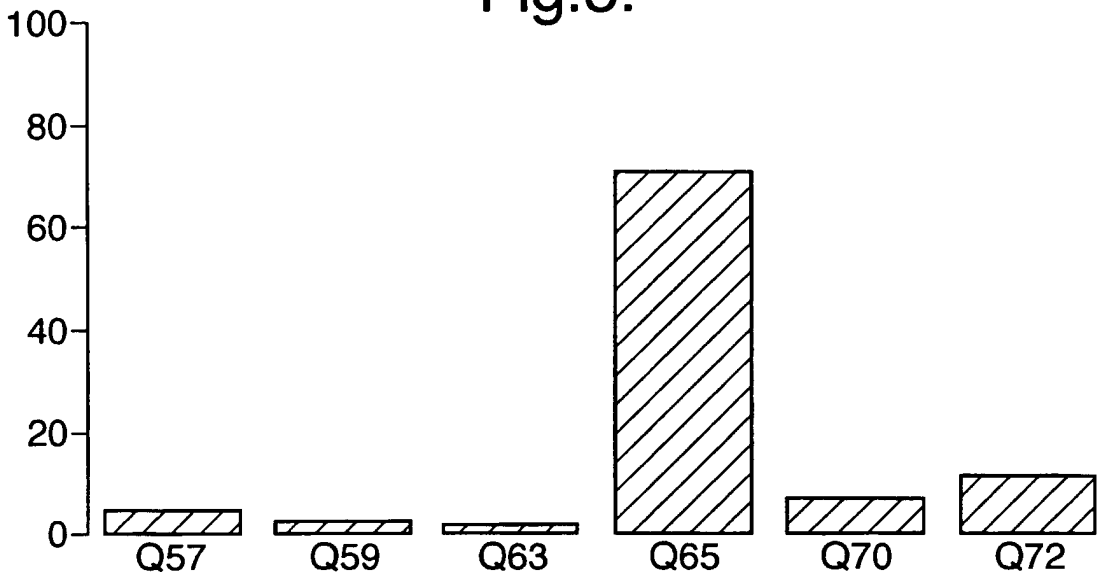

100
80
60
40
20
0
Q57
Q59
Q63
Q65
Q70
Q72

Fig.6.

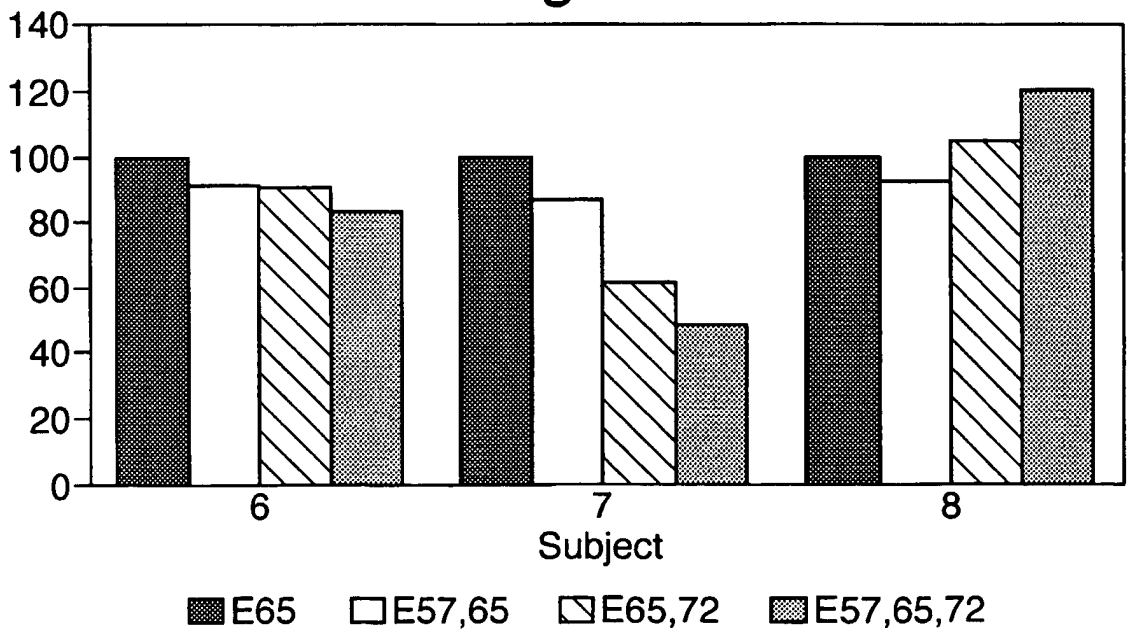

140
120
100
80
60
40
20
0
6
7
8
Subject
E65
E57,65
E65,72
E57,65,72

Fig.7a.

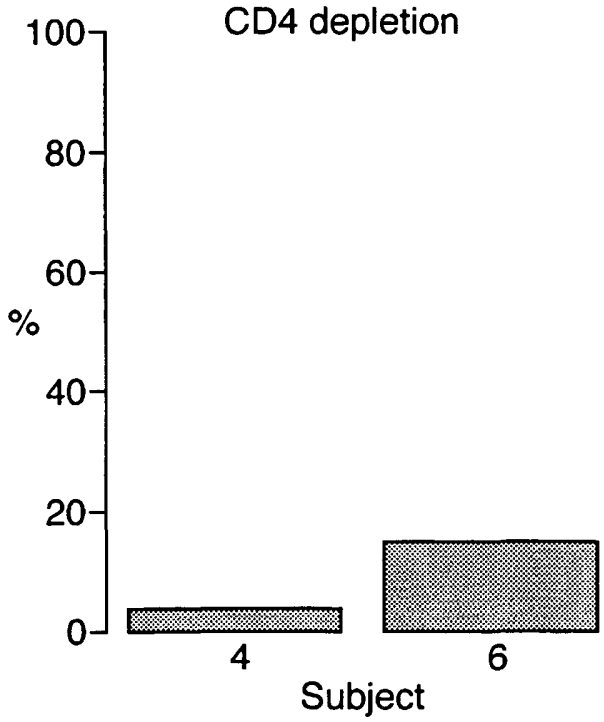

CD4 depletion
100
80
60
%
40
20
0
4
6
Subject

Fig.7b.

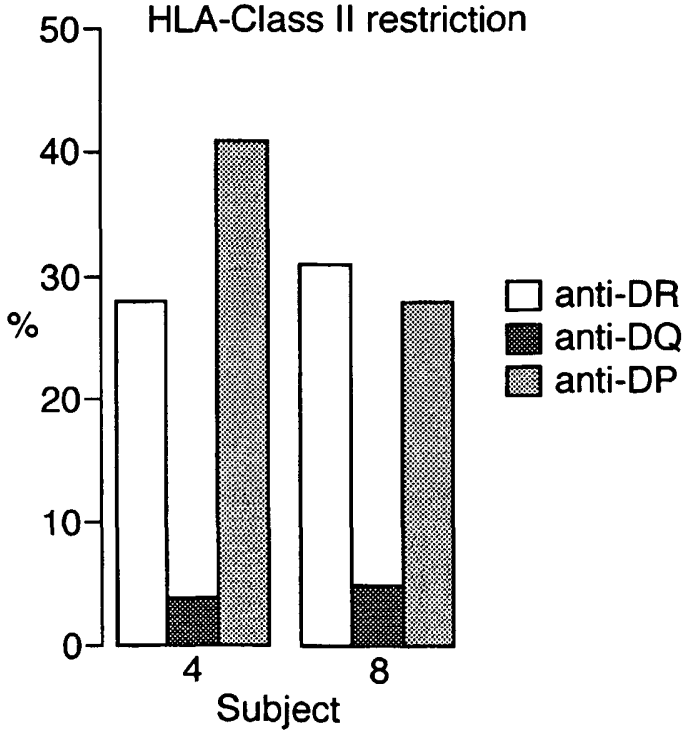

HLA-Class II restriction
50
40
30
%
20
10
0
4
8
Subject
anti-DR
anti-DQ
anti-DP

Fig.8.

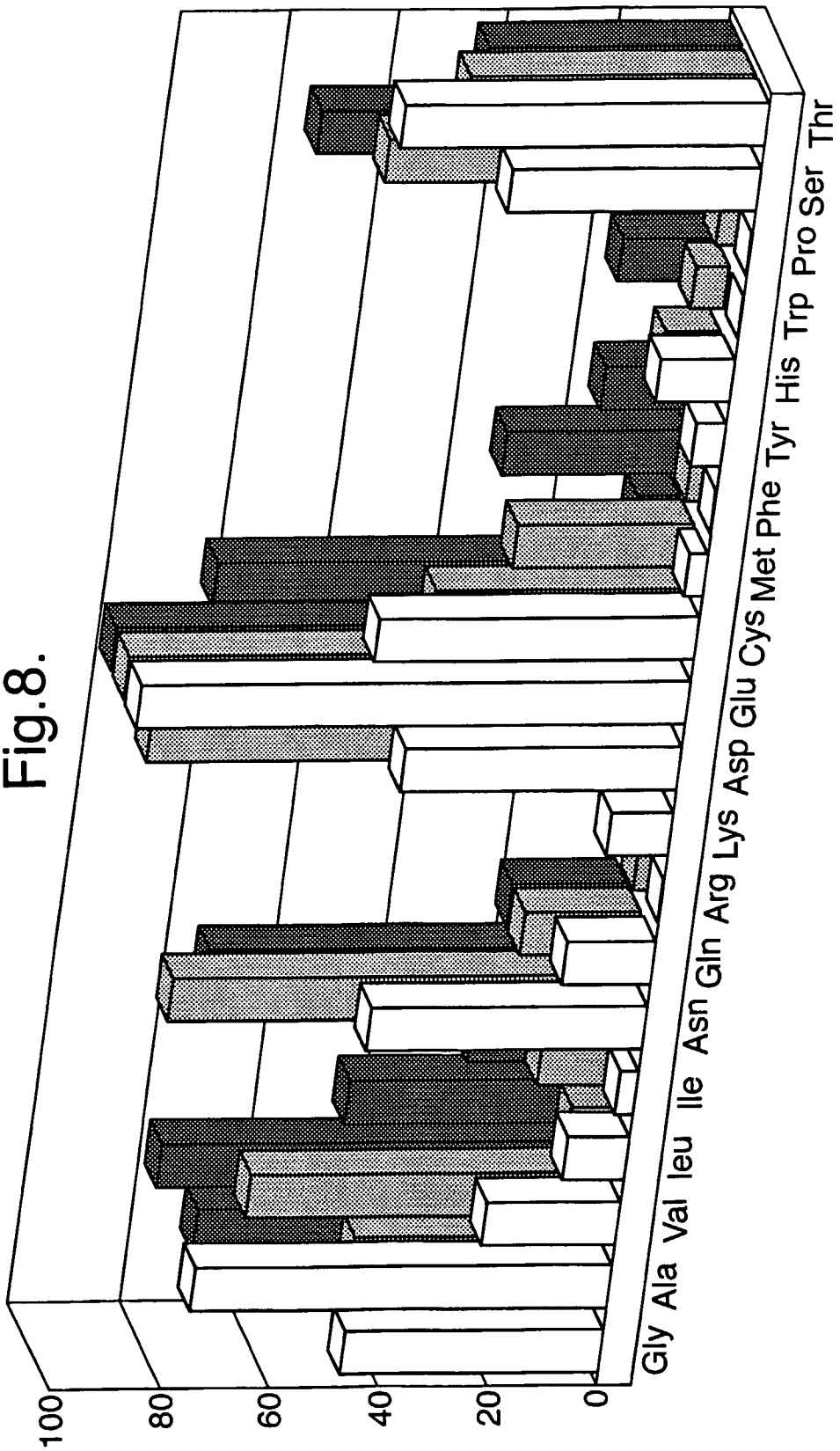

100
80
60
40
20
0
Gly Ala Val Ieu Ile Asn Gln Arg Lys Asp Glu Cys Met Phe Tyr His Trp Pro Ser Thr

Fig.9.

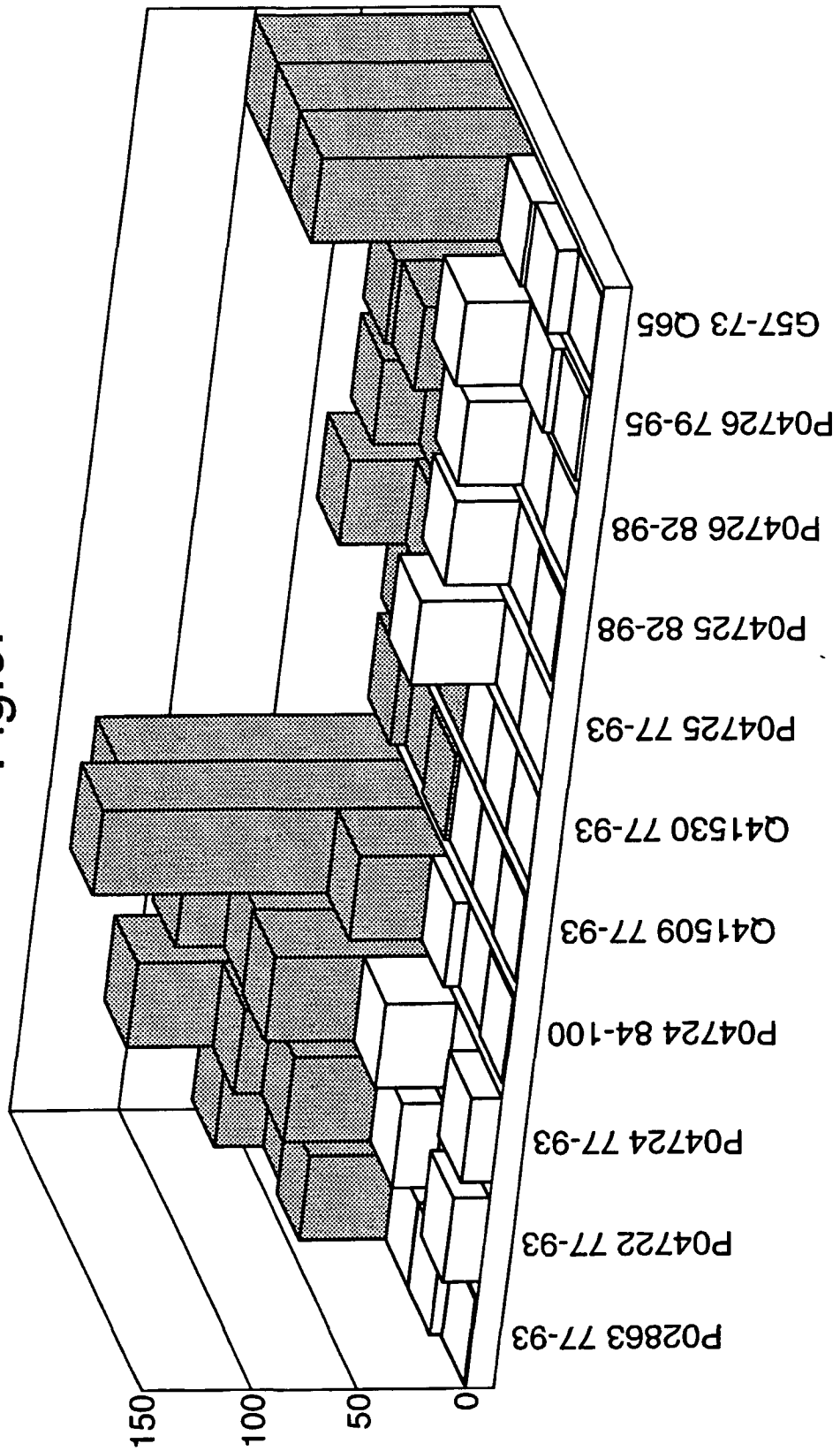
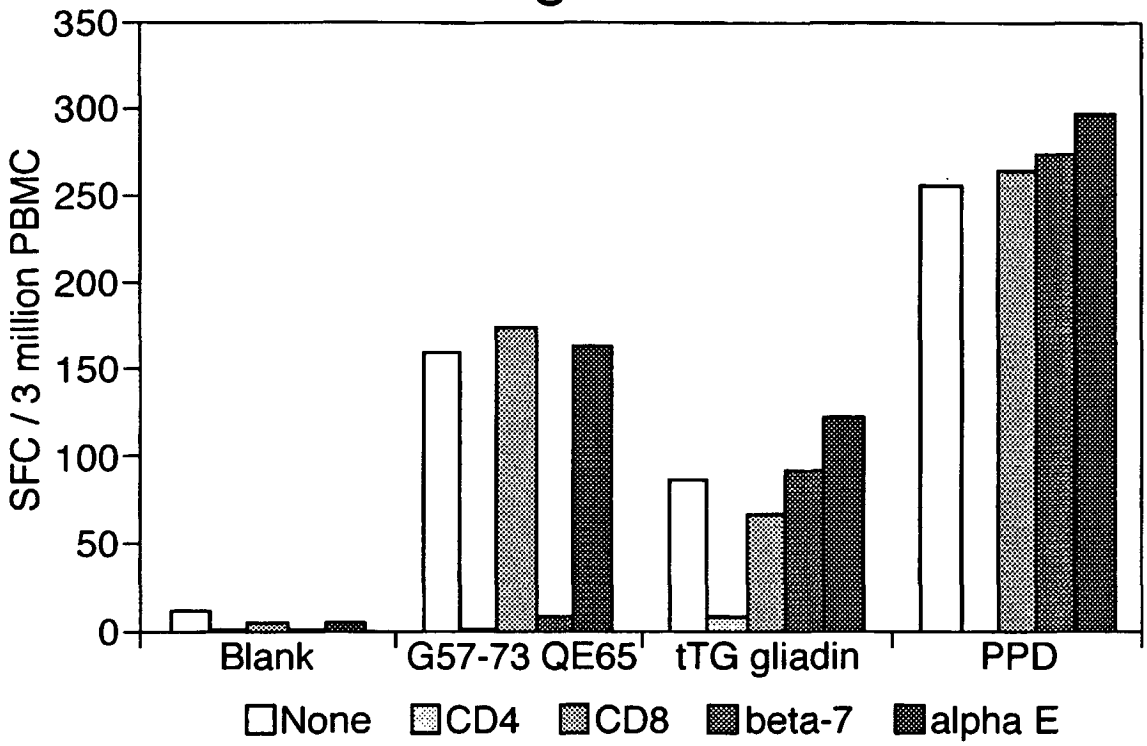

P02863 77-93
P04722 77-93
P04724 77-93
P04724 84-100
Q41509 77-93
Q41530 77-93
P04725 77-93
P04725 82-98
P04726 82-98
P04726 79-95
G57-73 Q65
150
100
50
0

Fig.10.


350
300
250
200
150
100
50
0
SFC / 3 million PBMC
Blank
G57-73 QE65
tTG gliadin
PPD
None
CD4
CD8
beta-7
alpha E

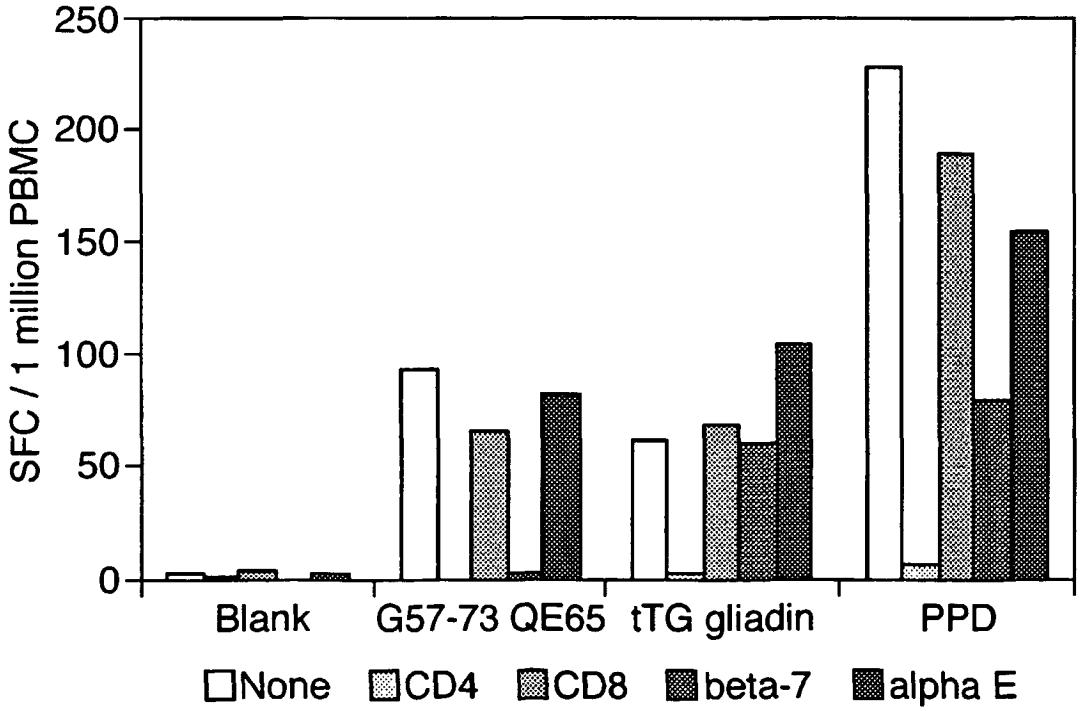

250
200
150
100
50
0
SFC / 1 million PBMC
Blank
G57-73 QE65
tTG gliadin
PPD
None
CD4
CD8
beta-7
alpha E

Fig.11.

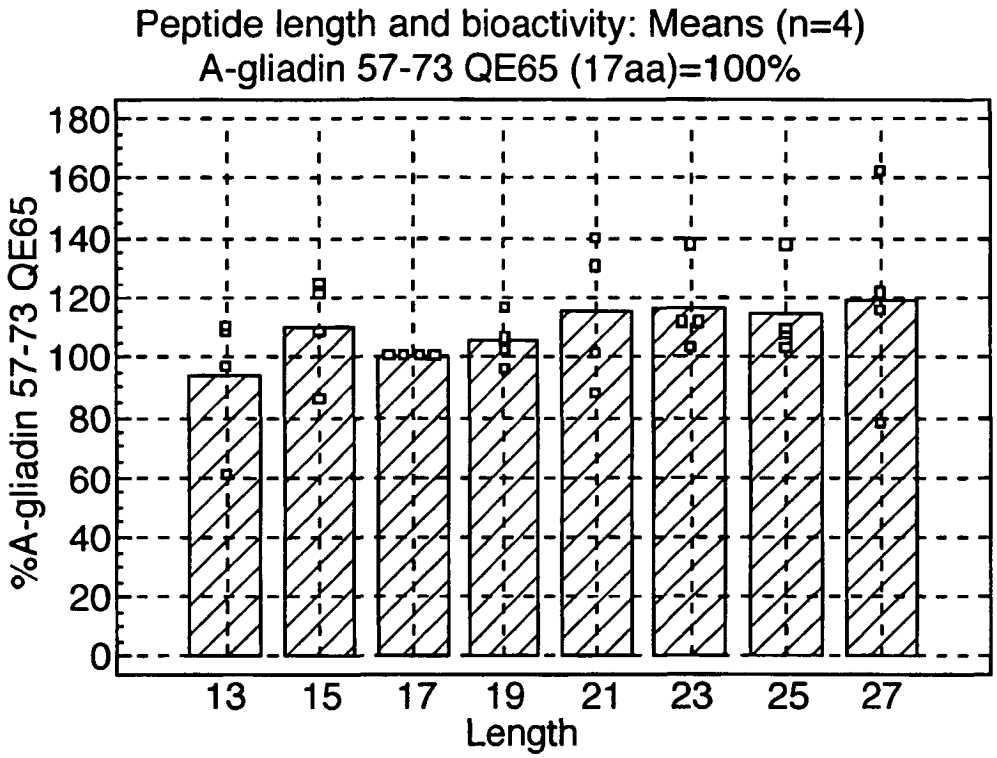

Peptide length and bioactivity: Means (n=4)
A-gliadin 57-73 QE65 (17aa)=100%
%A-gliadin 57-73 QE65
180
160
140
120
100
80
60
40
20
0
13
15
17
19
21
23
25
27
Length

Fig.12a.

Dose response to A-gliadin 57-73 QE65:
QLQPFPQPELPYPQPQS.

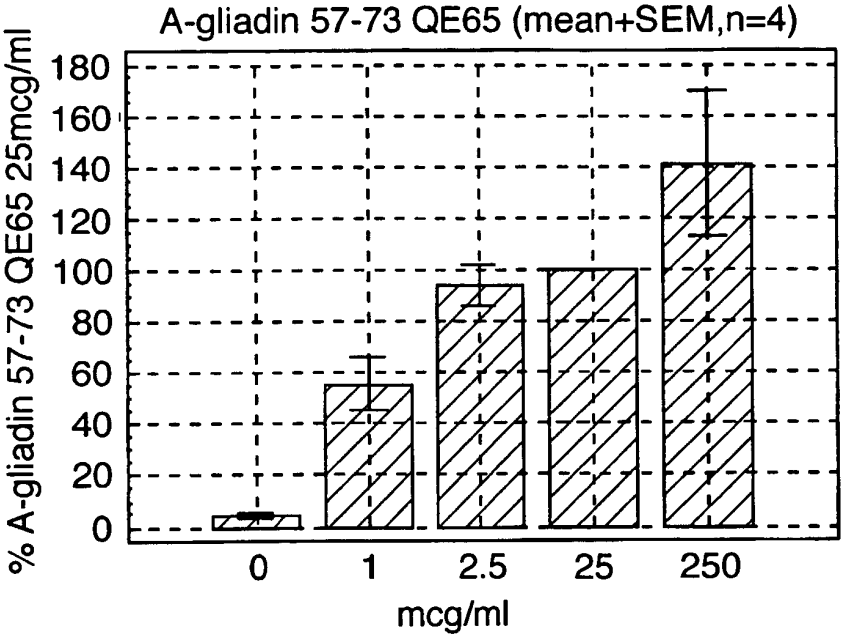


Fig.12b.

Dose response to GDA4_WHEAT P04724 84-100 QE92:
PQLPYPQPELPYPQPQP.

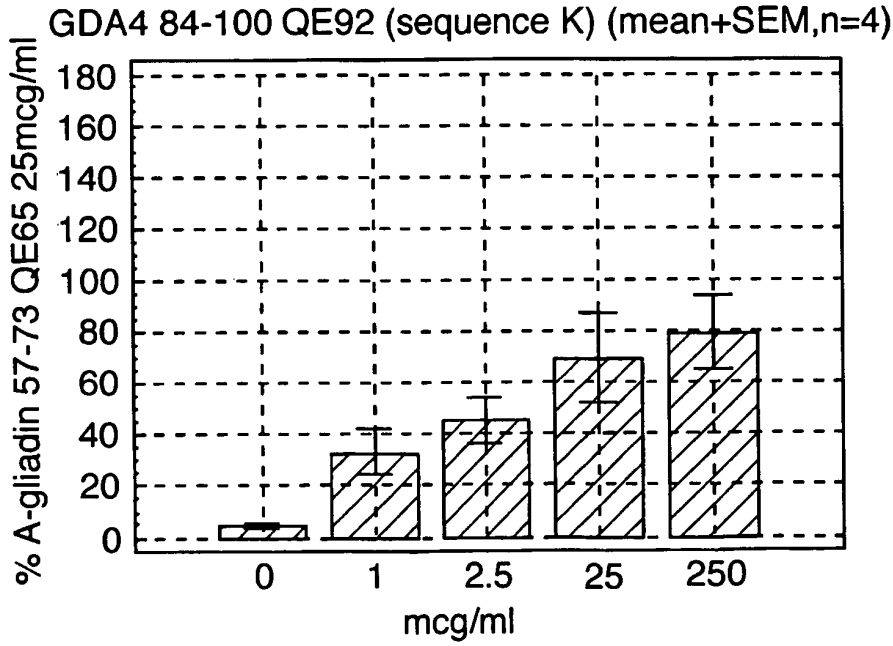


Fig.12c.

Dose response to A-gliadin 57-73: QLQPFPQPQLPYPQPQS (2.5, 25 & 250 mcg/ml), and A-gliadin 57-73 (25 mcg/ml) + tTG treatment.

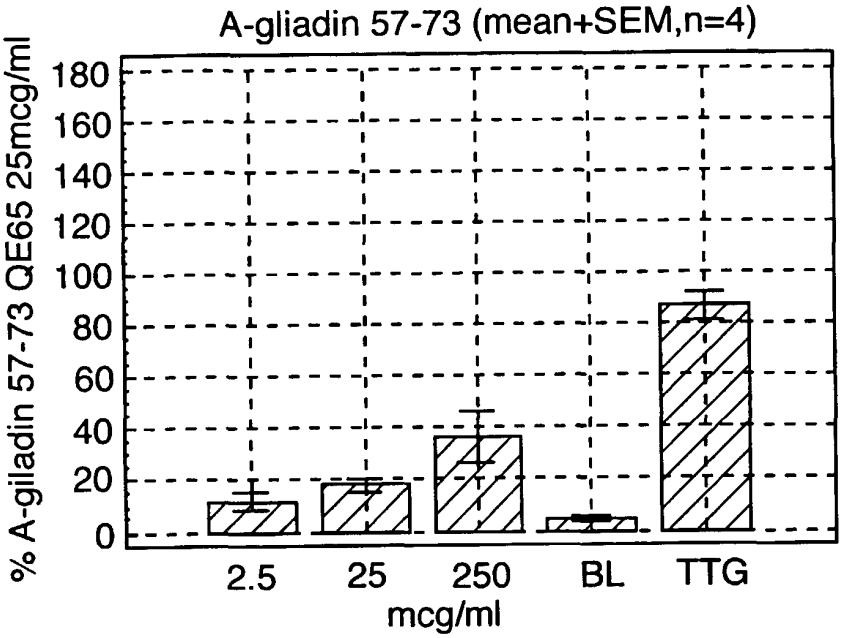


Fig.12d.

Dose response to GDA4_WHEAT P04724 84-100: PQLPYPQPQLPYPQPQP (2.5, 25 & 250 mcg/ml), and P04724 84-100 (25 mcg/ml) + tTG treatment.

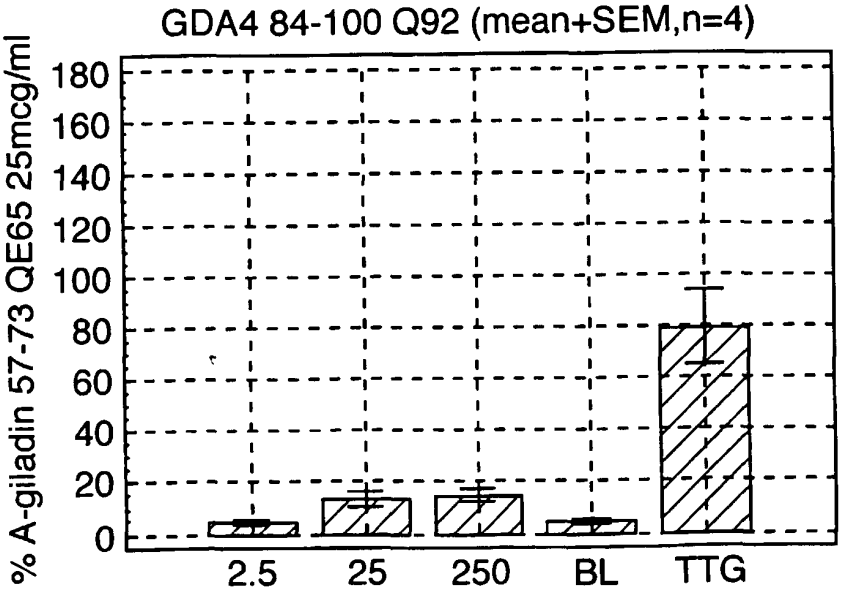


Fig.12e.

Dose response to the DQ2-restricted α gliadin T cell epitope A-gliadin 57-68 QE65: QLQPFPQPELPY (E65) (2.5, 25 & 250 mcg/ml), and A-gliadin 57-68: QLQPFPQPQLPY (Q65) (25 mcg/ml) +/- tTG treatment.

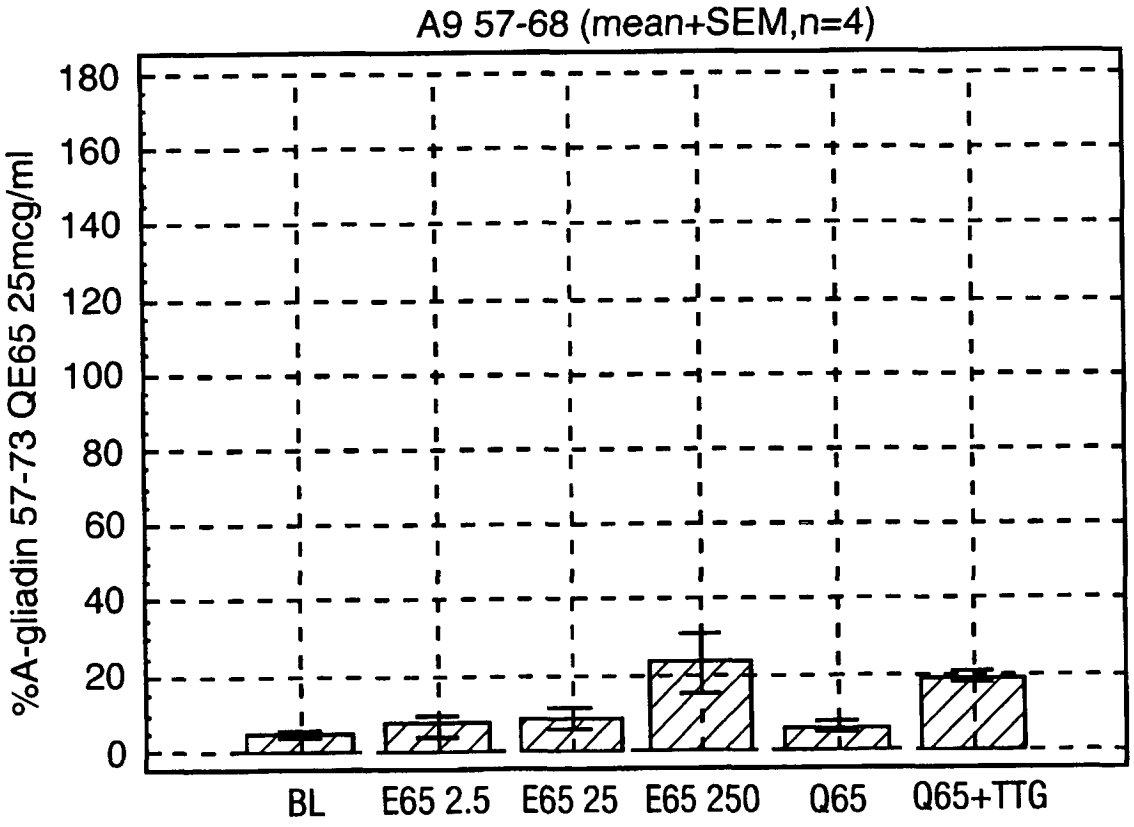


Fig.12f.

Dose response to the DQ2-restricted α gliadin T cell epitope α–2 62-75 QE65 & QE72: PQPELPYPQPELPY (E65) (2.5, 25 & 250 mcg/ml), and α–2 62-75: PQPQLPYPQPQLPY (Q65) (25 mcg/ml) +/- tTG treatment.

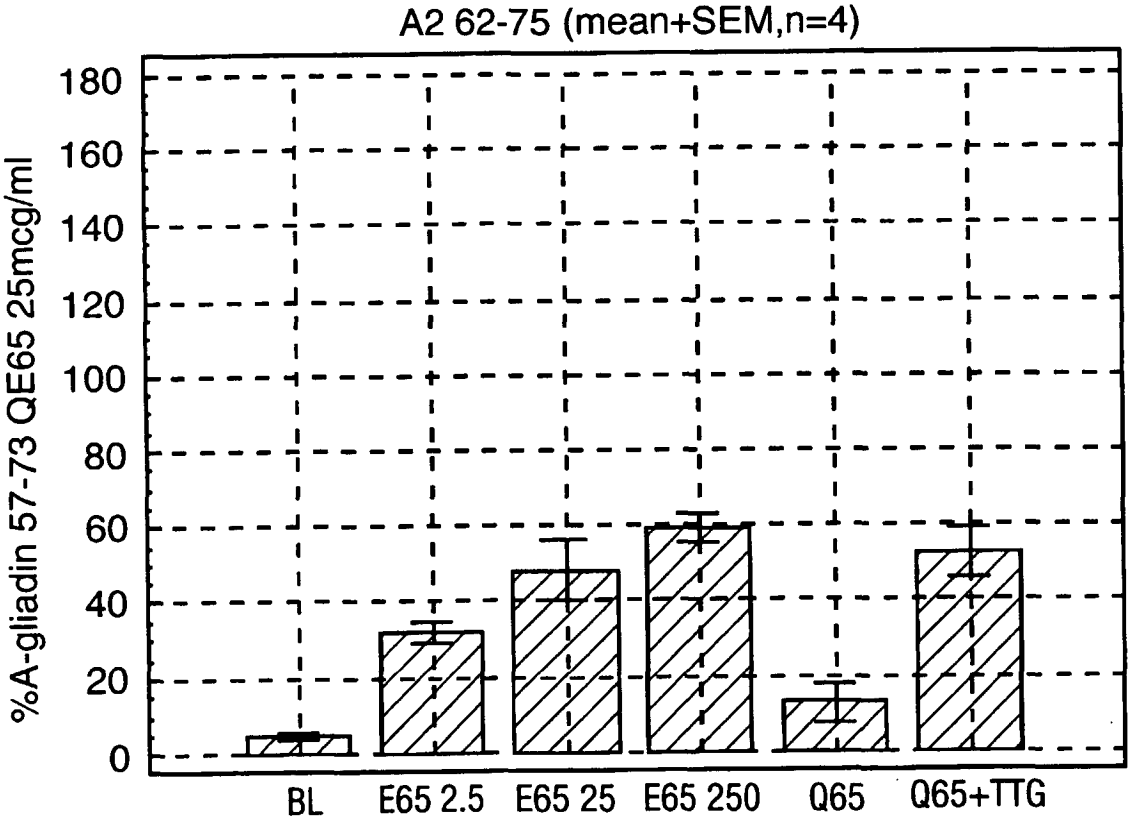


Fig.12g.

Dose response to the DQ8-restricted α gliadin T cell epitope GDA9 202-219: QE208 & 216: QQYPSGEGSFQPSQENPQ (E) (25 & 250 mcg/ml), and to GDA9 202-219 QQYPSGQGSFQPSQQNPQ (Q) (25 mcg/ml) +/- tTG treatment.

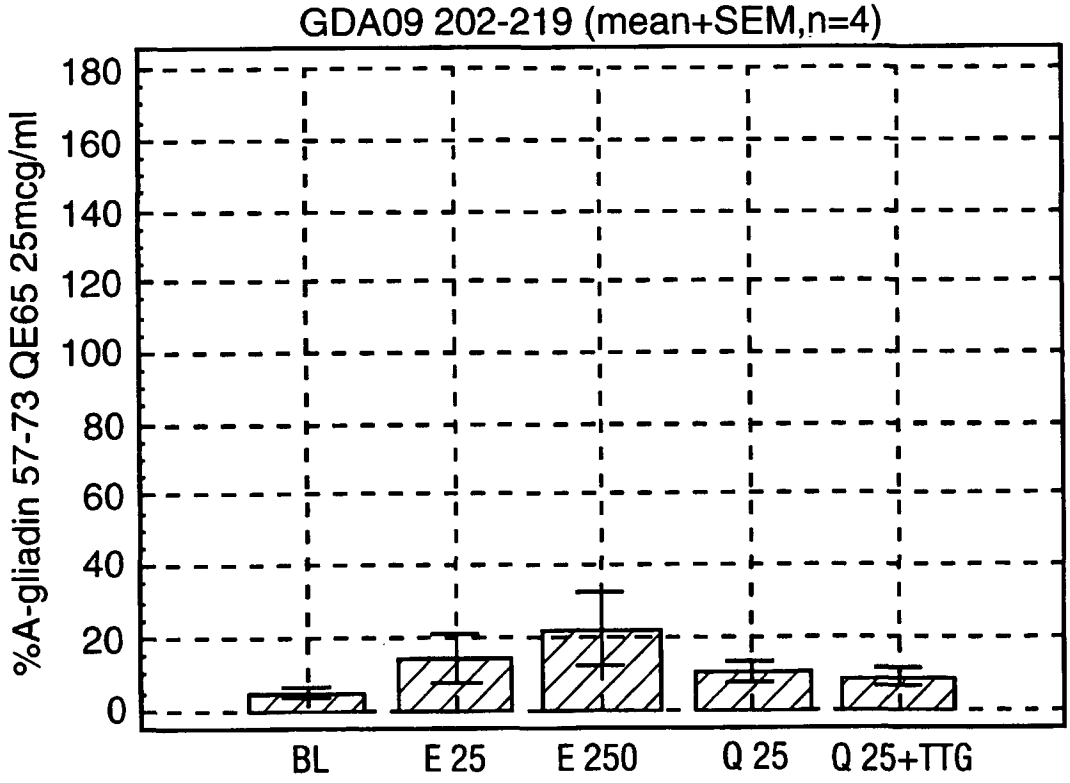


Fig.12h.

Dose response to the DQ2-restricted γ gliadin T cell epitope GDB2 134-153 QE140, 148,150: QQLPQPEQPQQSFPEQERPF (E) (25 & 250 mcg/ml), and to GDB2 134-153: QQLPQPQQPQQSFPQQQRPF (Q) (25 mcg/ml) +/- tTG treatment.

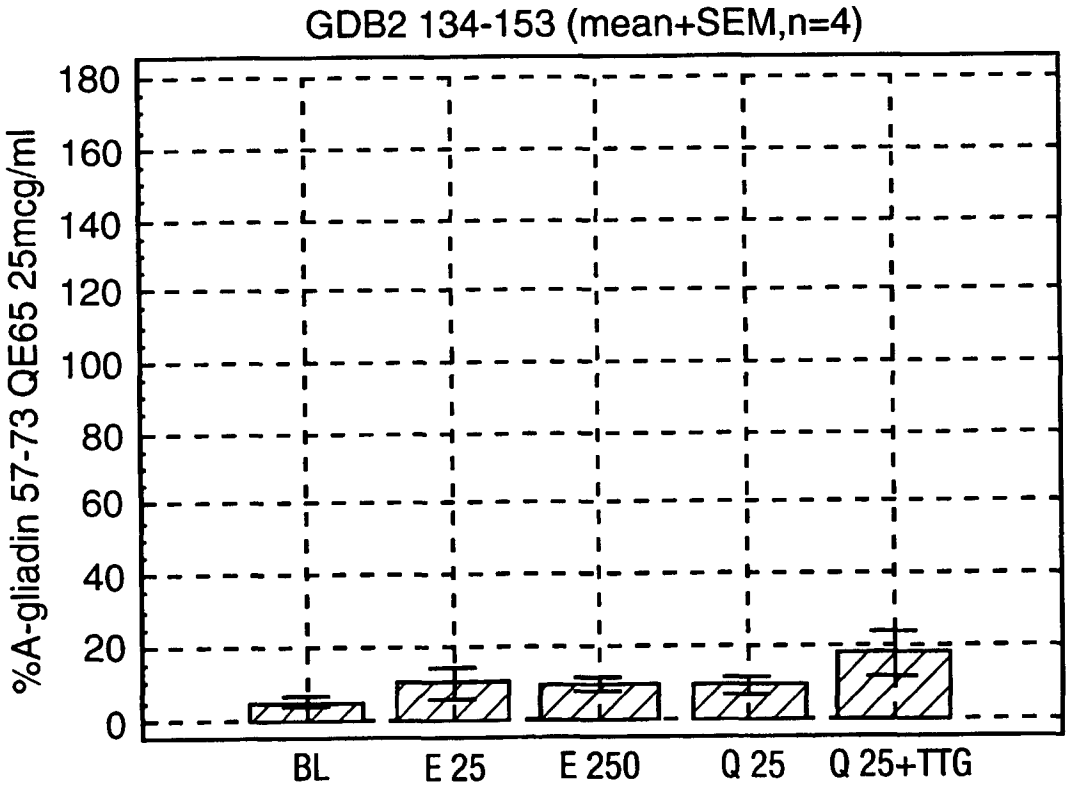


Fig.13a.

Dose response to gliadin digest by chymotrysin.

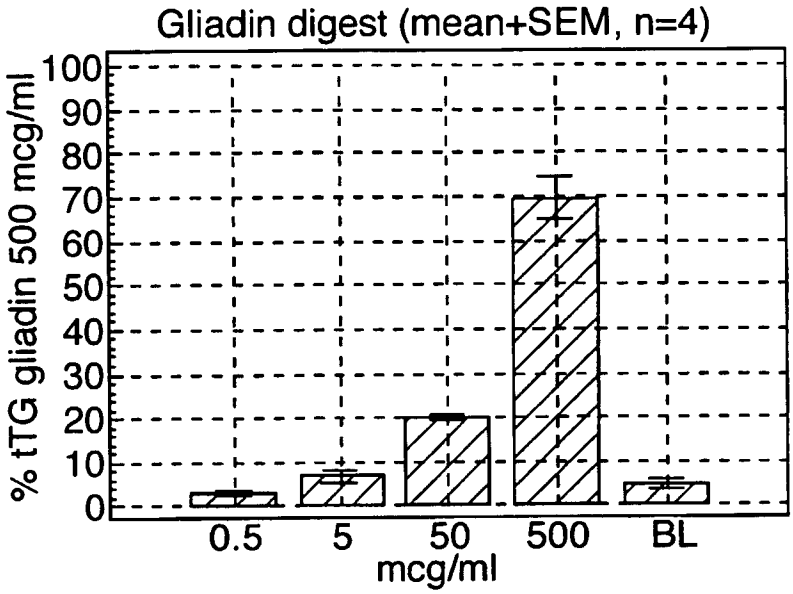


Fig.13b.

Dose response to gliadin digested by chymotrysin then treated with tTG.

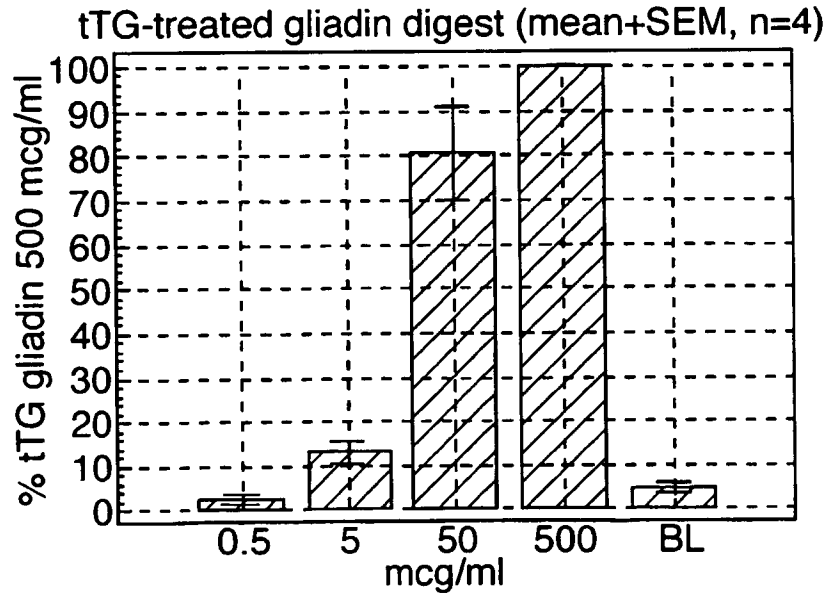
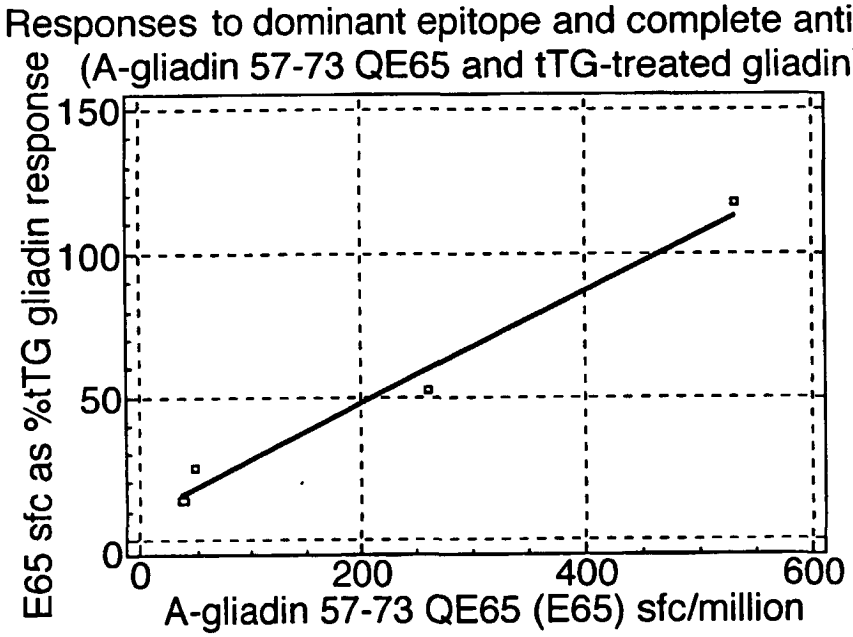


Fig.13c.

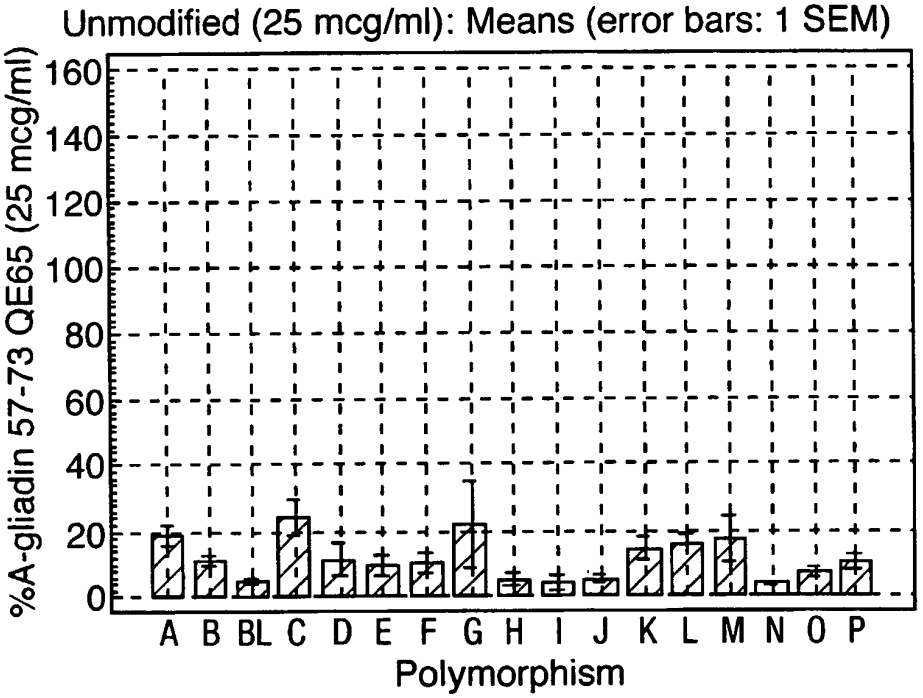
Total ELISpot responses to A-gliadin 57-73 QE65 (25mcg/ml) versus A-gliadin 57-73 QE65 responses as percent of tTG gliadin (500mcg/ml) responses.



(Fig.14.)

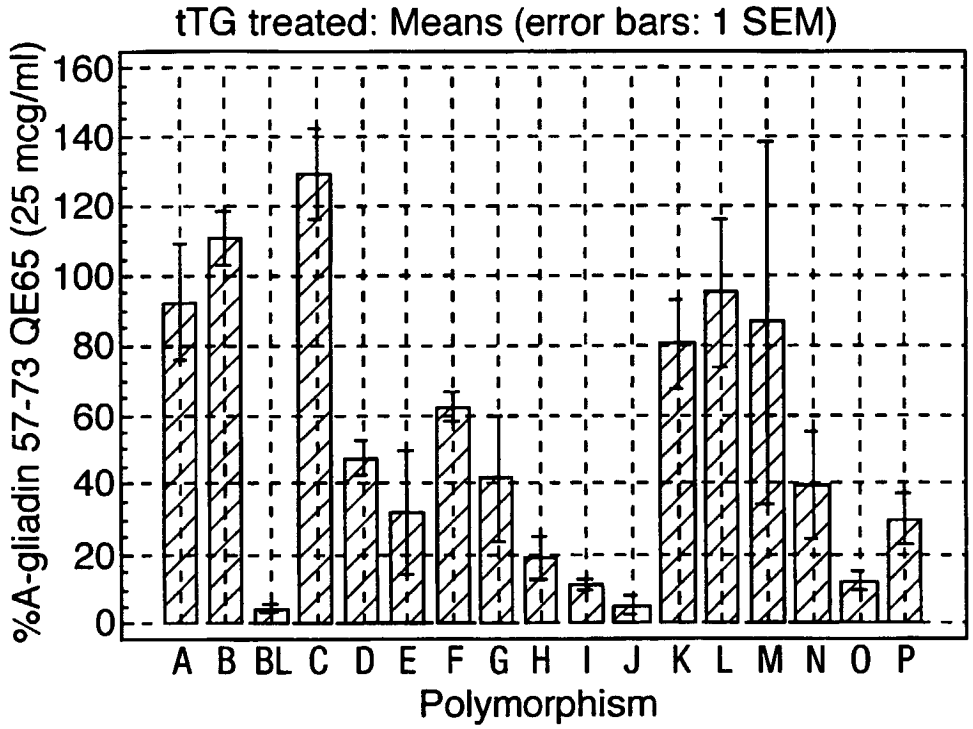
Bioactivity of gliadin polymorphisms of A-gliadin 57-73 (A) in coeliac subjects 6/7 days after gluten challenge (Gamma-Interferon Elispot) (n=4).

Fig.14a.



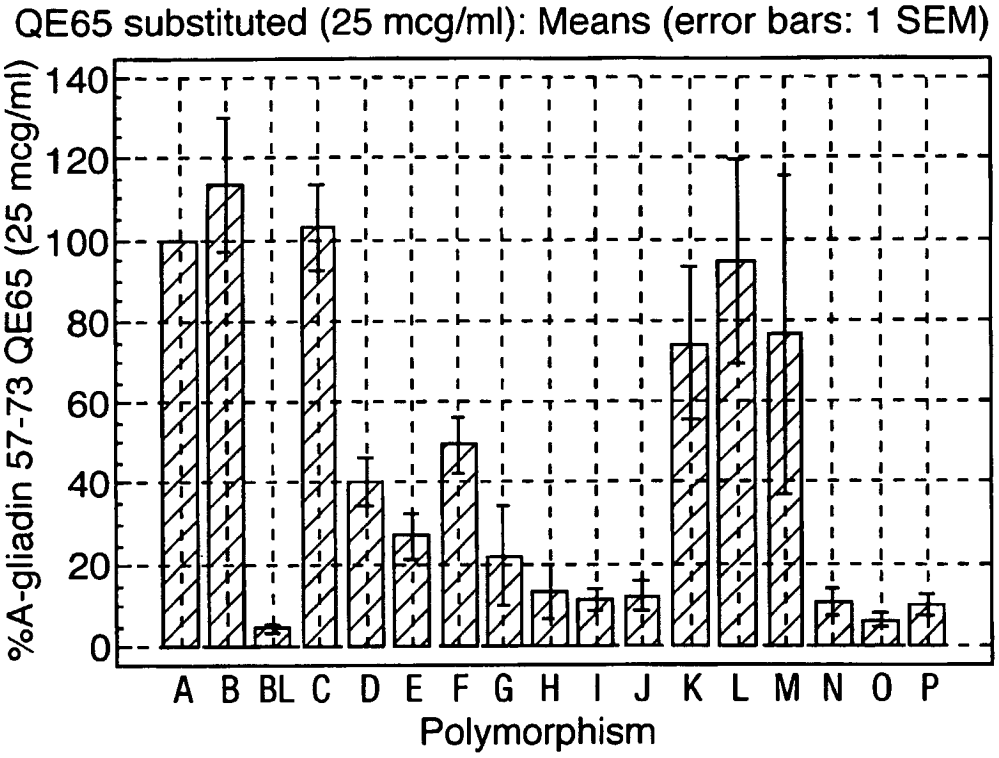
A	QLQPFPQPQLPYPQPQS	I	QLQPFPQPQLSYSQPQP
B	QLQPFPQPQLPYPQPQP	J	QPQPFPPPQLPYPQTQP
C	QLQPFPQPQLPYPQPQL	K	PQLPYPQPQLPYPQPQP
D	QLQPFPQPQLPYLQPQS	L	PQLPYPQPQLPYPQPQL
E	QLQPFPRPQLPYPQPQP	M	PQPQPFLPQLPYPQPQS
F	QLQPFPQPQLPYSQPQP	N	PQPQPFPPQLPYPQPQS
G	QLQPFLQPQLPYSQPQP	O	PQPQPFPPQLPYPQTQP
H	QLQPFSQPQLPYSQPQP	P	PQPQPFPPQLPYPQPPP

Fig.14b.



A	QLQPFPQPQLPYPQPQS	I	QLQPFPQPQLSYSQPQP
B	QLQPFPQPQLPYPQPQP	J	QPQPFPPQLPYPQTQP
C	QLQPFPQPQLPYPQPQL	K	PQLPYPQPQLPYPQPQP
D	QLQPFPQPQLPYLQPQS	L	PQLPYPQPQLPYPQPQL
E	QLQPFPRPQLPYPQPQP	M	PQPQPFLPQLPYPQPQS
F	QLQPFPQPQLPYSQPQP	N	PQPQPFPPQLPYPQPQS
G	QLQPFLQPQLPYSQPQP	O	PQPQPFPPQLPYPQTQP
H	QLQPFSQPQLPYSQPQP	P	PQPQPFPPQLPYPQPPP

Fig.14c.



A	QLQPFPQPQLPYPQPQS	I	QLQPFPQPQLSYSQPQP
B	QLQPFPQPQLPYPQPQP	J	QPQPFPPPQLPYPQTQP
C	QLQPFPQPQLPYPQPQL	K	PQLPYPQPQLPYPQPQP
D	QLQPFPQPQLPYLQPQS	L	PQLPYPQPQLPYPQPQL
E	QLQPFPRPQLPYPQPQP	M	PQPQPFLPQLPYPQPQS
F	QLQPFPQPQLPYSQPQP	N	PQPQPFPPQLPYPQPQS
G	QLQPFLQPQLPYSQPQP	O	PQPQPFPPQLPYPQTQP
H	QLQPFSQPQLPYSQPQP	P	PQPQPFPPQLPYPQPPP

Fig.14d.

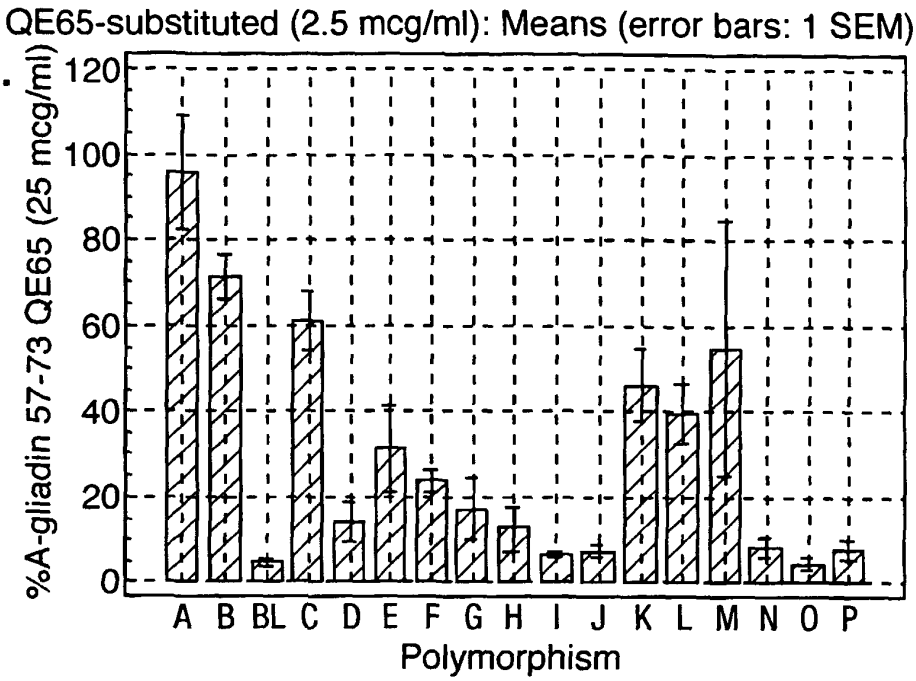
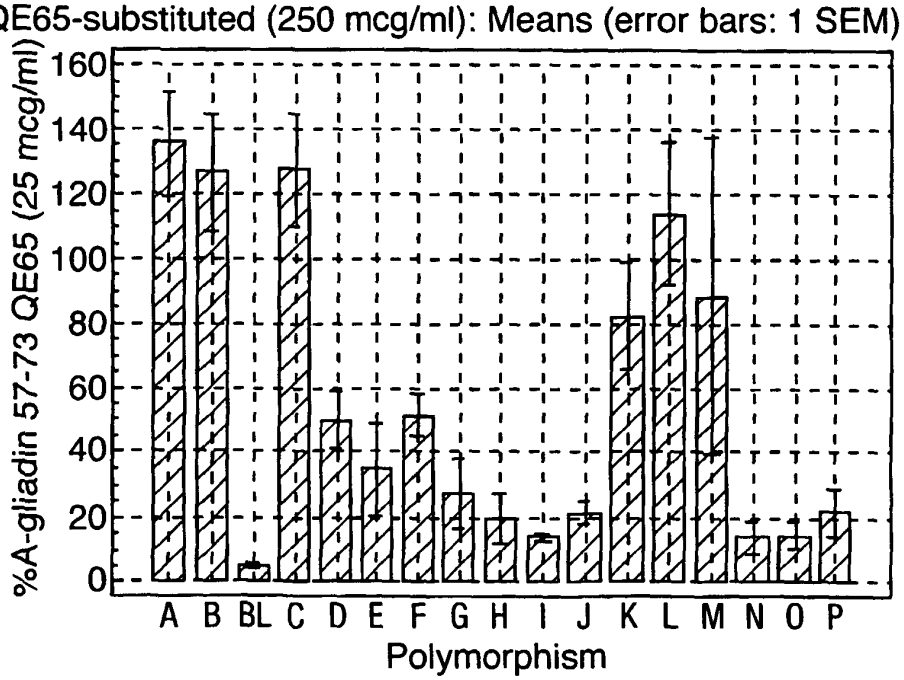


Fig.14e.



A	QLQPFPQPQLPYPQPQS	I	QLQPFPQPQLSYSQPQP
B	QLQPFPQPQLPYPQPQP	J	QPQPFPPPQLPYPQTQP
C	QLQPFPQPQLPYPQPQL	K	PQLPYPQPQLPYPQPQP
D	QLQPFPQPQLPYLQPQS	L	PQLPYPQPQLPYPQPQL
E	QLQPFPRPQLPYPQPQP	M	PQPQPFLPQLPYPQPQS
F	QLQPFPQPQLPYSQPQP	N	PQPQPFPPQLPYPQPQS
G	QLQPFLQPQLPYSQPQP	O	PQPQPFPPQLPYPQTQP
H	QLQPFSQPQLPYSQPQP	P	PQPQPFPPQLPYPQPPP

Fig.15.

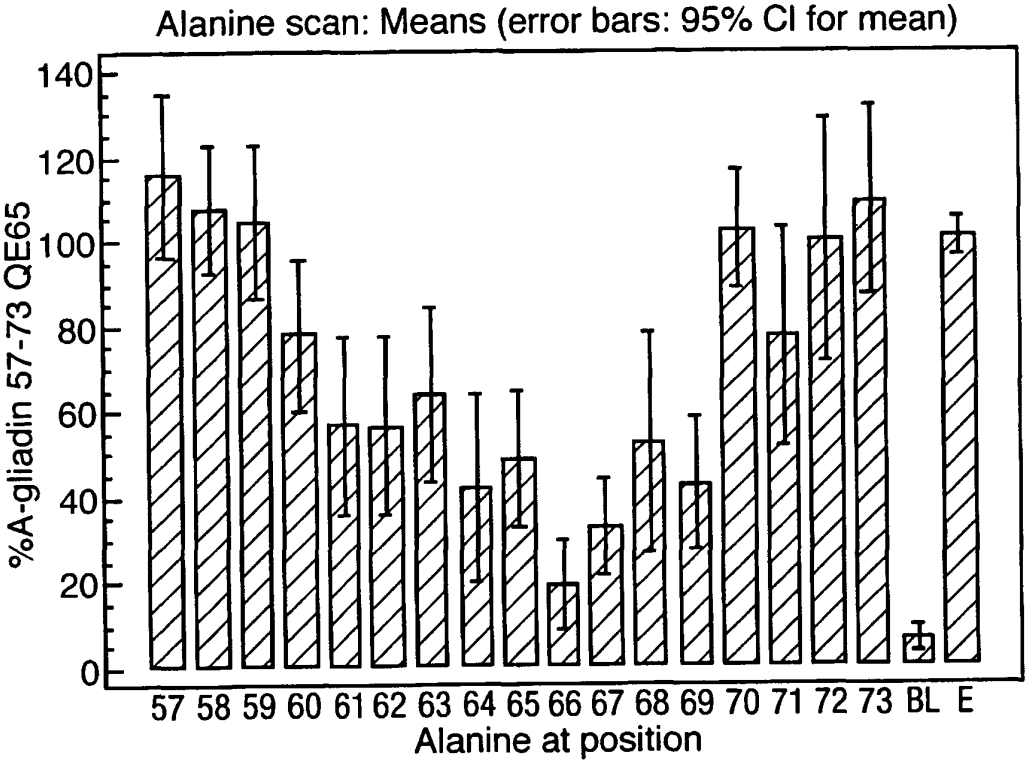

Alanine scan: Means (error bars: 95% CI for mean)
%A-gliadin 57-73 QE65
0
20
40
60
80
100
120
140
57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 BL E
Alanine at position

Fig.16.

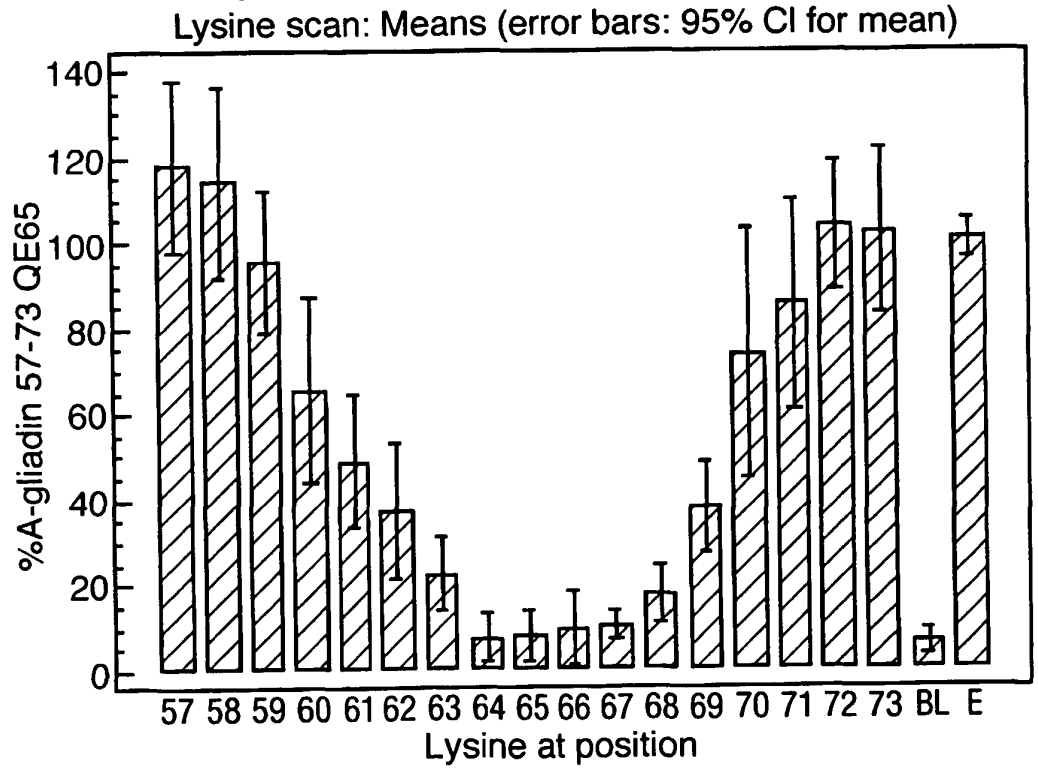

Lysine scan: Means (error bars: 95% CI for mean)
%A-gliadin 57-73 QE65
0
20
40
60
80
100
120
140
57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 BL E
Lysine at position

Fig.17.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

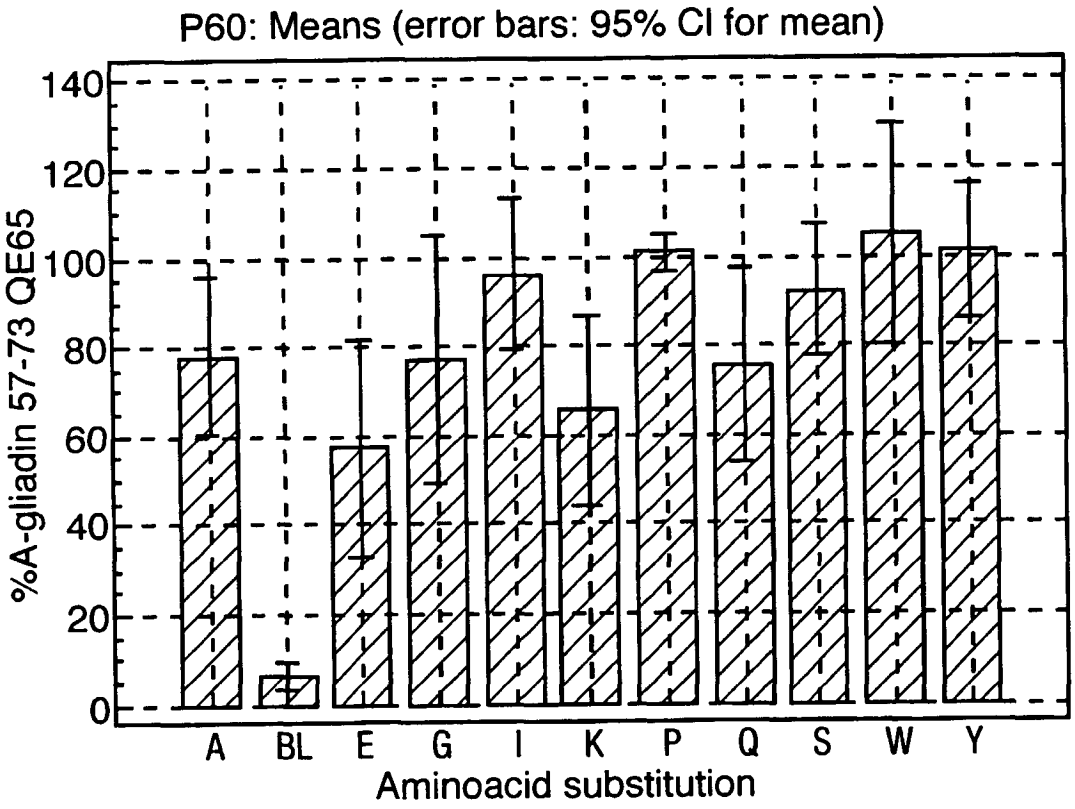


Fig.18.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

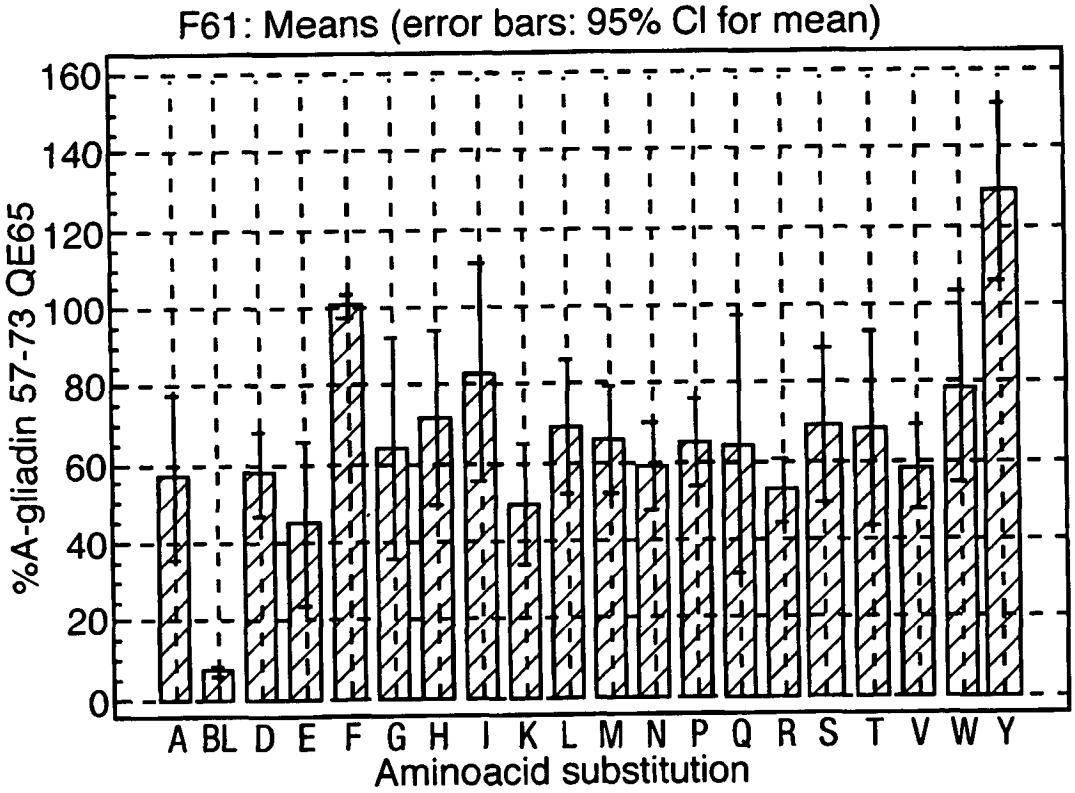


Fig.19.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

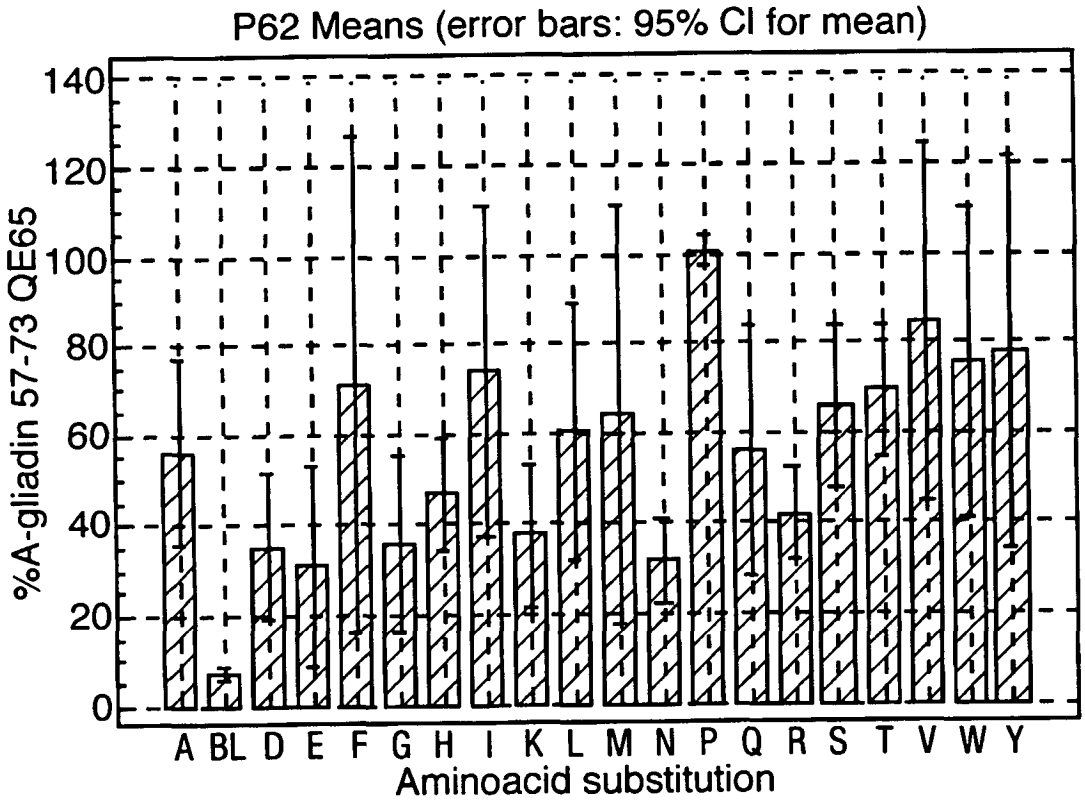


Fig.20.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

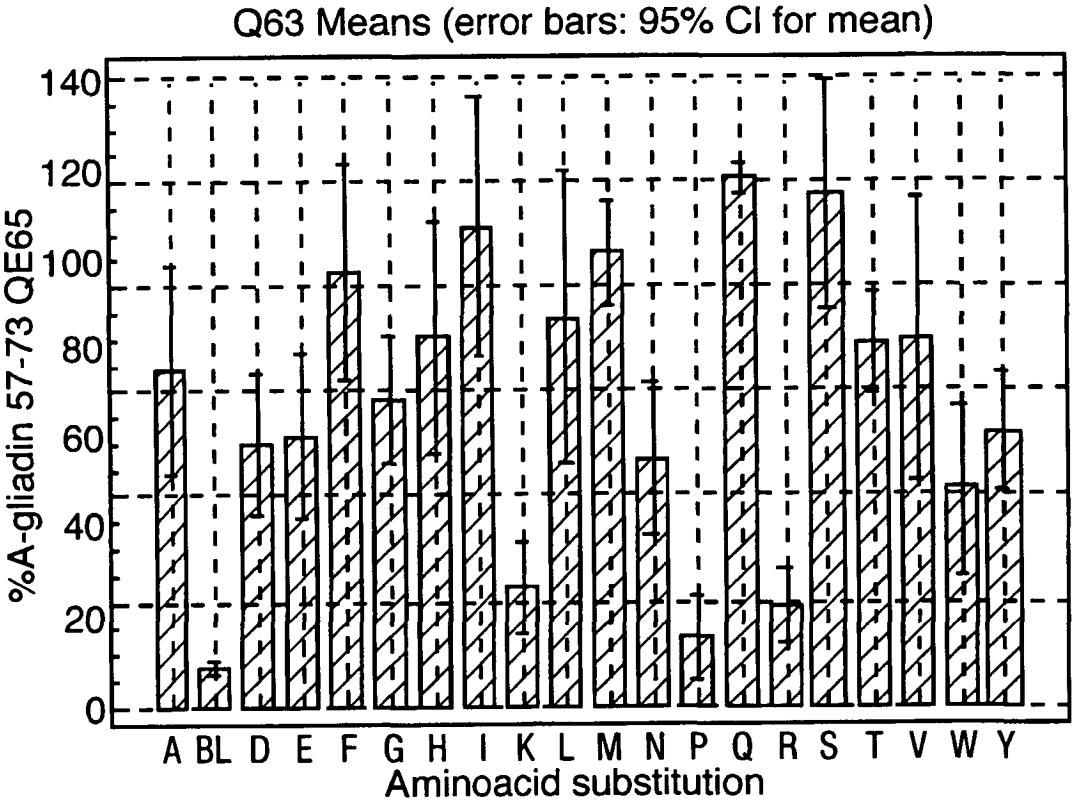


Fig.21.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

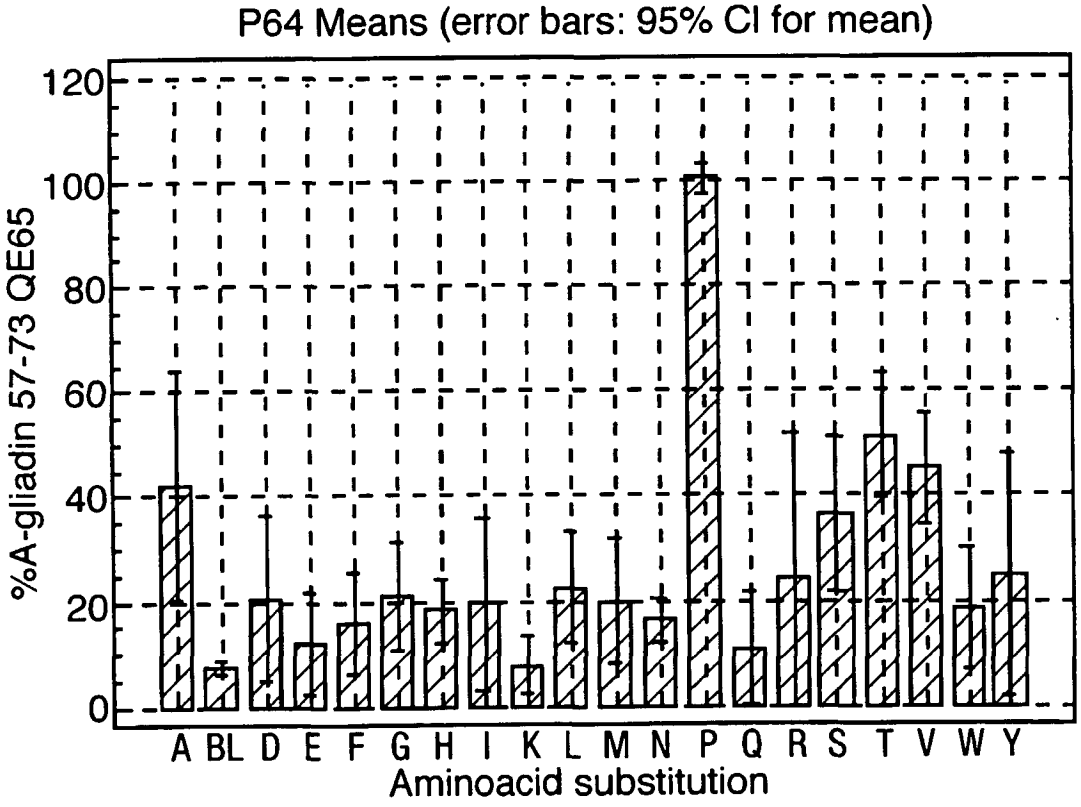


Fig.22.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

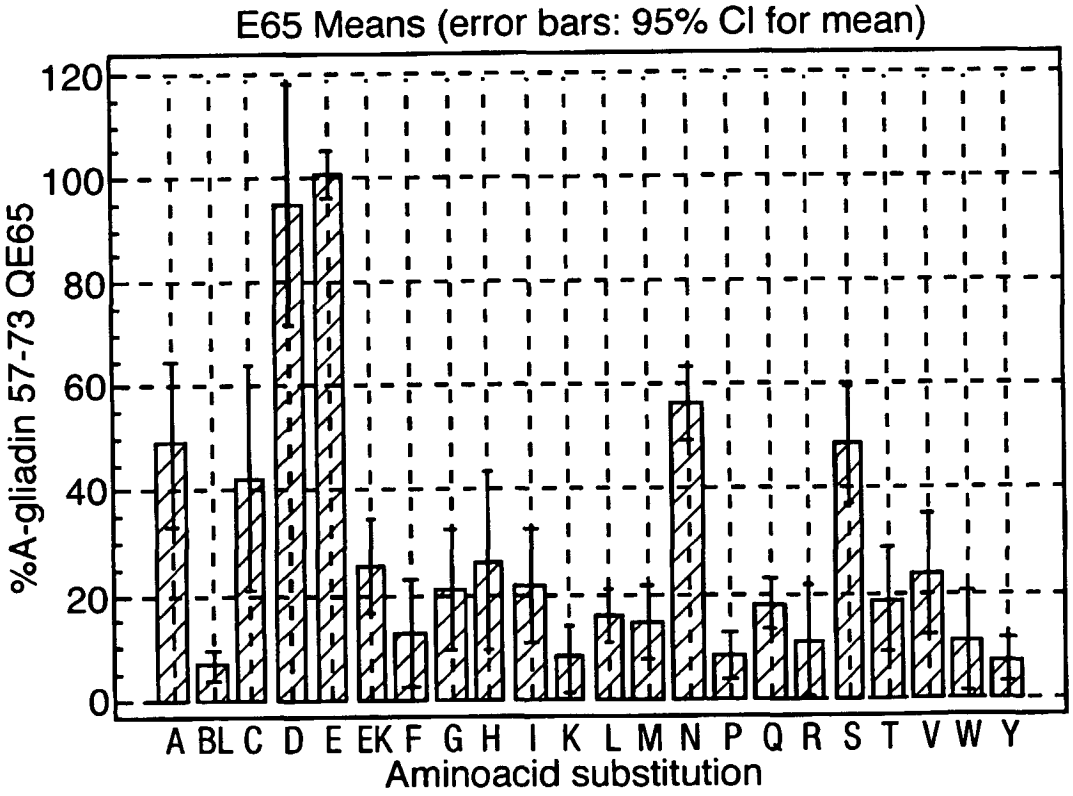


Fig.23.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

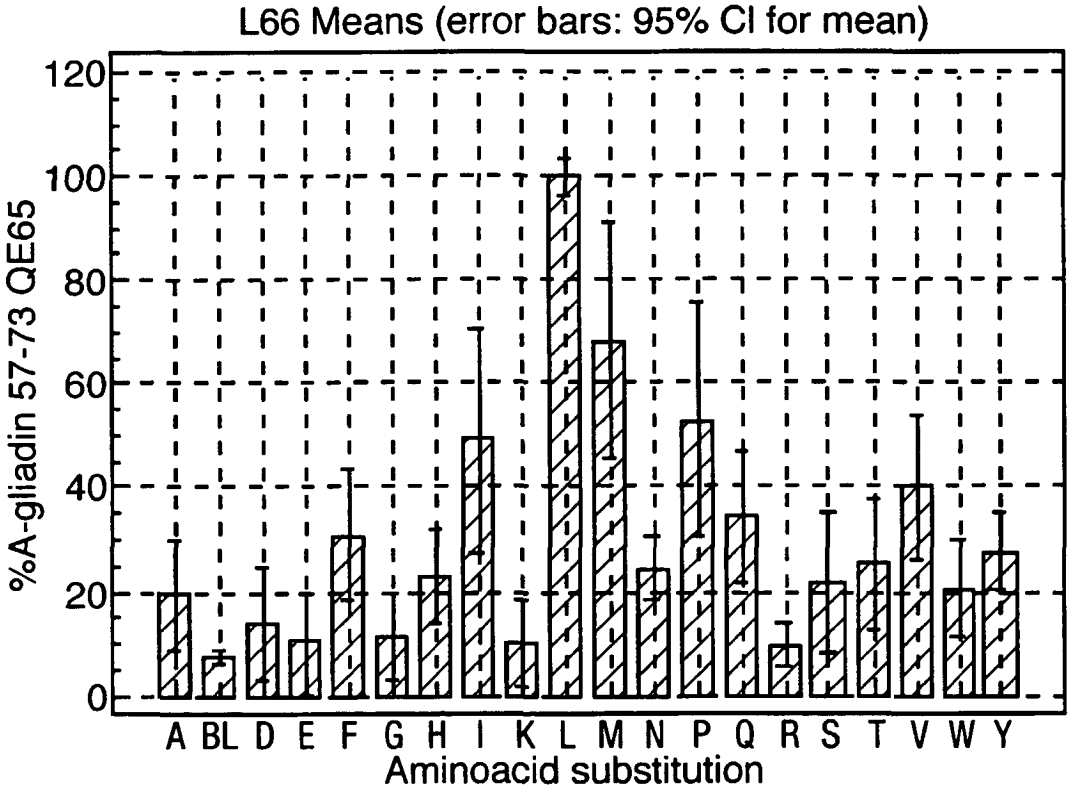


Fig.24.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

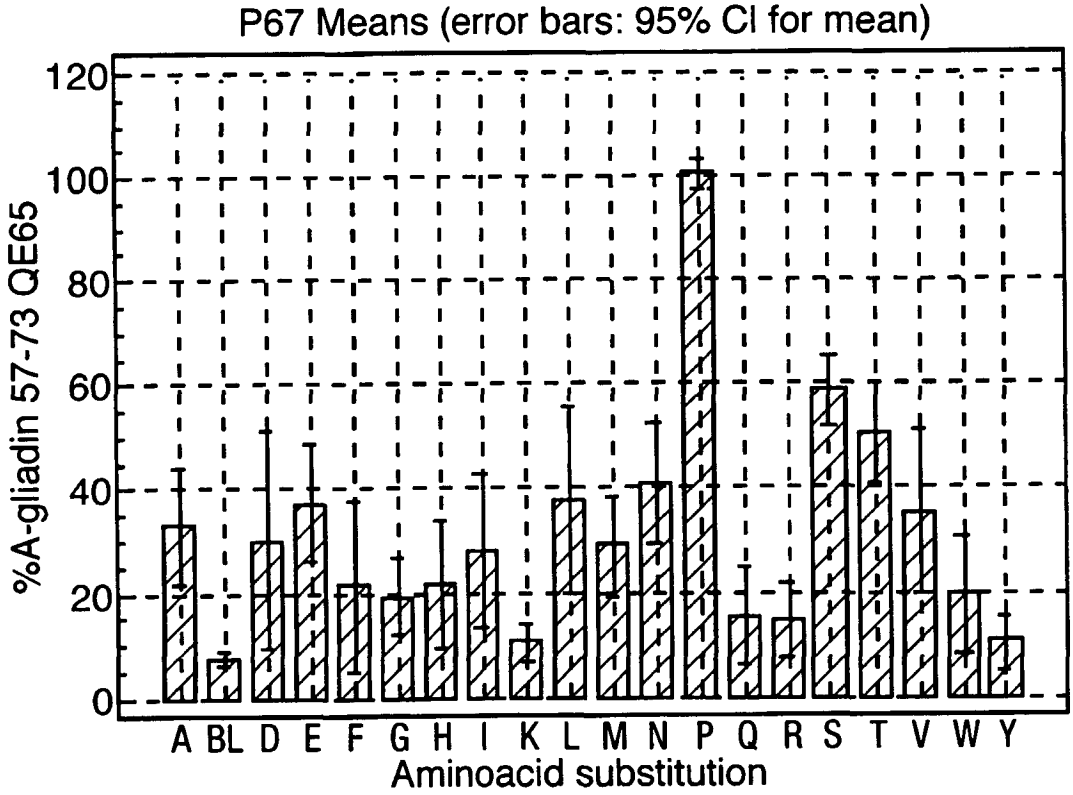


Fig.25.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

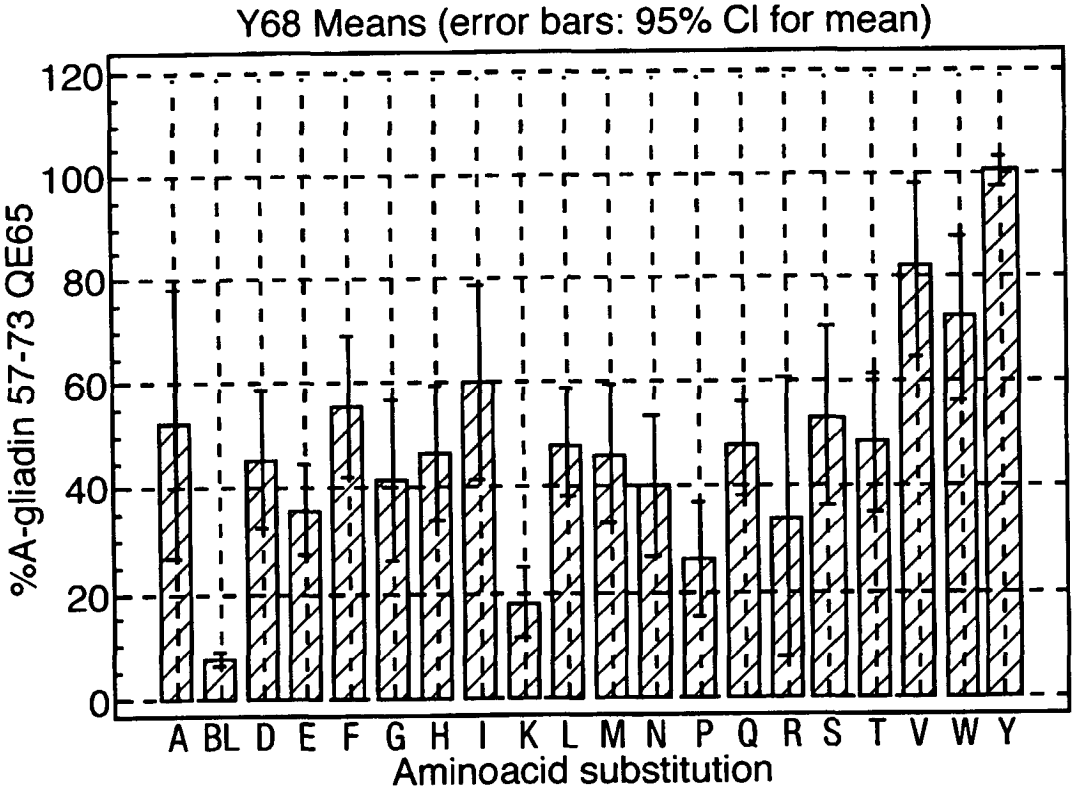


Fig.26.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

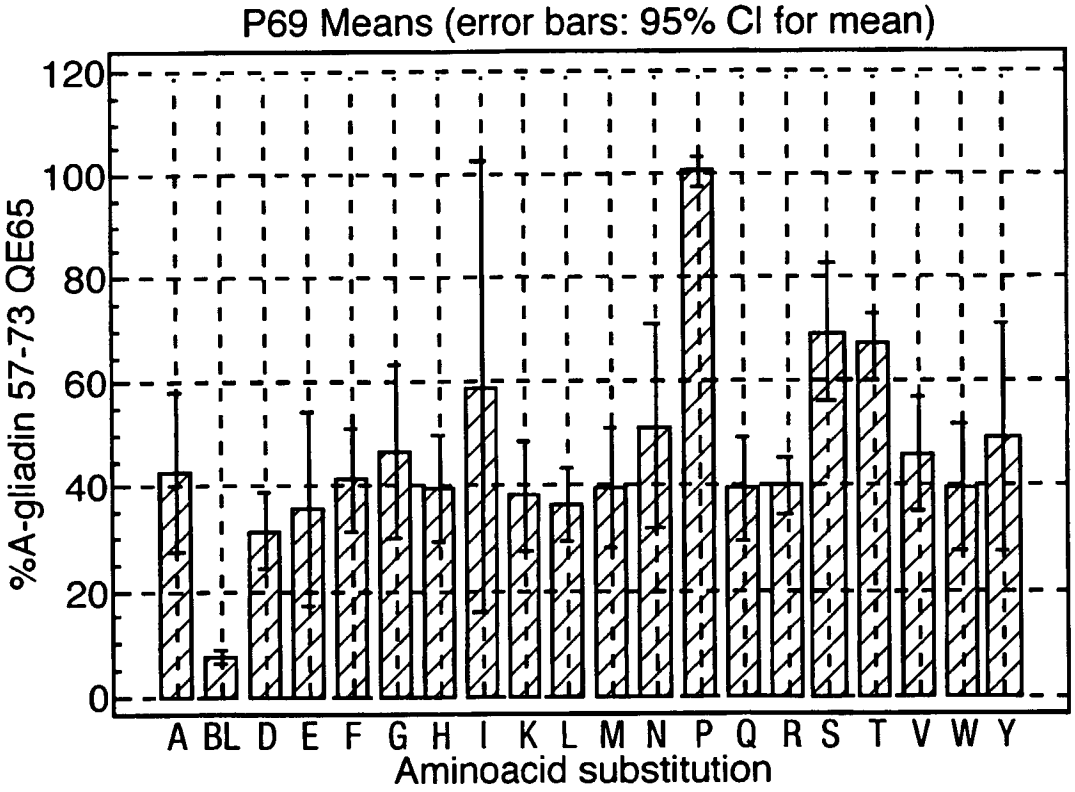
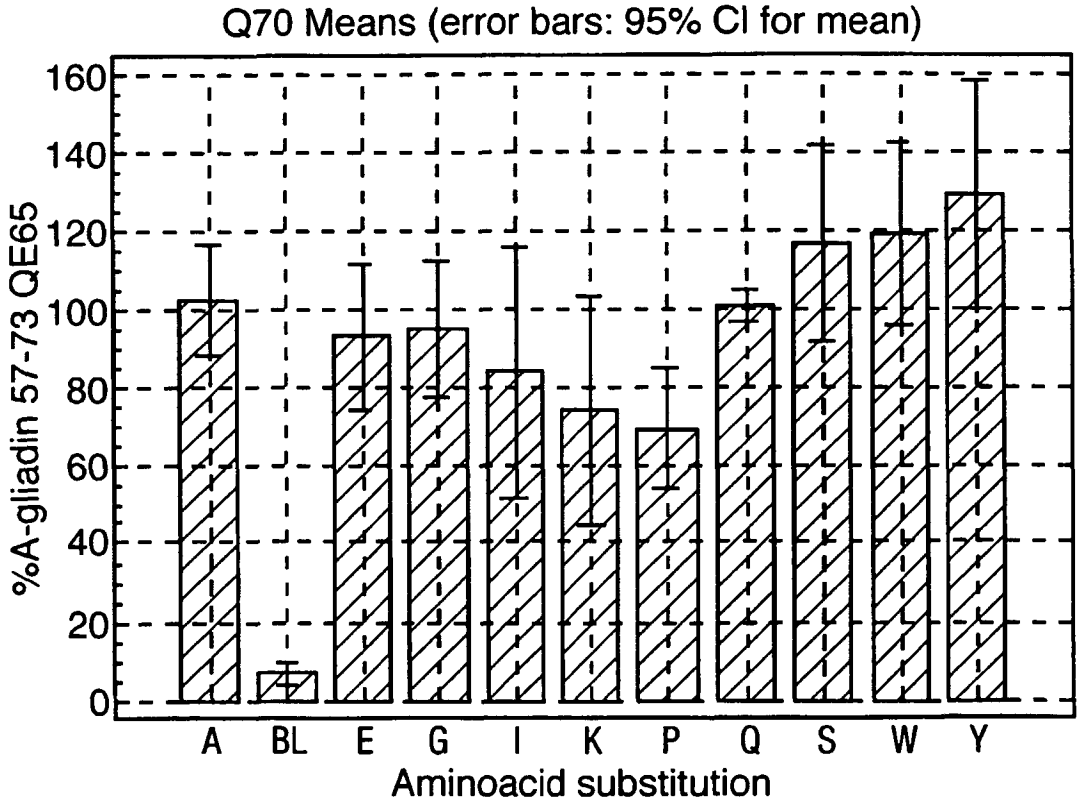


Fig.27.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70



(Fig.28.)

Interferon gamma ELISpot responses in newly diagnosed and treated coeliac subjects, before and after gluten challenge.

Fig.28a.

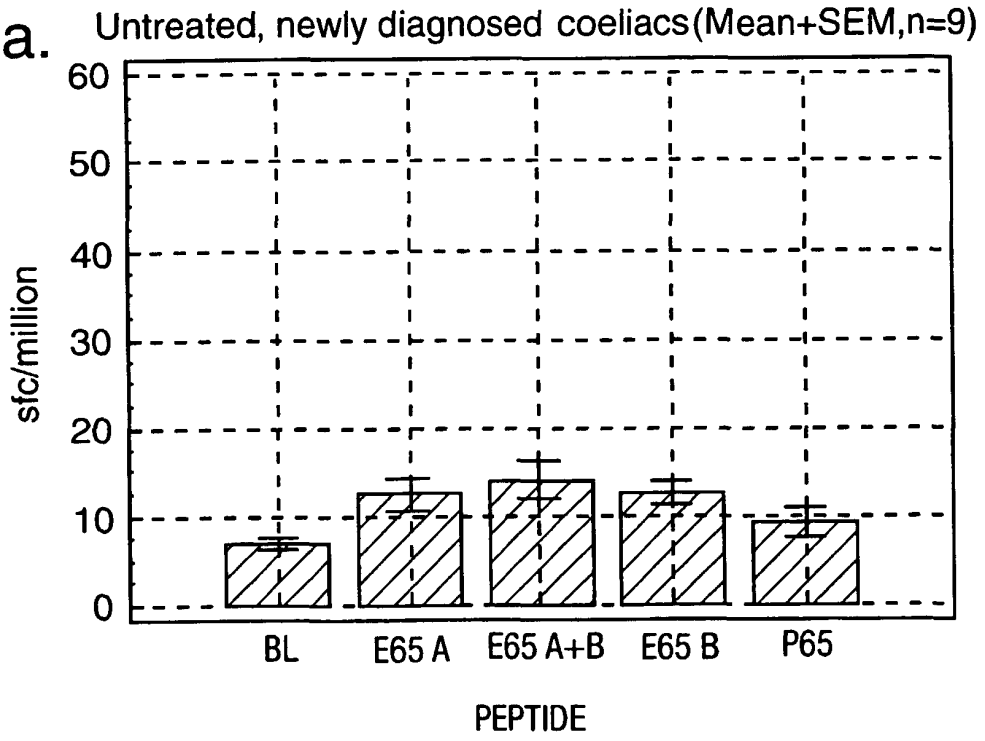


Fig.28b.

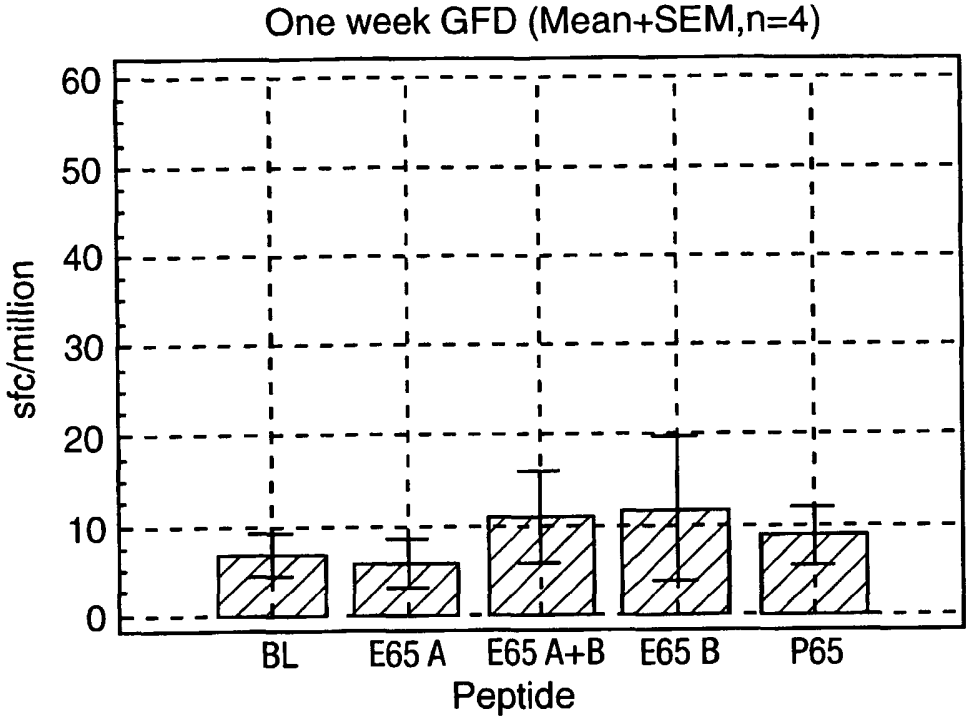


Fig.28c.

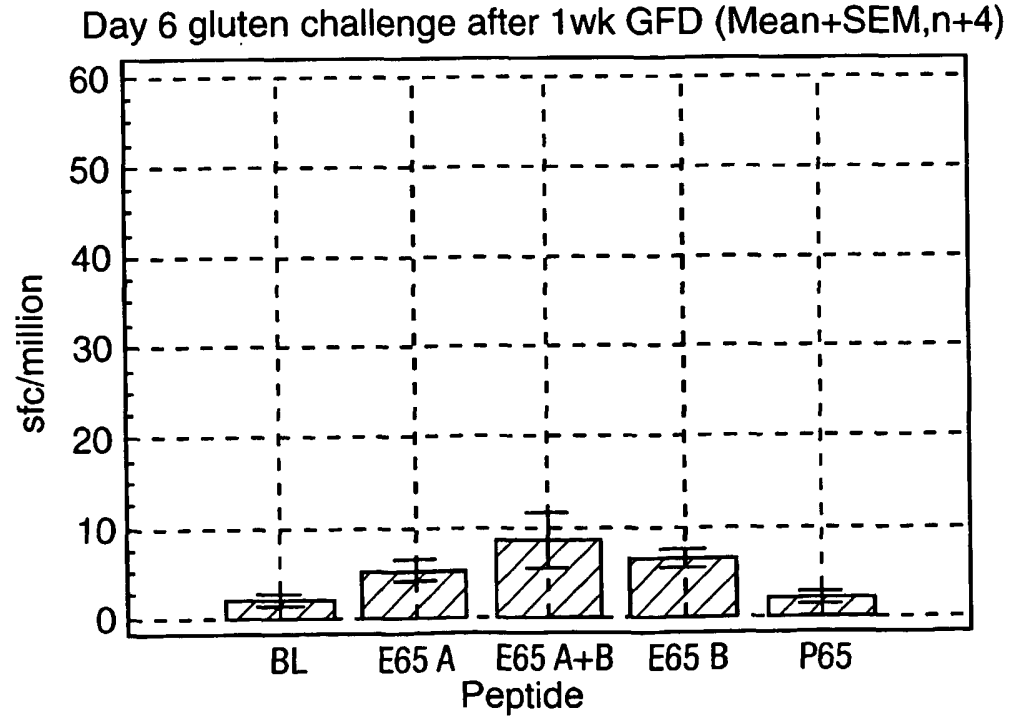


Fig.28d.

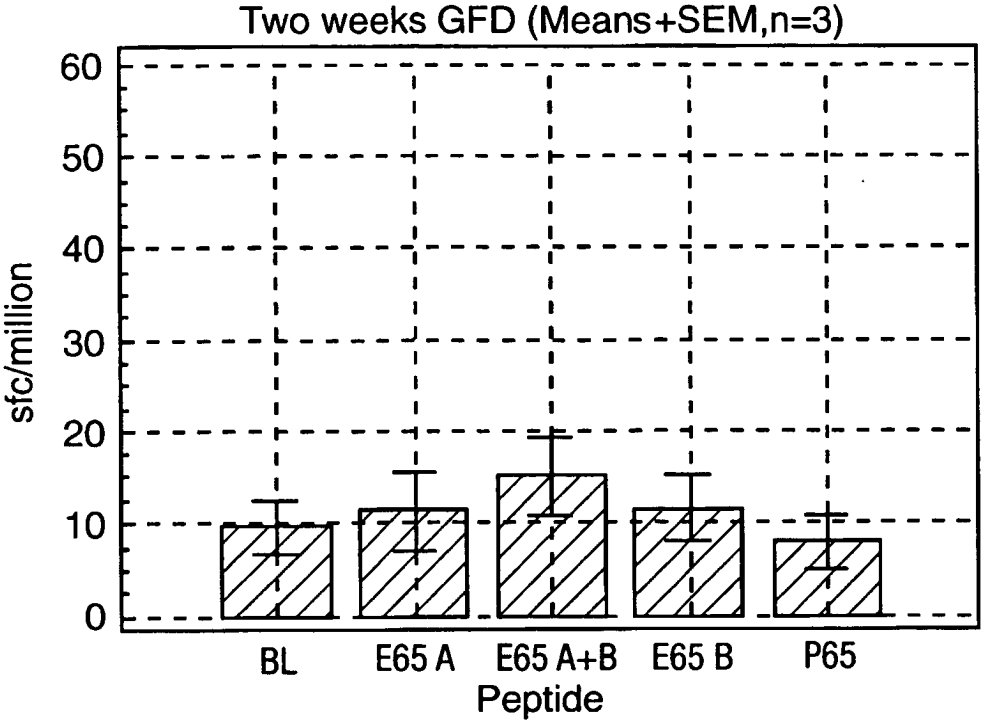


Fig.28e.

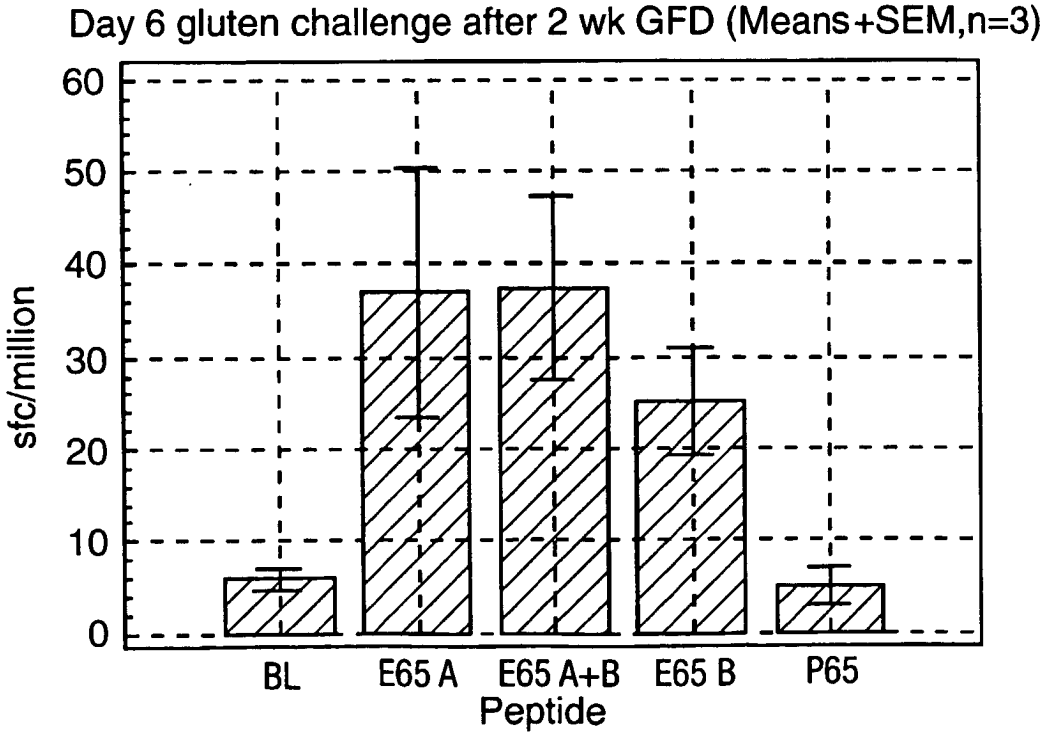


Fig.28f.

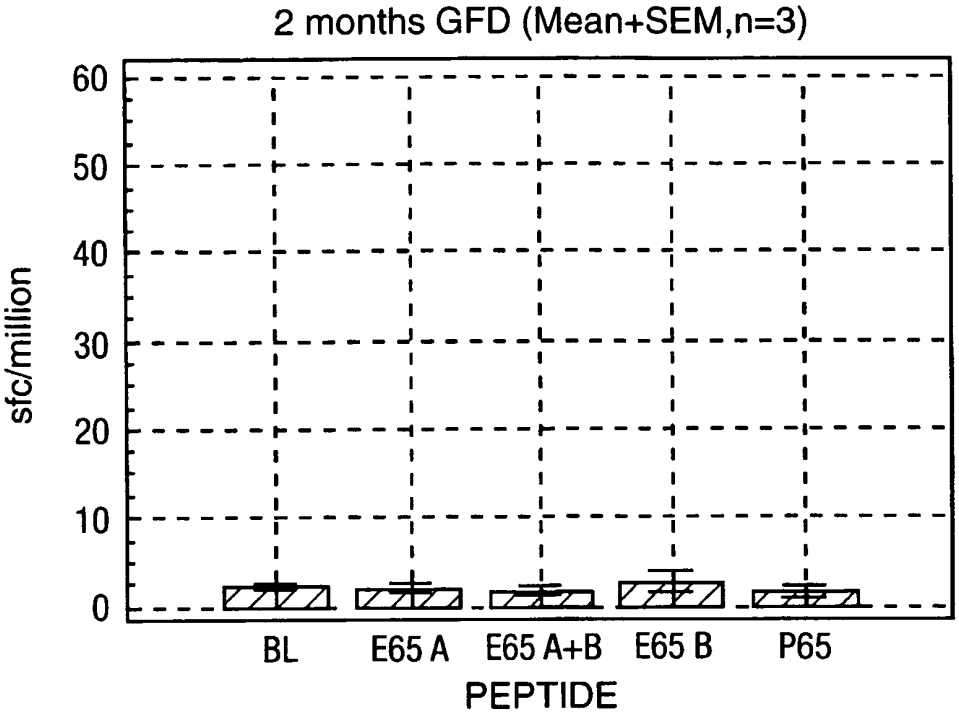
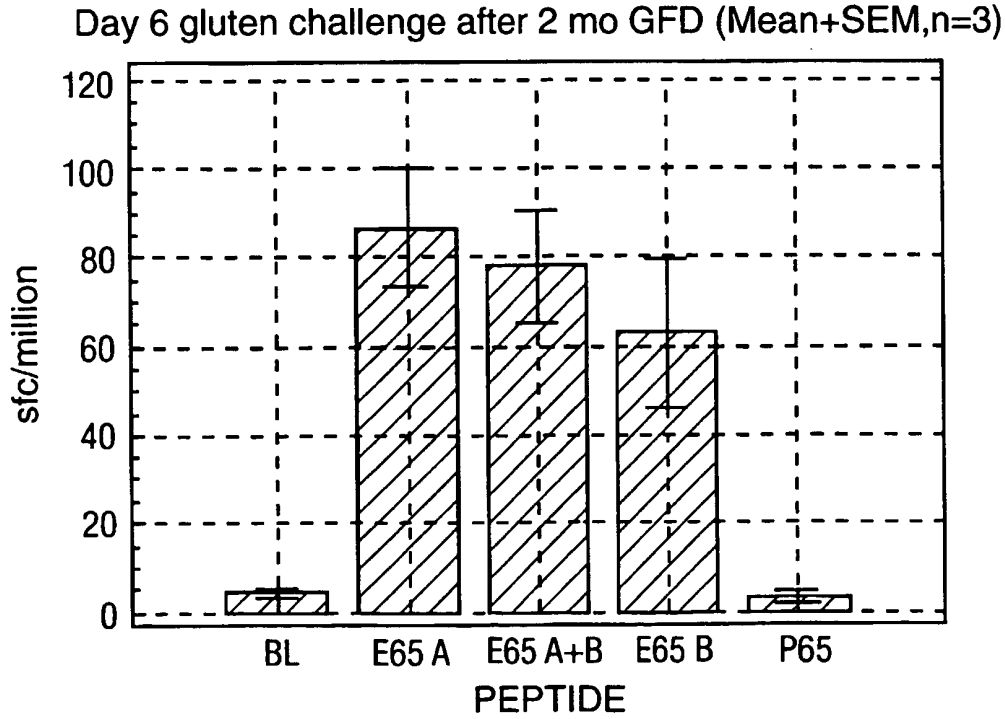


Fig.28g.



专利名称(译)	使用麦醇溶蛋白表位诊断乳糜泻		
公开(公告)号	EP1218751B1	公开(公告)日	2006-03-01
申请号	EP2000964460	申请日	2000-10-02
[标]申请(专利权)人(译)	ISIS创新有限公司		
申请(专利权)人(译)	ISIS创新有限公司		
当前申请(专利权)人(译)	ISIS创新有限公司		
[标]发明人	ANDERSON ROBERT PAUL MOLECULAR IMMUNOLOGY HILL ADRIAN VIVIAN SINTON WELLCOME TRUST CENT JEWELL DEREK PARRY GASTROENTEROLOGY UNIT		
发明人	ANDERSON, ROBERT PAUL, MOLECULAR IMMUNOLOGY HILL, ADRIAN VIVIAN SINTON, WELLCOME TRUST CENTRE JEWELL, DEREK PARRY, GASTROENTEROLOGY UNIT		
IPC分类号	G01N33/68 C07K14/415 A61P37/08 A01H5/00 A01H5/10 A01K67/027 A61K39/00 A61K45/00 A61P37/06 A61P43/00 C12N15/82 G01N33/15 G01N33/50 G01N33/53 G01N33/564		
CPC分类号	A61P1/00 C07K14/415 C12N15/8257 G01N33/505 G01N33/564 G01N33/6863 G01N2333/9108 Y10S435/975 A61K49/0006 C12N15/8258 G01N33/6854 G01N33/6878		
代理机构(译)	DOLAN，ANTHONY PATRICK		
优先权	1999023306 1999-10-01 GB		
其他公开文献	EP1218751A2		
外部链接	Espacenet		

摘要(译)

一种在个体中诊断乳糜泻或乳糜泻易感性的方法，包括：（a）使来自宿主的样品与选自（i）包含以下序列的表位的试剂接触：SEQ ID NO：1或2，或者来自SEQ ID NO：3所示的麦醇溶蛋白的天然存在的同源物的等同序列，（ii）包含序列的表位，所述序列包含：SEQ ID NO：1，或来自SEQ ID NO：1的天然存在的同源物的等同序列。 ID NO：3，该表位是衍生自麦醇溶蛋白的分离的寡肽，（iii）（i）或（ii）的类似物，其能够被识别（i）或（ii）的T细胞受体识别。在肽类似物的情况下，其长度不超过50个氨基酸，或（iv）包含两种或更多种如（i），（ii）或（iii）中定义的试剂的产物，和（b）确定体外样品中的T细胞是否识别药剂; T细胞识别表明个体患有或易患乳糜泻。还提供了包含表位和不引起乳糜泻的麦醇溶蛋白的治疗组合物。