INTERACTION OF NATURAL AND SYNTHETIC PEPTIDES DERIVED FROM GLIADIN WITH DIFFERENT CELL LINES

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INTRODUCTION

The mechanism by which cereal protein gluten affects the small intestine mucosa in coeliac disease is still unknown. Weiser and Douglas[1] proposed that gluten contains a toxic lectin which binds to altered membrane glycoproteins of coeliac enterocytes. This hypothesis was supported by Kottgen *et al.*[2] who confirmed that gluten has lectin-like properties and that gliadin peptides can bind to glycoproteins present on immature crypt cells of rat intestine. Auricchio *et al.*[3] reported that peptic-tryptic-digest of bread wheat gliadin and A-gliadin agglutinated the undifferentiated K562 human cell line.

In our work different cell lines of lymphoblastoid and epithelial origin were compared in order to study the interaction between gliadin peptides and cell membranes.

MATERIALS AND METHODS

Peptides Derived from Wheat Gliadin and Reagents

Natural gliadin peptides used in our experiments were Frazer's Fraction III i.e., peptic-tryptic digest of gliadin (FFIII), α-gliadin, β-gliadin, γ-gliadin, A-gliadin and Pepsin-Trypsin-Pancreatic digest of α-gliadin (PTP) isolated as described by Kocna *et al.*[4]

Synthetic peptides were produced by continuous flow solid-phase multiple peptide synthesis. They are dodecapeptides amides derived from the amino acid sequence of alpha-gliadin: P-8-6, P-8-7, P-14-9, P-20-3 and P-20-4.[5]

Sugars and their derivatives for inhibition studies were N-acetyl-D-galactosamine, D-fucose, mannan, phosphomannan, fetuin, alpha-D-glucose, D-galactose, D-mannose, D-mannitol, α-D-Lactose, D-glucosamine and N-acetyl-D-glucosamine.

Cell Lines

Following cell lines were used: K562 chronic myelogenous leukemia, RAJI B-lymphoblastoid, HT-29 colon carcinoma (epithelial), YAC-1 mouse AS/N with Moneti virus, DCH-5 dendritic cells, EL-4 lymphoma, S2 and RAW macrophages, L929

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fibroblast, P19X and P19S1801A1 teratocarcinoma cells and their derivative mutants[6]: 2C3/D5, 3A6/G7, ricin cells and RBL-2H3 macrophages were kindly provided by Dr. Draber and Draberova from Institute of Molecular Genetics, Prague.

Agglutination test was performed as described by Auricchio *et al*[3] using 1.2-2x10^7 cells/ml. In order to study the specificity of the binding in the agglutination test, subagglutinating dose of FFIII (3.5 µg/ml and PTP (7.5 µg/ml), synthetic peptides at a concentration 30 µg/ml and several sugars (2mM) were used as inhibitors of the agglutinating activity. The inhibition was done as follows: cell lines were incubated with the inhibitor for 1 h at 37°C, washed 3 times with PBS and assayed with different concentrations of gliadin peptides.

RESULTS AND CONCLUSIONS

The agglutinating activity involving a variety of different cell lines of lymphoblastoid and epithelial origin with two digested peptides of α-gliadin (FFIII and PTP) have generally shown similar interaction as ConA. K562, RAJI, YAC-1 and RBL-2H3 were also tested with α, β, γ and A-gliadin but only K562 and RAJI reacted with α and A-gliadin.

All cell lines tested were agglutinated by Frazer's Fraction III and by pepsin-trypsin pancreatic digest of α-gliadin in a concentration-dependent manner. Lymphoblastoid cell lines were very sensitive to low amount of gliadin, teratocarcinoma cells requested high concentrations (Fig. 1).

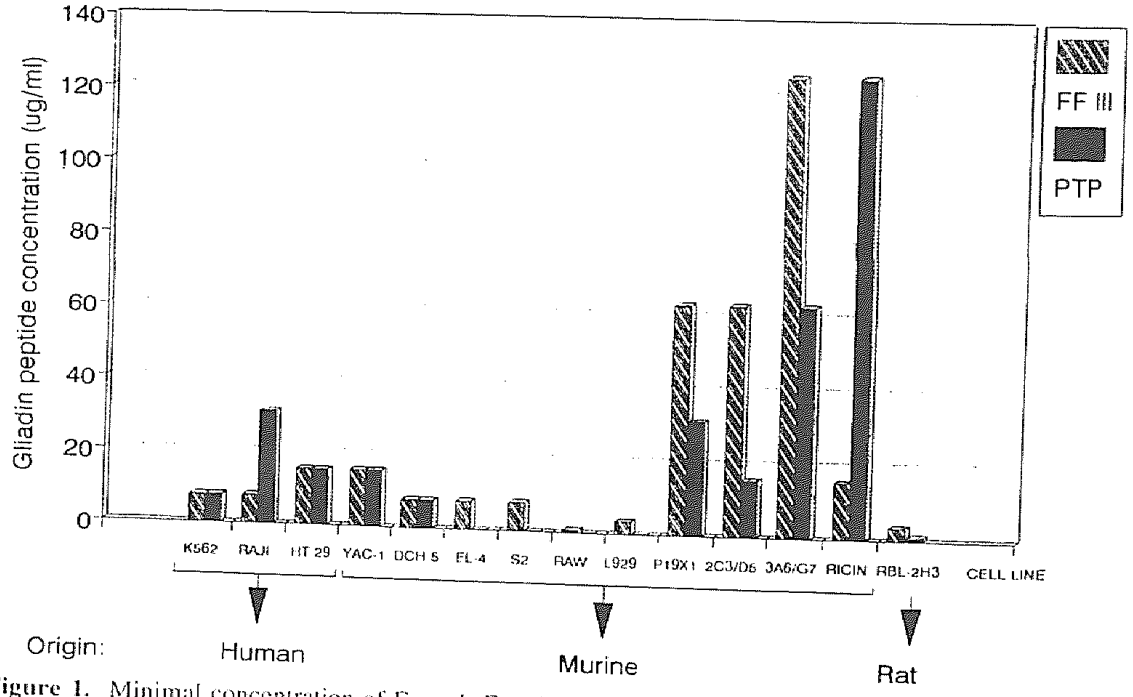

Figure 1. Minimal concentration of Frazer's Fraction III of gliadin (FFIII) and pepsin-trypsin-pancreatic digest of α-gliadin (PTP) causing the agglutination of cell lines.

The parenteral teratocarcinoma cell lines and their derivative mutants which are defective in carbohydrate epitopes showed agglutinating activity by FFIII and PTP. None of the gliadin-derived synthetic peptides agglutinated K562 cell lines nor inhibited the agglutination of these cell lines induced by FF III of gliadin and pepsin-trypsin-pancreatic digest of α-gliadin. The inhibition was not seen when natural peptides (FFIII and PTP) in

subagglutinating doses (3.5 and 7.5 μg/ml, respectively) were used with K562 and YAC-1 cells.

When we used different sugars at 2mM concentration for inhibition of agglutination, only fetuin, D-mannitol and α-D-lactose were active and inhibited agglutination of K562 with FFIII at concentrations 7.5 and 30μg/ml.

These data as well as the lack of inhibition by natural peptides (FFIII and PTP), synthetic peptides, and sugars suggest that the cell agglutination is a result of a nonspecific binding of gliadin peptides to the cell membrane.

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