In vitro influence of *Phaseolus vulgaris*, *Griffonia simplicifolia*, concanavalin A, wheat germ, and peanut agglutinins on HCT-15, LoVo, and SW837 human colorectal cancer cell growth

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Abstract

**Background/Aims**—Compared with normal colonic mucosa, lectin receptor expression is increased in hyperplastic and neoplastic tissues; some lectins have been shown to influence human colonic epithelial cell proliferation. The aim was to assess further the influence of five lectins (*Phaseolus vulgaris* (PNA), *Griffonia simplicifolia* (GSA), concanavalin A (Con A), wheat germ (WGA), and peanut (PHA-L) agglutinins) on cellular growth in three human colorectal cancer cell lines (LoVo, HCT-15 and SW837).

**Methods**—Cells were cultured in four lectin concentrations (0.1, 1−, 10, and 100 µg/ml) and growth assessed at days 2, 3, 5, and 7. The experiments were performed in media supplemented with either 1% or 10% fetal calf serum (FCS). Growth was assessed using the MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay.

**Results**—Growth in each cell line was greatly affected by at least two of the lectins tested. There was some variation in the effect of a given lectin on different cell lines. Lectin effects showed a dose-response and the greatest effects generally resulted from the highest concentrations at the longest culture time. WGA and Con A induced large effects in all cell lines; the effects of Con A were partly blocked by the higher concentration of FCS. PNA had modest and uniform stimulatory effects overall. The effects of GSA and PHA-L varied between cell lines.

**Conclusions**—The lectins studied all have the potential to affect colonic cancer growth in vitro. Many dietary lectins are resistant to digestion and may have important effects in vitro but the definition of their role in human colonic cancer biology must take into account the variability in lectin response.

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Lectins are non-immune proteins or glycoproteins that bind to specific carbohydrates expressed on the cell surface, commonly affecting cellular physiology. Little is known about the potential relation between dietary lectins and the development of cancer of the colon.1 3 Peanut butter consumption was identified as a risk factor for rectal cancer,3 but the epidemiological relation between dietary factors and colon cancer remains controversial.4 The protective effect of dietary fibre seems to be associated more with cruciferous vegetables than legumes;5 and possibly more with low vegetable fibre than cereal fibre.5 Vegetable fibre typically has several times more galactose content than cereal fibre6 7 and it is possible that a high galactose content will serve to inhibit binding of galactose binding mitogenic lectins such as peanut agglutinin (PNA).8

Some investigators have described lectin binding to the colonic epithelium. Dolichos biflorus agglutinin (DBA) and soybean agglutinin (SBA) are markers of differentiation in normal appearing colonic epithelium. *Anthemus caudatus* agglutinin (ACA) binds a cytoplasmic glycoconjugate expressed at the colonic crypt base, serving as a possible proliferation marker in the distal, but not the proximal, colon.9 Normal adult colonic epithelium expresses receptors for wheat germ agglutinin (WGA).1 Receprors for concanavalin A (Con A), are present in the proximal, but not the distal, colon.7 *Ulex europaeus-I* agglutinin (UEA-I), and PNA show little or no binding in the normal colon, but commonly bind to hyperplastic and neoplastic colonic epithelium.10 11

Increased lectin binding is often found in inflammatory and neoplastic colonic epithelium.10 11 Lectins are present in a wide range of dietary constituents14 and are highly resistant to digestion.1 8 It is therefore likely that the colonic epithelium is exposed to many lectins.1

In vitro studies have examined the effects of UEA-I, Con A, PNA, and WGA on HT29 and Caco2 human colorectal cancer cell lines,1 PNA on normal human colonic epithelium and HT29 human colorectal cancer cell lines,4 and *Griffonia simplicifolia I-A4* (GSA), lima bean lectin (LBP) and DBA on LS174t and SW1116 human colon cancer cell lines.15

Information regarding the effect of various lectins on cell culture growth is limited; media conditions, lectin concentrations, and the appropriate time in culture to assess the effects all remain undefined variables, and may be dependent on the specific lectin or cell line studied. Our aim was to obtain more infor-
mation on the effect of lectins on human colon cancer cell lines; in particular to consider some of the variables related to culture conditions.

We studied PNA, WGA, Con A, Phaseolus vulgaris agglutinin (PHA-L), and GSA. Of these, the effects of PNA are perhaps the best described. WGA is a dietary lectin and binds to colonic receptors but the in vitro effects have not been fully characterised. Con A affects fibroblast apoptosis and binds to colonic receptors; it has varying concentration dependent effects on HT29 and Caco2 human colorectal cancer cell growth. PHA-L is a common dietary lectin, isolated from red kidney beans, which is toxic but inactivated by heat. GSA was shown to be cytotoxic to LS174t and SW1116 human colon cancer cell lines.

We measured growth in the HCT-15, LoVo, and SW837 human colorectal cancer cell lines in response to four lectin concentrations (0-1, 1, 10, or 100 μg/ml). The experiments were performed in either 1% or 10% fetal calf serum (FCS). Cell growth was assessed by the colorimetric MTT (3-(4,5)-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay.

**Methods**

**CELL LINES AND CULTURE MEDIUM**

The HCT-15 (ATCC CCL-225), LoVo (ATCC CCL-229), and SW837 (ATCC CCL-237) cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The HCT-15 cell line was derived from a human colonic carcinoma. The LoVo cell line was initiated from a histologically established colon adenocarcinoma metastasis. The SW837 cell line was isolated from a rectal adenocarcinoma. The LoVo and SW837 cell lines produce carcinoembryonic antigen.

The three cell lines were adapted to grow as monolayers cultivated at 37°C in closed Falcon plastic dishes (Nunc, Poly Labo, Strasbourg, France) containing Eagle’s minimum essential medium (MEM, Gibco, Cergy Pontoise, France) supplemented with 10% FCS (Gibco) heat inactivated for one hour at 56°C. Media were supplemented with a mixture containing 0-6 mg/ml glutamine, 200 IU/ml penicillin, 200 μg/ml streptomycin, and 0-1 mg/ml gentamicin (all Gibco).

Cells received from ATCC were considered passage “0” and were amplified through two passages after which they were stored in liquid nitrogen. All experiments were performed using cells removed from this repository after an additional four passages.

**LECTINS**

The five lectins studied were Phaseolus vulgaris (PHA-L, which binds to N-acetylgalactosamine β1,2 mannose triantenate-complex oligosaccharide), Griffonia simplicifolia I-Ag (GSA, which binds to terminal α-N-acetylgalactosaminyl (GalNAc) groups), peanut (PNA, which binds to terminal galactose-β1,3-N-acetylgalactosamine), wheat germ (WGA, which binds to N-acetylgalcosamine and neuraminic acid), and Concanavalin A (Con A, which binds to mannose and glucose). All were obtained from Vector Laboratories Inc (Burlingame, CA, USA).

**EXPERIMENTAL SCHEDULE FOR CELL CULTURE**

In each experiment the three cell lines were incubated in lectin free medium for 24 hours to ensure adequate plating. After this, cells were incubated for two, three, five, and seven days in a control condition (lectin free), and one supplemented with either 0-1, 1-0, 10, or 100 μg/ml of each of the five lectins. Each experiment was separately performed in MEM supplemented with 1% and 10% FCS.

Confluent growth for the three cell lines began nine days of culture (data not shown).

Each experiment was repeated six times.

**ASSAYS OF GROWTH**

Growth was assessed using the MTT assay, for which a detailed protocol has been described. Growth assessment is based on the capability of living cells to reduce the yellow product (MTT) to a blue product, formazan, by a mitochondrial reduction reaction. The number of living cells is directly proportional to the intensity of the blue colour, which is quantitatively measured by spectrophotometry at 570 nm.

In view of criticism of this method we ensured the accuracy of our technique by the use of appropriate controls, by comparison of our results with those obtained by genomic incorporation of tritiated thymidine, and by direct cell nuclei counting. These results were highly concordant (see Fig 2); similar results in previous studies corroborate the validity of our technique.

An internal control of the methodology was carried out as detailed later.

**CYTOCHEMICAL CHARACTERISATION OF LECTIN BINDING PATTERN**

Aliquots of a suspension containing exponential growth phase cells were plated in Petri dishes containing a glass coverslip and cultured for 72 hours in MEM containing 1% FCS, after which they were immersed in buffered formalin. Biotinylated lectin was applied to the coverslip and counterstained as previously described. Each culture condition was replicated five times. For each combination of cell line and lectin, a control was created in which the biotinylated lectin was incubated in a solution containing 0-2-0-3 M of its specific inhibitory sugar (Janssen Chemica, Beere, Belgium) before its application to the coverslip.

Lectin staining was quantified in 200 cells per coverslip (1000 cells per cell line) using a SAMBA 2005 cell image processor (Alcatel-TTIN, Grenoble, France). A JVC (KY15) colour camera mounted on a Leitz Diaplan microscope equipped with a 20× magnification lens interfaces the processor. For each
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Results

CYTOCHEMICAL CHARACTERISATION OF LECTIN BINDING PATTERN
Each cell line exhibited significant binding to the five lectins, with a binding pattern globally similar in the three cell lines (Fig 1).

CONTROL EXPERIMENT FOR CELL GROWTH ASSESSMENT
The experiments performed to validate the MTT method by comparison with counting Feulgen stained nuclei showed comparable (p>0.05) results with both the MTT colorimetric assay (Fig 2A) and cell counting (Fig 2B); we conclude the MTT method used is valid.

CHARACTERISATION OF THE LECTIN INDUCED MODIFICATION OF CELL GROWTH
Figures 3 (HCT-15 cells), 4 (LoVo cells) and 5 (SW837 cells) show the lectin induced effects on growth in MEM supplemented with 1% FCS. In control conditions, SW837, HCT-15 and LoVo cells exhibited log growth until the fifth day of culture. On the ninth day of culture (data not shown), growth was confluent.

Phaseolus vulgaris agglutinin (PHA-L)
PHA-L in high concentrations tended to inhibit growth in the three cell lines; this was least noticeable in the SW837 cell line, in which lower concentrations promoted growth. Increasing the media concentration of FCS

Figure 2: Assessments of cell growth in the HCT-15, LoVo, and SW837 cell lines by means of the MTT colorimetric assay (Fig 2A) and the direct cell count of Feulgen-stained nuclei (Fig 2B). The influence exerted on the cell growth by 100 µg/ml Con A (grey bars) and 100 µg/ml PHA-L (black bars) in comparison with control (open bars) was assessed by both techniques. No significant difference (p>0.05) was found between the techniques.
The influence of five lectins, PHA-L, Con A, PNA, GSA, and WGA, on HCT-15 cell growth was determined at four concentrations, 0.1 (□), 1.0 (■), 10 (▲), and 100 (●) µg/ml. Cell growth was determined on the second, third, fifth, and seventh days and compared with control conditions. Each experimental condition was replicated six times. The control condition for each day of culture is represented by the horizontal line labelled “0%.” The SEM of the each controls mean is represented by the dashed lines parallel to this line. Modification of growth for each day of culture is expressed as a percentage gain or loss relative to the respective control, permitting a global comparison of all experimental conditions. The SEMs of the means percentage change are shown by error bars; in most cases this deviation is so small as to be imperceptible on the graph.

Figure 3: Cell growth by means of the MTT assay in the HCT-15 human colorectal cancer cell line. The influence of five lectins, PHA-L, Con A, PNA, GSA, and WGA, on HCT-15 cell growth was determined at four concentrations, 0.1, 1.0, 10, and 100 µg/ml. Cell growth was determined on the second, third, fifth, and seventh days and compared with control conditions. Each experimental condition was replicated six times. The control condition for each day of culture is represented by the horizontal line labelled “0%.” The SEM of the each controls mean is represented by the dashed lines parallel to this line. Modification of growth for each day of culture is expressed as a percentage gain or loss relative to the respective control, permitting a global comparison of all experimental conditions. The SEMs of the means percentage change are shown by error bars; in most cases this deviation is so small as to be imperceptible on the graph.

tended to increase the growth response seen in the lower concentrations used.

Low concentrations of PHA-L induced no significant modifications in HCT-15 cell growth. High concentrations significantly decreased growth (p≤0.001). This effect was not seen in 10% FCS media (data not shown). The highest concentration of PHA-L significantly inhibited LoVo growth (p≤0.001). In MEM supplemented with 10% FCS, there was a tendency to mitigate the modest inhibitory effects of the lower concentrations (data not shown). The highest concentration of PHA-L suppressed SW837 cell growth, whereas lower
Concentrations induced a significant (p ≤ 0.05 to p ≤ 0.001) increase. In MEM supplemented with 10% FCS, there was exaggeration of both stimulatory effects of lower concentrations and the inhibitory effects of higher concentrations (data not shown).

**Concanavalin A agglutinin (Con A)**

High Con A concentrations consistently inhibited growth, whereas lower concentrations had little effect. Increasing the FCS concentration reversed the observed inhibition.

Con A significantly (p ≤ 0.001) decreased HCT-15 cell growth at both 10 and 100 μg/ml concentrations in cultures containing 1% FCS; this effect was reversed in the 10 μg/ml Con A concentrations when cells were cultured in 10% FCS (data not shown). At low concentrations - 0.1 and 1.0 μg/ml - Con A transiently stimulated (p ≤ 0.05 to p ≤ 0.001) growth of HCT-15 cells from the fifth to the seventh day of culture in a medium with low FCS concentration; this effect was not seen in medium supplemented with 10% FCS (data not shown).
In the LoVo cell line, Con A induced a pronounced (p<0.001) decrease in growth at both 10 and 100 μg/ml concentrations as early as the third day of culture in medium containing 1% FCS. Medium supplemented with 10% FCS negated the effect in the 10 μg/ml concentration (data not shown).

SW837 cell growth was inhibited at both 10 and 100 μg/ml concentrations in 1% FCS supplemented MEM; lower concentrations (0.1 and 1.0 μg/ml) greatly stimulated SW837 cell growth. Increasing FCS supplementation from 1% to 10% abolished the stimulatory effects of low Con A concentrations on SW837 cell growth as well as the 10 μg/ml induced inhibitory effect (data not shown).

Peanut agglutinin (PNA)
The effects of PNA on cell growth in these three cell lines were globally modest and alteration of the media supplementation with FCS had little effect.

PNA slightly stimulated (p≤0.05 to p≤0.01) the growth rate at the fifth day of culture, becoming inhibitory at the end of the

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Figure 5: Cell growth in the SW837 human colorectal cancer cell. The rest of the legend is identical to that of Fig 3.
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experiment for HCT-15 cells cultured in 1% FCS supplemented medium. All statistically significant growth modifications were obliterated by increasing the FCS concentration (data not shown).

In the LoVo cell line, PNA induced no significant stimulatory growth effect regardless of the FCS supplementation concentration. PNA induced only weak inhibitory effects at the highest concentration tested (100 μg/ml) in both 1% and 10% (data not shown) FCS supplemented MEM.

PNA stimulated (p≤0.05 to p≤0.001) SW837 cell growth in both 1% and 10% (data not shown) FCS supplemented media, with a more pronounced effect in 1% than 10% FCS. High PNA concentration (100 μg/ml) was without effect at either concentration of FCS supplementation.

Griffonia simpligifolia I-A4, agglutinin (GSA)
GSA had varied effects, most pronounced in the LoVo cell line. Changes in the concentration of FCS in the media had little influence on growth response.

GSA induced modest (p≤0.05) stimulation of HCT-15 cell growth at low concentrations. At 100 μg/ml, growth was inhibited, an effect negated by increasing the concentration of supplemental FCS (data not shown).

The highest GSA concentrations (10 and 100 μg/ml) significantly (p≤0.05 to p≤0.01) stimulated LoVo cell growth at the beginning of culture, and became inhibitory at the end of culture in 1% FCS supplemented MEM; the lower concentrations were without apparent effect (p>0.05). Increasing the concentration of FCS supplementation had little modulatory effect (data not shown).

The 0.1 and 10 μg/ml concentrations of GSA significantly (p≤0.05 to p≤0.001) stimulated SW837 cell growth in both 1% (Fig. 5A) and 10% FCS supplemented MEM. The 100 μg/ml GSA concentration was without effect at either concentration used.

Wheat germ agglutinin (WGA)
WGA at high concentrations (10 and 100 μg/ml) was highly (p≤0.001) inhibitory to HCT-15 growth in both 1% and 10% (data not shown) FCS supplemented media. Low concentrations (0.1 and 1-10 μg/ml) only slightly modified HCT-15 growth.

WGA at low concentrations (0.1-10 μg/ml) significantly (p≤0.05 to p≤0.001) stimulated LoVo cell growth at the beginning of culture in both 1% and 10% (data not shown) FCS supplemented media. At the end of the experiment in media supplemented with 1% FCS, WGA significantly (p≤0.01 to p≤0.001) inhibited LoVo cell growth. The 100 μg/ml WGA concentration was highly (p≤0.001) inhibitory.

WGA at 0.1 or 1-10 μg/ml concentrations significantly (p≤0.05 to p≤0.001) stimulated SW837 cell growth at the end of the experiment in both 1% and 10% (data not shown) FCS supplemented media. At 10 and 100 μg/ml concentrations, WGA was highly (p≤0.001) inhibitory to SW837 growth.

Discussion

Many lectins are potent exogenous growth signalers, mimicking the action of metabolic hormones. This biological activity is a function of lectin structure, through recognition and binding to specific carbohydrate receptors expressed on cellular membranes. In some instances, internalisation of the lectin may be required.

Lectin receptor expression is increased in hyperplastic and neoplastic colonic mucosa, allowing interaction with certain dietary lectins which are resistant to digestion and which would otherwise pass through the normal colon without binding. Assuming that these lectins possess a mitogenic effect, changes in lectin receptor expression could effect regulation of epithelial growth and subsequent malignancy. Thus PNA, the receptor (the Thomsen-Friedenreich antigen) of which shows increased expression in hyperplastic and neoplastic colonic epithelium promotes growth of the human colon cancer cell line HT29. However, whereas data obtained from FITC (fluorescein isothiocyanate) labelled lectins show that PNA receptor expression in the Caco2 neoplastic colon cell line is greater than in HT29, PNA has no apparent mitogenic effect on the Caco2 cell line. It must be emphasised that in their study, Ryder et al showed that the PNA binding cell surface glycoproteins on Caco2 are of different molecular size to those found in HT29 cells and in a resected human colon cancer, which might explain the apparent discrepancy.

Our data show that PNA increased the growth rate in only one (SW837) of the three cell lines studied. Increased growth effects attributable to PNA in HT29 (data not shown) FCS-supplemented (HCT-15 and LoVo) was marginal or absent.

Whereas PNA induced modifications to HT29 growth have been reported as lacking an inhibitory effect even at high concentrations, our results suggest that PNA at high concentrations inhibits growth in LoVo and HCT-15 cell lines. This inhibitory PNA mediated effect was blunted when FCS supplementation was increased in the culture media. The possibility remains that the FCS contained significant amounts of galactose; we noticed that PNA induced modifications to SW837 growth could be prevented by the addition of 10 mM lactose (data not shown). This supports a finding made by Sanford and Harris-Hooker, who studied the mitogenic effect of PNA in smooth muscle and pulmonary arterial cells. The Rhodes group also showed that 25 μg/ml of the edible mushroom Agaricus bisporus lectin (ABL) inhibited the growth of HT29 and Caco2 colon cells. Lower concentrations (10 μg/ml) completely negated the stimulatory effect of PNA (25 μg/ml) on HT29 growth. ABL is a dietary Gal β1,3-GalNAc binding lectin differing from PNA in its ability to bind to sialeylated Gal β1,3-GalNAc.
Concluding our results and those of others, it seems that PNA exerts a mitogenic effect in fewer than half the cell lines tested. Ryder et al. showed a similar growth effect of PNA on cultured human colonic mucosa. This implies that it may be useful to investigate further the difference in PNA binding glycoproteins of the different cell lines as this may provide additional information concerning the proliferative response. In addition, the amount of PNA receptors in a given colon cancer varies greatly during its development, a finding we made with human colorectal cancers grafted on to nude mice. Sialyl residues may occupy the terminal position in both N-linked and O-linked oligosaccharides, masking the penultimate β-Gal or Gal β1,3-GalNAc and thus mitigating the potential for lectin binding. No difference (p>0.05) in PNA lectin binding was found, however, before or after treatment of our three cell lines with 10 mM neuraminidase.

Chen et al. showed that GSA inhibited human LS174t and SW1116 colorectal neoplastic cell development. They also reported that two other lectins recognising α-GalNAc residues (DBA and LBL (lima bean agglutinin from Phaseolus lunatus)), were significantly less cytotoxic to colon cancer cells than GSA. In the present study we noted that GSA stimulated cell growth in the SW837 cell line at both low and high concentrations. The LoVo model it promoted growth at low concentrations but inhibited growth at high concentrations. For HCT-15 cell growth, GSA had no effect at low concentrations, inhibiting growth at high concentrations dependent on FCS concentration. These GSA mediated effects were specific as they were inhibited by the presence of 10 mM GalNAc (but not 10 mM N-acetylgalcosamine).

Considering our results and those of Chen et al., GSA significantly inhibits growth at high concentrations (≥10 μg/ml) in three of five cell lines (LS174t, SW1116, and LoVo), weakly inhibiting one (HCT-15), and a similar concentration stimulated one (SW837). Chen et al. did not study low concentrations (≤1 μg/ml) of GSA, which we found significantly stimulated growth in both the LoVo and the SW837 models.

Human colorectal cell lines behave heterogeneously with respect to the GSA mediated influence on their growth rate, as indeed they do with respect to PNA. These results are not surprising considering that many colorectal cell lines display large growth variations in response to treatment with various hormones or growth factors.

With respect to the growth effects of WGA and Con A, our results are similar to those reported by Ryder et al. using HT29 and Caco2 cell lines. Considering both studies, WGA and Con A systematically inhibited growth in the five cell lines independently of the FCS media supplementation, when high concentrations (≥10 μg/ml) were used. At low concentrations, WGA stimulated growth of HT29, LoVo, and SW837 cells, while having no effect on Caco2 and HCT-15 cells. Similar concentrations of Con A affected the HT29, HCT-15, and SW837 cell lines, whereas Caco2 and LoVo exhibited no sensitivity.

PHA-L, the fifth lectin under study, induced modifications in HCT-15, LoVo, and SW837 cell growth similar to those found in the case of WGA.

In conclusion, our data with that published by the Rhodes and Boland groups, PNA stimulates growth in some, but not all, neoplastic colon cell lines. Whereas GSA is exclusively reported as an inhibitor of neoplastic colon cell growth, low concentrations of GSA stimulated growth in two of the three cell lines used in our study. The remaining lectins, Con A, WGA, and PHA-L, exhibited a similar positive effect on neoplastic colonic cell lines; they either stimulated or had no effect at low concentrations, but systematically exhibited inhibitory effects in high concentrations.

The possibility that high lectin concentrations could be directly cytoxic is a topic of a current investigation using image cytometry of Feulgen stained nuclei to define cell cycle kinetics. Preliminary results defining the relative proportions of the cell cycle in the cell lines represented here suggest that high concentrations of Con A suppress growth, but increase S phase activity compared with controls in the surviving cell population. GSA, PNA, PHA-L, and WGA tend to inhibit growth by accumulation of the surviving cell population in the G1 phase. Additional studies are required to define the relation between cellular proliferation and lectin mediated apoptotic effects.

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