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Comparison of Lectin Induced Chemotactic Selection and Chemical Imprinting in *Tetrahymena pyriformis*

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Summary. In case of hormonal (chemical) imprinting, the first encounter of a mixed cell population with a bioactive molecule provokes changes in the receptor-signal transduction system. In case of chemotactic selection, from the mixed cell culture the chemotactically most affine cells are selected and maintained. In the present experiments chemotactic effects elicited by three lectins (Concanavalin-A = Con A, lens, *Helix*) were studied in *Tetrahymena pyriformis* GL unicellular model, for comparing the effect of imprinting and selection. *Tetrahymena* (without selection or imprinting) showed a molecule-dependent (positive) chemotactic reaction to lectins. Subpopulations gained by chemotactic selection were inducible in a ligand-specific scale with preference of [a-Man-a-Glu] or N-Ac-Gal specificity lectins, producing a significant affinity to *Helix* and non-significant affinity to Con A, and negative chemotaxis to lens). Similar results were shown in case of selection and imprinting with Con-A (except the significant affinity of mixed population), nevertheless in case of *Helix* and lens lectins (having similar, however not identical sugar specificity) differences had been observed between selection and imprinting. These accordance's and deviations of results in imprinting and selection studies suggest that chemotaxis receptors share functional moieties with lectin receptors of unicellular organisms. The experiments call attention to the possible role of selection in the development of imprinting however, this is dependent on the nature of the imprinter molecule.

Key words: chemotactic selection, chemotaxis, hormonal imprinting, lectin, *Tetrahymena pyriformis* GL.

INTRODUCTION

Lectins are multifunctional monovalent or complex peptide-type substrates possessing carbohydrate specificity. Wide ranges of biological entities (plants, animals, bacteria or viruses) have the ability to synthesize these molecules. Their roles are very diverse as some of these ligands are essential intracellular regulatory components of the protein traffic and sorting in the rER (L-type lectins, calnexin) or in the Golgi/post-Golgi system (P type lectins), while others have role in the ER associated degradation of glycoproteins (M-type lectins). Some lectins have the ability to influence the turnover of glycoproteins; others are effector molecules of enzyme targeting (R-type lectins). Other distinct group of lectins have cell surface or extracellular matrix associated functions: several types of lectins contribute to the cell adhesion (e.g. C- and I-type lectins); others are members of the innate immunity (cell lectins) or promote

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cross-linking between glycan components (galectins) of the extracellular matrix (Drickamer and Taylor 1998).

Extracellular activities of lectins with their specific recognition of saccharide components provides a primordial, "immune system"-like significance of these molecules in organisms possessing no genuine humoral and cellular immune network (e.g. sponges, tunicates). Other basic cell-physiological functions like phagocytotic behaviour, the target reaction of chemotaxis, and growing of cells are also influenced by lectins at higher and lower levels of phylogeny (Agrell 1966). Chemoattractant or chemorepellent moieties of lectins are essential even on these levels; distinct subpopulations of cells are mobilized or localized by them. This activity of lectins is also detectable at unicellular level; animal and plant type lectins - *Helix*, Con A etc., can induce or suppress chemotactic responses of the eukaryotic ciliate *Tetrahymena pyriformis* (Kőhidai and Csaba 1996*)*. Ciliary membrane of these cells possess numerous binding sites for lectins (Pagliaro and Wolfe 1987, Csaba and Kovács 1991, Kovács *et al*. 1995, Driscoll and Hufnagel 1999), in one of the experiments sixteen Con-A binding polypeptides were demonstrated (Dentler 1992), in an other experiment the Con-A binding protein seemed to be a 66 kDa glycoprotein (Leick *et al.* 2001). Lectins are also applied to follow changes of carbohydrate composition or membrane fluidity in the ciliate surface membrane as a result of treatments with bioactive substances (Kőhidai *et al*. 1986).

Tetrahymena is a frequently used model cell in the study of cellular signaling (Csaba 1985,1994). Its homologies to the higher vertebrates is present at membrane receptor level (i.e. insulin receptor) (Kovács and Csaba 1990a, b, Christopher and Sundermann 1995, Leick *et al*. 2001, Christensen *et al*. 2001) in intracellular second messenger systems as cAMP (Csaba and Lantos 1976), cGMP (Kőhidai *et al.* 1992), Ca-calmodulin system (Schultz *et al.* 1983; Kovács and Csaba 1987a, b), inositol lipids (Kovács and Csaba 1990a, b) and in homologies of hormonally influenced metabolic responsiveness (Kőhidai and Csaba 1985). On the basis of the mentioned characteristics was observed the hormonal imprinting, which develops at the cell's first encounter with a biologically active molecule and persists also after hundreds of generations. As a result of imprinting the binding capacity of receptors is influenced permanently as well, as signal transduction and responsiveness (Csaba 1994, 2000).

Chemotactic activity of these unicellular ciliates is one of the most essential cell-physiological properties. They can distinguish slight diversities of small and relatively big ligands i.e. amino acids (Levandowsky *et al.* 1984) and insulins (Csaba *et al.* 1994). Chemotactic selection, a recently developed technique provides the possibility to select these cells upon their chemotactic responsiveness towards bioactive molecules (e.g. hormones or chemokines) (Kőhidai and Csaba 1998, Kőhidai *et al.* 2000). Selected subpopulations are dedicated groups of cells for studying backgrounds of chemotaxis, short- and long-term signalling in eukaryotic models (Kőhidai 1999).

On the basis of the above described phylogenetical and cell-physiological characteristics of lectins in the present study our objectives were: (i) to study ability of lectins to select subpopulations *via* their chemotactic potency; (ii) to study the effect of pretreatments (imprinting) with lectins on the chemotactic responsiveness of subpopulations; (iii) to compare the results of imprinting and chemotactic selection.

MATERIALS AND METHODS

Cells and culturing

Cells of *Tetrahymena pyriformis* GL strain were maintained in axenic cultures containing 1% tryptone (Difco, Michigan, USA) and 0.1% yeast extract, without addition of antibiotics, at 28 °C. According to the certificate analysis, the two basic components of media were free of lectins. However, the potential of interference of the used lectins with the media composing peptides is given, the non-synthetic type of media was used as our earlier experiments (Kőhidai and Csaba 1998, Kőhidai 1999) and reference works of the chemotaxis-literature of *Tetrahymena* were done in such systems (Almagor *et al.* 1981, Francis and Hennessey 1995, Kuruvilla and Hennessey 2001). Cultures were in logarithmic phase of growth; density of samples was 10^4 cells/ml.

Chemicals and buffers

The studied lectins were purified from plants *Canavalia ensiformis* (Con A); *Lens culinaris* (lens) and snail *Helix pomatia* (*Helix*). All the three lectins were obtained from Sigma Chemical Co., St. Louis, MO, USA. In the experiments (PBS), 0.05 M phosphate buffer containing 0.9% NaCl at pH 7.2 was also used.

Chemotaxis assay

The chemotactic ability of cells was determined in a two-chamber capillary assay system modified by us (Kőhidai *et al.* 1995). According to this setup tips of a multi-8-channel automatic pipette served as an inner chamber to minimize the standard error of sampling, while microtitration plates were used as outer chambers. The outer chamber was filled with the cells to be tested; the inner one contained the test substance of the lectins. In control experiments culture medium was

used as attractant (absolute control). For validation of the assay two chemoattractants 10^{-9} M f-Met-Leu-Phe (f-MLF; Sigma, St. Louis, USA) and 10^{-11} M interleukin-8 (IL-8; Promega, Madison, USA) were also tested as positive controls. After 15 min incubation the samples of inner chambers, containing the chemotactically positive responder cells, were fixed in 4% formaldehyde containing PBS. The samples were evaluated in a Neubauer hemocytometer.

Chemotactic selection

This technique deals with the chemotactic capacity of different signal molecules to form sub-populations from mixed cultures of cells. First we applied the chemotaxis assay (see above). The inner chamber of the system contained the test substance of the lectins (10^{-9} M in case of Con-A and 10^{-6} M in case of lens and *Helix*). In control experiments culture medium was used as attractant (absolute control). At the end of incubation, the positive responder cells were transferred to fresh culture medium for cultivation. Both cultures selected with a lectin (in general L) and controls (C) were consecutively transferred in s determined every 48 h. The chemotactic response of cultures was studied again after one week in the following combinations: L/L - cells selected with the lectin in the first run and assayed to the signal substance in the second run; L/C - cells selected with the lectin in the first run and assayed to the control substance in the second run; C/L - cells selected with the control substance in the first run and assayed to the signal substance in the second run; C/C - cells selected with the control substance in the first run and assayed to the control substance in the second run.

Pretreatment (imprinting) with lectins

Tetrahymena cells were pretreated with 10^{-6} M lectins (Con A, lens and *Helix*) for 1 h at room temperature. Controls were treated with fresh culture medium. After treatments the samples were washed trice with PBS and then aliquots of cell-suspensions were transferred into fresh culture medium. Pretreated and control cultures were transferred consecutively in every 48 h. After one week chemotactic responsiveness of cultures was tested by the chemotaxis assay described above. Similar combinations were applied as in the chemotactic selection study: C/C, C/L, L/C, L/L (abbreviations are given above).

Statistical evaluation

Each lectin was tested in ten replica assays, the figures demonstrate the averages of these results. The statistical analysis was done by ANOVA of Origin 4.0.

RESULTS AND DISCUSSION

In an earlier experiment (Kőhidai and Csaba 1996) all the three investigated lectins proved to have chemoattractant ability, however the concentration optimum was characteristic to each chemoattractant ligand (Table 1). The effective chemoattractant concentration was wide in the case of both plant lectins. In the case of the Con A - possessing a-mannose and a-glucose specificity - a two-peak (10^{-11} and 10^{-6} M) dose-dependency was detected with the peak at 10^{-9} M. In contrast, the a-mannose specific lens lectin had a wide range chemoattractant character, its maximal chemoattractant effect was elicited only in the higher concentration range at 10^{-6} M. Profile of concentration dependency of chemotaxis elicited by the N-acetyl-galactose-amine specific *Helix* lectin was similar to lens, its maximal chemoattractant effect was observed also at the highest tested concentration (10^{-6} M). In this study *Helix* proved to be the only chemorepellent lectin, this negative responsiveness of the model cells was elicited at 10^{-11} M. The most positively effective concentration was chosen for the chemotactic selection assay in each case.

Table 1. Concentration course study on chemotactic activity of three lectins in *Tetrahymena pyriformis* GL cells. (x- $p<0.05$; y- $p<0.01$; z- $p<0.001$)

Conc. log molar [M]	Con A [%]	Lens [%]	Helix [%]
-12	120^{x}	127^{y}	106
-11	109	109	79
-10	128^{x}	154^{y}	98
-9	159^{y}	172^{y}	131^{x}
-8	144^{y}	209^{z}	137^{x}
-7	131^{y}	190^{y}	167^{y}
-6	150^{y}	227^{z}	181^{y}

Chemotactic selection provides the possibility to distinguish signaling mechanisms used by different ligands. In classical way chemotaxis is a result of a specific interaction of ligands and their receptors in the plasma membrane. As a consequence of the downstream signaling in the cytoplasm reorganisation of cytoskeletal network is triggered and migratory behaviour of cells is changed. Dynamic presence of chemotaxis-receptor components can determine consistence of chemotactic responsiveness in progeny generations, unstable structure or fast turn-over of some binding sites make them responsible for "short-term" chemotactic responsiveness, while other fixed, constant and genetically well preserved units of the plasma membrane might work as receptors of "long-term" acting chemoattractant ligands. During chemotactic selection the most responsive cells are selected and maintained, forming a special group of the original cell population.

In the present study the three lectins could select subpopulations chemotactically in different ways (Fig. 1). Chemotactic responses were detected in sub-

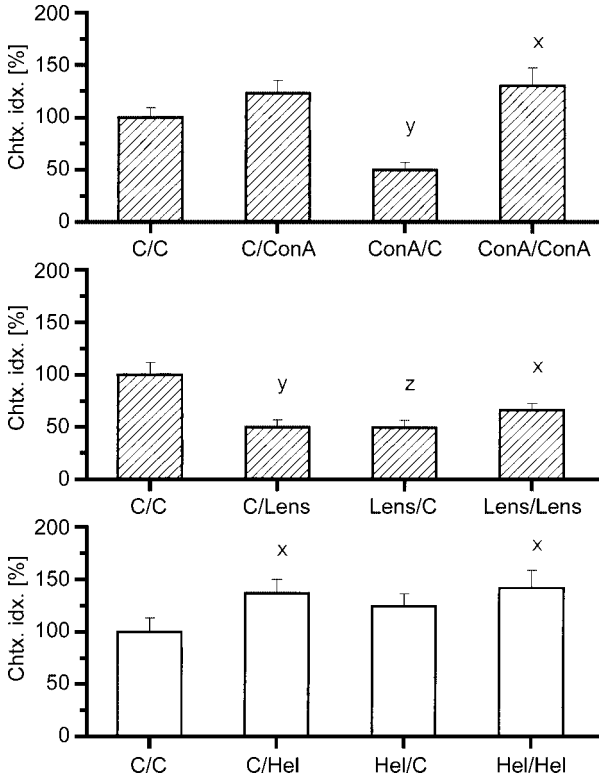

Fig. 1. Chemotactic selection of *Tetrahymena* cells with lectins (Con A, lens, *Helix*). Chemotactic responsiveness of cells selected with the identical lectin (L) or the control substance (C) was assayed in four combinations: the first letter refers to the type of ligand applied at selection; the second letter refers to the ligand used at the second chemotaxis assay 1 week after selection. (x- $p<0.05$; y- $p<0.01$; z- $p<0.001$).

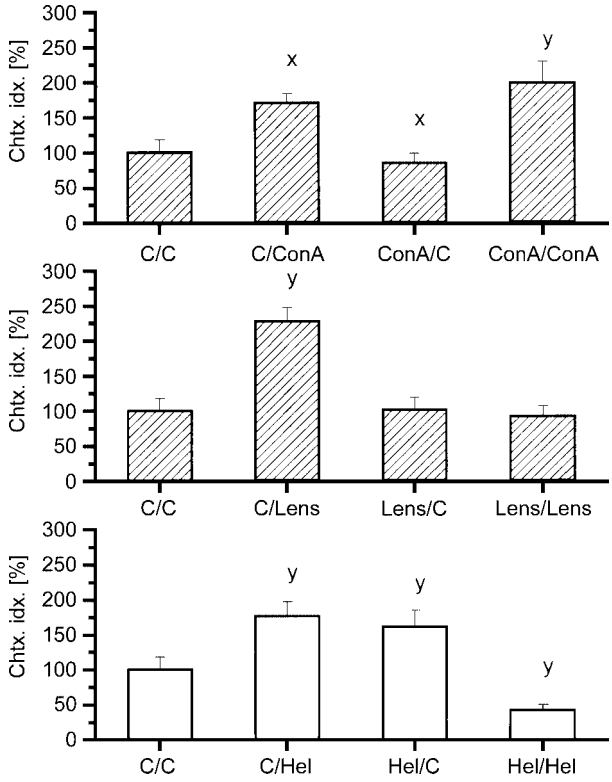

Fig. 2. Effect of imprinting with 10^{-6} M lectins (Con A, lens, *Helix*) on *Tetrahymena*. Chemotactic responsiveness of pretreated cells with the identical lectin (L) or the control substance (C) was assayed in four combinations: the first letter refers to the type of ligand applied at pretreatments; the second letter refers to the ligand used at the second chemotaxis assay 1 week after pretreatments. (x- $p<0.05$; y- $p<0.01$; z- $p<0.001$).

populations *selected with the plain culture medium*. We considered these results as relative controls of the successful chemotaxis assay with the identical lectin. However, it was found that the chemotactic potency and activity of the subpopulations selected with chemotaxis was characteristically different. In control-medium-selected cells, the control medium itself (composed of tryptone and yeast extract) was observed to have a potent selector effect on *Tetrahymena*. Comparing the results of our selection a study determining different chemotactic responses of *Tetrahymena* with varying concentration courses of the same lectins shows that the response of cells selected with this control substance was ligand-dependent. In the present experiment the responsiveness was decreased with Con A (159% *vs.* 122.9%; not significant) and lens (227 *vs.* 49.8; $p<0.01$) and increased with *Helix* (136.9%; *vs.* 181.2% $p<0.05$).

Subpopulations selected with lectins expressed also ligand-dependent responsiveness. Chemotactic responsiveness of subpopulations selected with Con A or lens sharing in ligand-specificity to a-mannose was significantly decreased (Con A/C and lens/C), while a moderate, increased chemotactic ability (124.7%) was detected in subpopulations selected with the oligosaccharide specific *Helix* lectin (Hel/C).

Results of the repeated encounter with the selector lectin showed that the diverse membrane level changes responsible for different forms of chemotactic responsiveness in selection studies are detectable even for lectin-type ligands. In Con A selected group (Con A/Con A) the identical lectin could elicit a statistically significant and increased chemotactic response, while lens lectin (lens/lens) could not induce positive response, in contrast, the selected subpopulation showed negative chemotactic behaviour to the lens lectin. Subpopulations selected with *Helix* lectin expressed positive chemotaxis towards the selector ligand, however this activity of cells was not enhanced compared to their relative controls

(Hel/Hel = 142% *vs*. Hel/C = 124.7% or C/Hel = 136.9%). The considerable range in the effect of the two monosaccharide specific lectins (Con A/Con A = 129.9% *vs*. lens/lens = 66.2%) suggests that in the fine adjustment of chemotactic signaling carbohydrate moieties of the membrane play also important role. Data obtained suggest that in our ciliated model a concurrent a-mannose and a-glucose binding is required for induction responses of long-term selection, while the single a-mannose specific binding fails to do so. On the other hand, relative positive effectiveness of *Helix* lectin raise the possibility that plasma membrane is furnished with constant, however non-inducible constituents e.g. N-acetyl-galactose-amine residues.

While in case of chemotactic selection a rapid incubation is used to assort cells from a mixed population with high responsiveness, in case of imprinting (pretreatment) with biologically active substances are still applied to a mixed population to tune/adjust cells in a time- and concentration-dependent way, however the investigated cultures are still mixed. Series of former experiments proved that *Tetrahymena* is a sensitive model for imprinting; long-lasting or transient modifications of the plasma membrane were characterized in binding studies with hormones (Kovács *et al.*1984) or lectins (Csaba and Kovács 2000). In the present experiment our goal was to evaluate whether the lectin induced chemotactic selection and imprinting have homologies or deviations in signaling.

In this setup chemotactic responsiveness of lectin-pretreated cells was tested to the identical lectin used for imprinting or to the fresh culture medium as control. To validate the experiments an absolute control (C/C) and a second control (C/L) were applied, in which the chemoattractant ability of the used lectin was tested. Proper sensitivity of our system was confirmed by the significant positive chemotactic indices of all the three second controls (C/Con A, C/lens and C/Hel) (Fig. 2).

In case of Con A, imprinting had dual effect: the pretreated cells expressed an increased chemotactic responsiveness to Con A at the second encounter (Con A/Con A=198,4%), while the plain culture medium was recognized as chemorepellent (Con A/C = 84,6%).

Lens lectin could not induce long-lasting positive effects like Con A. Pretreatment with lens abolished the positive chemotactic responsiveness of *Tetrahymena* cells to the lectin (lens/lens = 92,3%), nevertheless a decrease in the native chemotactic ability of the lens pretreated cells (lens/lens = 92,3% or lens/C = 101,2% *vs*. C/lens = 227,2%) was also detected.

Pretreatments with *Helix* lectin presented also a unique and characteristic outcome. In contrast Con A and lens lectins the native chemotactic activity of the *Helix* pretreated cultures (Hel/C = 160,7%) was enhanced, whereas we failed to apply the identical lectin as a chemoattractant ligand, it worked as a very strong chemorepellent substance (Hel/Hel = 42%), what means that a negative imprinting developed.

As it was presented above, interaction of the three lectins with the target cells has a long lasting effect considering their chemotactic ability. Results of repeated encounters with the ligand applied at the pretreatment suggest that lectins possessing slight diversities in carbohydrate specificity (see variance of a-glucose specificity of Con A to lens) can result an increase or decrease of chemotaxis receptors however, the diversity in the protein configurations of lectins also have to be considered, as this can also influence the affinity or repellence. This means that only the comparison of the results gained by imprinting or selection leads to clear and exact conclusions, the results of inter-lectin comparison suggest only possible explanations. One of this explanations is that while selection of sensitive cells might have a role in the development of imprinting (e.g in case of Con-A), this is not regular, as other factors also could play a role, which turned to negative imprinting of the positive selection (e.g. in case of *Helix* lectin).

Cultures of the present study were maintained for one week after pretreatments, the reexposed cells, due to the short cell cycle of *Tetrahymena*, were ~70th generation of the culture, this made possible to study long-term persisting receptors and the associated activities. Potential significance of lectin receptors in chemotaxis- working as a probable subclass of chemotaxis receptors - is underlined in two reasons (i) the investigated group of lectins could characteristically induce the expression of saccharide components of the plasma membrane capable as components of the responsible binding sites (e.g. coexistence of a-mannose and a-glucose is required for the positive outcome) and (ii) can elicit chemotactic responses in a high carbohydrate-specific manner (e.g. the amino-sugar N-acetyl-galactose-amine linked signaling could "freeze" the required chemotactic pathways).

Comparison of results gained by chemotactic selection and pretreatment show that ligands e.g. lectins, possessing deep phylogenetical backgrounds have molecule-specific potency to act as chemotactic agents. Chemotactic responsiveness induced by imprinting has similarities and diversities to chemotactic selection: con-

gruence of positive Con A/Con A results of the two different experiments raises the possibility that imprinting might have a selector capacity, too; however other ligand (*Helix* lectin) could work also as a good selector but a reversed, negative effect was registered after imprinting. It is not unusual that different lectins having similar sugar affinity disparately influence cell functions: this was observed in case of cell aggregation (Csaba and Kovács 1991), and conjugation (Ofer *et al.* 1976).

In conclusion, present data point out that three lectins, that are phylogenetically ancient signal molecules, can elicit molecule-specific chemoattractant responses in unicellular model. Investigations of chemotactic selection and imprinting with the lectins call attention that chemotaxis is based upon fine receptor-ligand interactions and that carbohydrate moieties are potent functional components of chemotaxis receptors. In earlier experiments similar phenomenon was demonstrated in case of insulin receptors (Kovács *et al.* 1987, 1996). The experiments demonstrate that in contrast to the similarities in the provocation of reaction (treatment with a bioactive molecule and study of later response), the selected population can show similar or disparate reaction in chemotactic assay, depending on the sugar affinity (and maybe on the protein structure) of the provoking molecule.

Molecular-genetic backgrounds of the described phenomena is still obscure. As both selection and pretreatments have the potency to interfere with the genetic mechanisms of this protozoon, causing changes in long-term cell-physiological responsiveness, we intend to broaden our analysis into genetic aspects.

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