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SHORT COMMUNICATION

PRESENCE, UPTAKE AND LOCALIZATION OF AN IMMUNOREACTIVELY INTERLEUKIN 6 (IL-6)-LIKE MOLECULE IN *TETRAHYMENA PYRIFORMIS*

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The unicellular *Tetrahymena* and its medium contain immunoreactively interleukin 6 (IL-6)-like molecules (hereinafter IL-6) in a measurable quantity in the 24 h-old cultures. This protozoan takes up exogenously supplied IL-6 very quickly, and this can be found in similar amounts in both the cells and the media after 1 h. After 24 h (48 h cultures), an equal amount of IL-6 is present in the control and IL-6-treated cells and their media. By 120 h, cells which have not had their medium changed retained the same quantity of IL-6 as the control; however less than half was found in IL-6-treated cells. In the medium of 120 h-old cultures, there was a reduction of IL-6 content relative to the 24 h content in the control; however, in the IL-6-treated cell culture medium, less than half of the level in the controls was found. Confocal microscopy demonstrated the localization of IL-6 in/on the oral apparatus and basal bodies, and the nuclear envelope also showed moderate labelling. IL-6 antibody binding was enhanced after IL-6 pretreatment (hormonal imprinting). The experiments call attention to the presence of an IL-6-like molecule and its uptake at a very low level of phylogeny.

KEYWORDS: IL-6; hormonal imprinting; *Tetrahymena*; uptake; localization; secretion.

INTRODUCTION

It was first demonstrated in the 1970s that *Tetrahymena pyriformis* reacted to hormones of higher animals (Csaba and Lantos, 1973; Csaba, 1980, 1994), an observation which called attention to the presence of binding sites (receptors) on its plasma membrane (Csaba, 1980, 1985). The specificity of these receptors was later confirmed (O'Neil *et al.*, 1988; Christopher and Sundermann, 1992, 1995). In addition, *Tetrahymena* also produces, stores and secretes hormone-like molecules characteristic of higher vertebrates (LeRoith *et al.*, 1983; Csaba, 1994; Csaba *et al.*, 1998; Kõhidai and Csaba, 1995; Csaba and Kovács, 1999), and possesses signal transduction mechanisms which are similar to those of the higher animals (Kuno *et al.*, 1979; Kovács and Csaba 1997). The first encounter between an exogenously given signal molecule and *Tetrahymena* increased the binding capacity of the receptor (hormonal imprinting), and this property could be transmitted to hundreds of subsequent progeny (Csaba 1980, 1994). With regard to insulin, this 'imprinting' also influenced the hormone-producing capacity of the cell, which persisted for many cell generations (Csaba and Kovács, 1995; Csaba *et al.*, 1999).

The cytokine, interleukin 6 (IL-6) significantly increases the growth rate of *Tetrahymena* and an imprinting response was also observed, manifested as a stimulation of growth rate upon a second encounter (Csaba *et al.*, 1995). This led us to study the presence and localization of IL-6-like molecules (IL-6 hereinafter) in *Tetrahymena* as well as the effect of imprinting on IL-6 production and binding.

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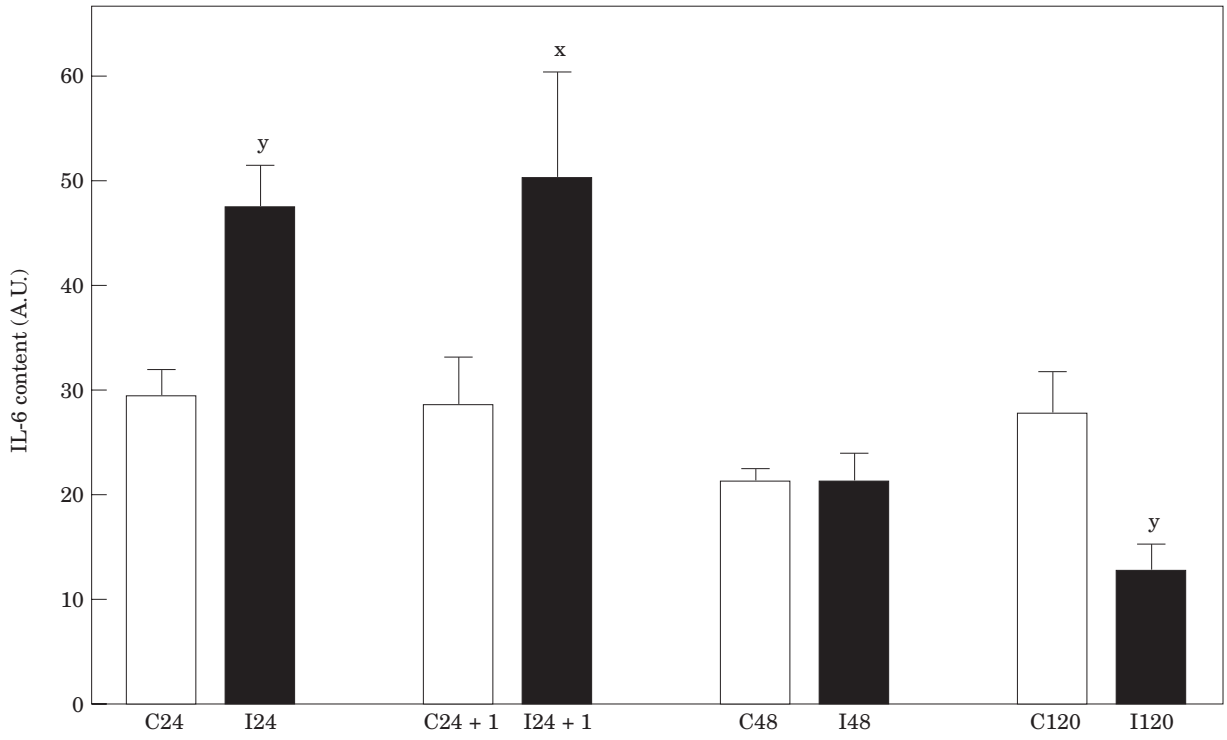

Fig. 1. IL-6 concentrations of *Tetrahymena* cells treated with 1 ng/ml IL-6. IL-6 content is calculated to the amount of protein and is given as arbitrary units. The real amount of IL-6 in C24 is 75 pg/10^5 cell. C=control, measured in untreated *Tetrahymena* parallel with the treated ones. The numbers demonstrate the age of the cultures. I= IL-6 treated *Tetrahymena*. I24 is measured 3 min after addition of IL-6 (time of centrifugation). x=$P<0.05$; y $P<0.01$.

MATERIALS AND METHODS

Cells and culturing

Cultures of *Tetrahymena pyriformis* GL were used in the logarithmic phase of growth. The cells were sustained in a medium containing 1% tryptone (Difco, Michigan, U.S.A.) and 0.1% yeast extract (Reanal) at 28°C for 24 h.

Measurement of IL-6 by ELISA

Treatment of cultures. Cultures were treated with 1 nM IL-6 (Sigma, St. Louis, MO, U.S.A.). Samples were taken from the cultures at 0, 1, 24 and 96 h after treatment, control groups were treated with equal volumes of the solvent/growth medium of IL-6. The samples were centrifuged ($1000 \times \boldsymbol{g}$ for 30 s) and the supernatant was removed from the cells. The sample containing the cells was washed with PBS. The fraction containing the cells in final volume of 500 µl in PBS was ultrasonicated (Vibra-Cell, Danbury, U.S.A.) for 15 cycles/sample.

Measurement. Solid phase, polyclonal anti-human IL-6 containing ELISA kit was used (Diagnosticum, Budapest, Hungary) to determine the immunoreactive IL-6 content of the supernatants and the cell homogenates. The detector system of the kit was a conjugate of anti-human IL-6-with HRP; the substrate used was TMB. The plates were evaluated at 450 nm. Protein contents of the cells and the medium were determined by Coomassie brillant-blue method.

Calculation and statistical evaluation. The ELISA values were related to the protein content of the samples. Since the pure proteose-tryptone medium did not contain proteins in a measurable quantity, the protein values refer to the amount of the cells and their secretions (in the medium). For statistical evaluation, Origin 2.8. and Statistica were used. These provided the values of Student's *t*-test, standard deviation and variance.

Study of the localization of IL-6 by confocal microscopy

Treatment of cultures. Tetrahymena were cultured as above and were in mid-exponential growth phase ($\sim 5 \times 10^5$ cells/ml) when used. The cells were treated with 1 ng/ml IL-6 (Sigma) for 60 min

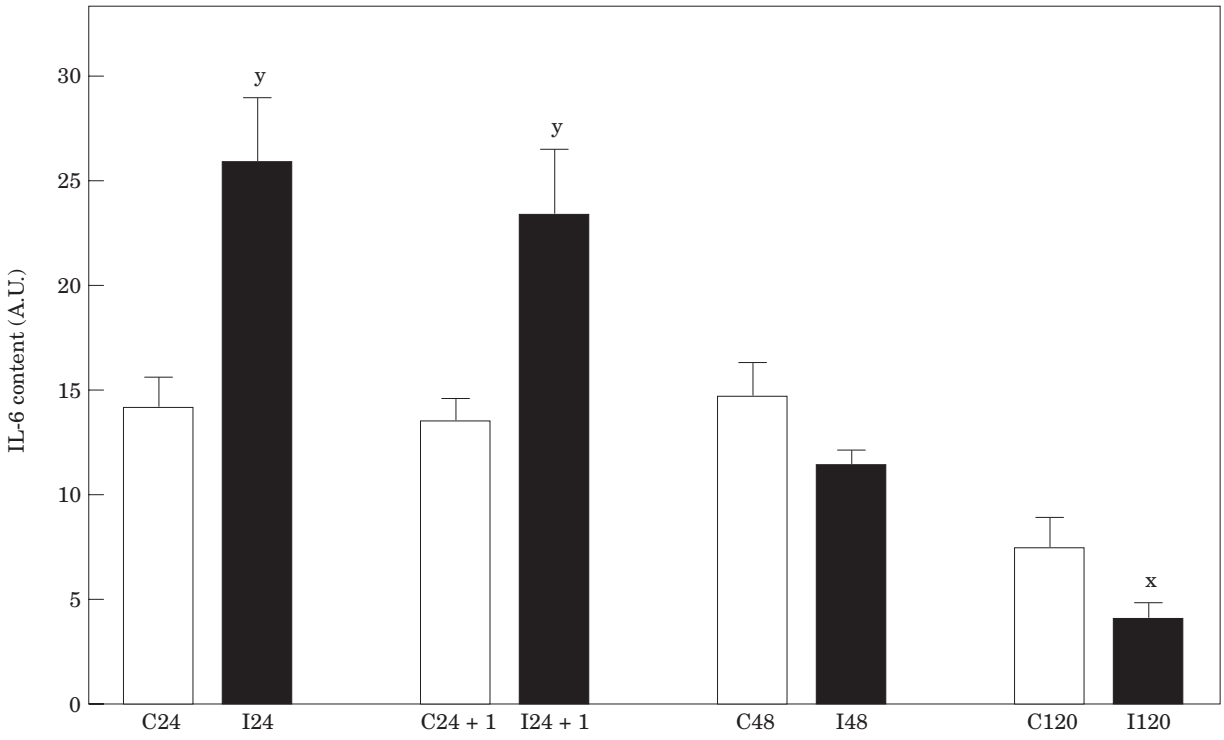

Fig. 2. IL-6 concentrations of culture medium of *Tetrahymena* treated with 1 ng/ml IL-6. IL-6 content is given in arbitrary units. C = control medium of untreated *Tetrahymena*, measured parallel with the medium of the treated ones. The numbers demonstrate the age of the cultures. I24 is measured 3 min after addition of IL-6 (time of centrifugation). x = $P<0.05$; y = $P<0.001$.

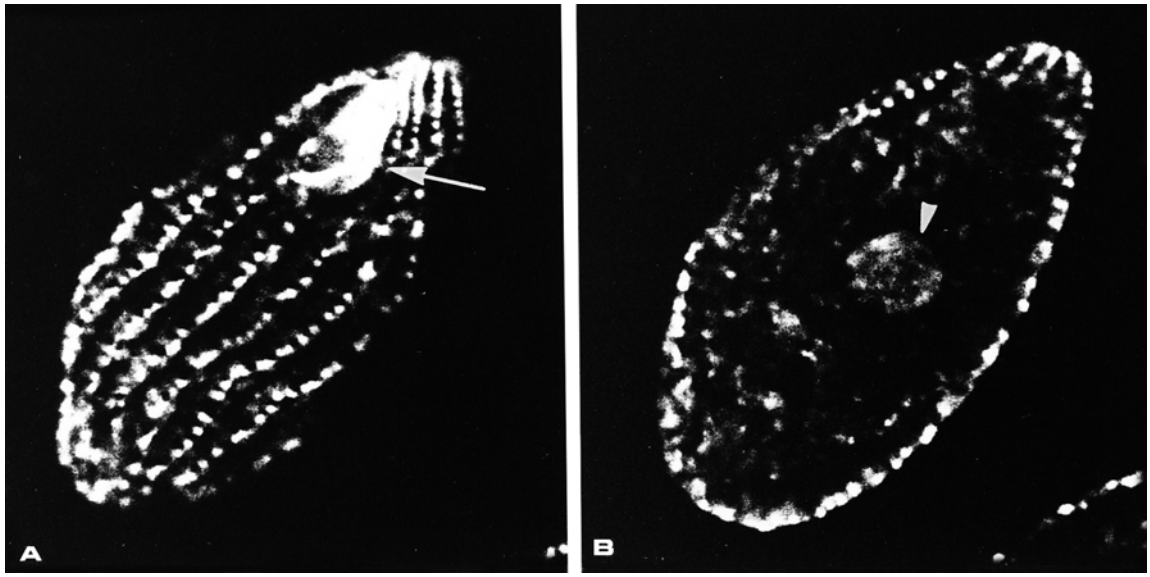

Fig. 3. Fluorescence micrographs (CLSM pictures) showing the fluorescence of basal bodies and oral apparatus (arrow) of control (untreated) *Tetrahymena*. The nuclear envelope (arrowhead) also shows weak fluorescence. A) optical section (1 μm) from the surface, 1500 × ; B) same cell, optical section of the mid-region, 1500 × .

and part of the cultures was studied. In other part of these cultures after the treatments the cells were thoroughly washed with fresh culture medium and cultivated for 24 h in it. Untreated cells served as controls. After 24 h treated and control cultures were divided into two parts: one part of the

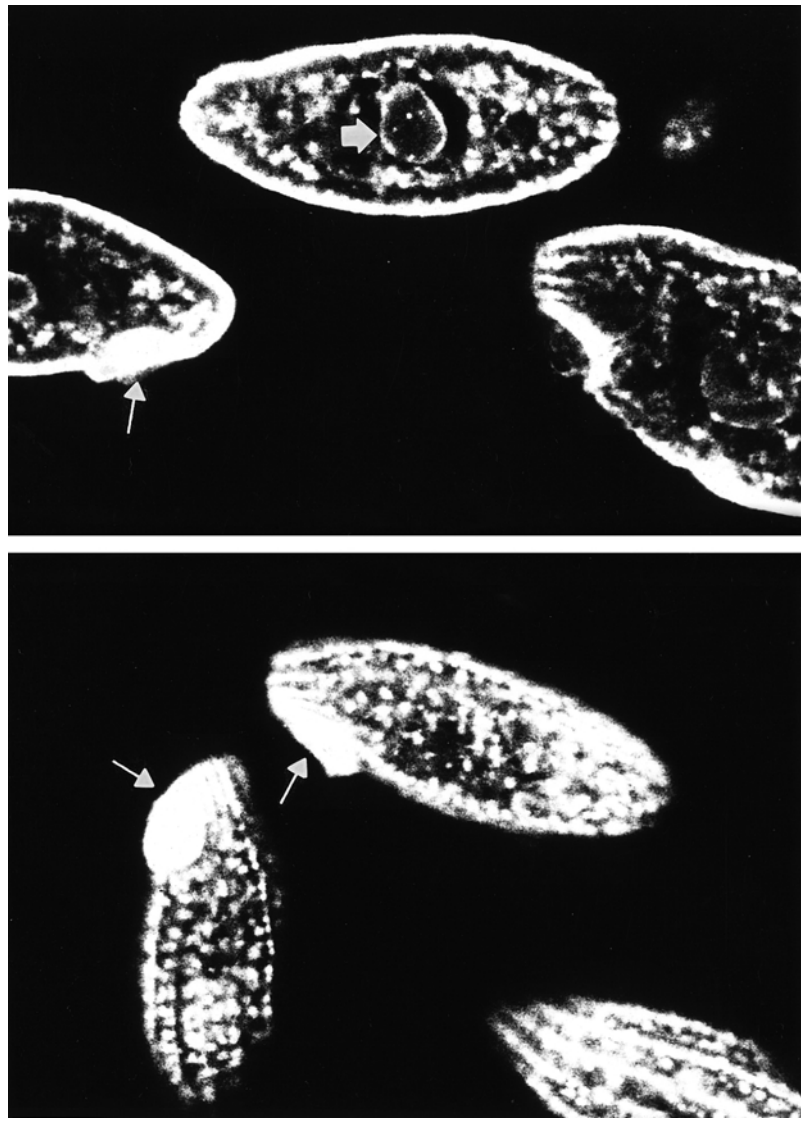

Fig. 4. Control *Tetrahymena* treated with 1ng/ml IL-6 after fixation and before treatment with labelled anti-IL-6 antibody. The oral apparatus (arrow), the basal bodies and the nuclear envelope (thick arrow) show intensive fluorescence. 1100 × .

cultures was treated with 1 ng/ml IL-6 for 60 min; another part of the samples remained untreated. A part of the cultures were studied. In the cultures which were sustained further, the medium was changed after 48 h.

Examination of cultures. After the treatments the cells were washed thrice with fresh culture medium and fixed with 8% paraformaldehyde solution (v/v) buffered at pH 7.2 in PBS. After fixation the cells were washed in wash buffer (20 mM Tris-HCl; 0.9% NaCl; 0.1% BSA; 0.05% Tween 20, pH 8.2) and subsequently concentrated by gentle centrifugation. After treatment with blocking buffer [1% rabbit serum in 20 mM Tris-buffered saline (TBS), pH 8.2] the samples (50 µl) were incubated with 50 µl anti IL-6 antibody (anti-human IL-6, developed in rabbit, Sigma) diluted 1:200 with antibody buffer (1% BSA in TBS, pH 8.2) for 60 min at room temperature. Finishing the incubation the cells were washed twice with wash buffer and twice with 0.1% goat serum containing TBS. Finally the samples were incubated with fluorescein isothiocyanate (FITC)-labelled secondary antibody (goat anti-rabbit IgG, whole molecule, Sigma) for 45 min and subsequently washed four times in wash buffer. Controls were not treated with primary antibody or the primary antibody was exhausted with IL-6. The analysis of fluorescence pattern of cells was performed with a BioRad (U.K.) MRC 1024 confocal

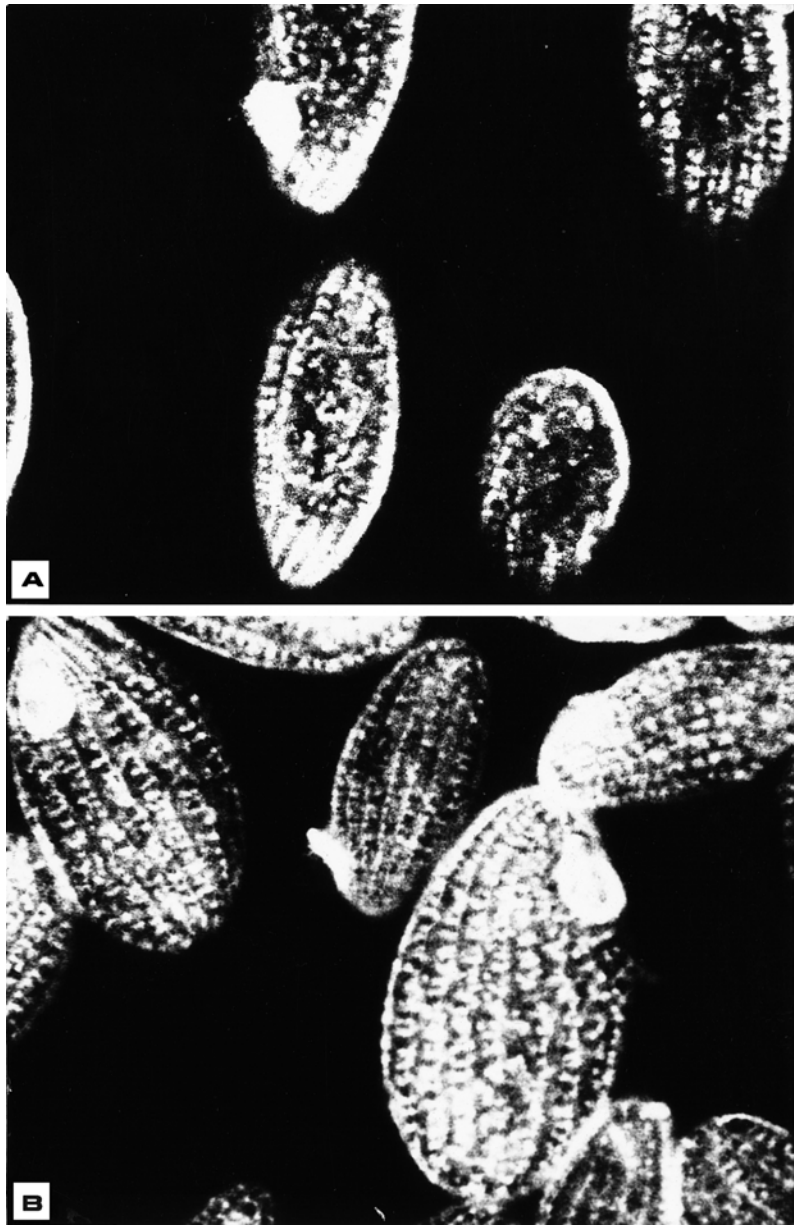

Fig. 5. IL-6-pretreated (imprinted) *Tetrahymena* cells. A without IL-6 treatment (after fixation) prior to the treatment with anti-IL-6 antibody; and B, after treatment with 1 ng/ml IL-6 prior to immunocytochemical labelling. 1200 × .

laser scanning microscope (CLSM) equipped with a krypton/argon laser. Analyses were performed in z-steps of 1 μm.

RESULTS

Quantitative studies with ELISA

In totally untreated *Tetrahymena,* there was a measurable quantity (75 pg/10^5 cell) of IL-6 (Fig. 1), which remains relatively constant over a 96 h period. There was a significant elevation of IL-6 content in the cells immediately after IL-6 treatment and this was also constant up to 1 h. By 48 h, this level was equal to the control, and at 120 h it was less than half of the control.

In the medium of the untreated 24 h cells there is a measurable quantity of IL-6 (Fig. 2). There is no IL-6 in the fresh medium without cells. In the media of 24+1- and 48-h-old cultures the same quantity of IL-6 was observed as at 24 h. At 120 h this quantity is significantly less.

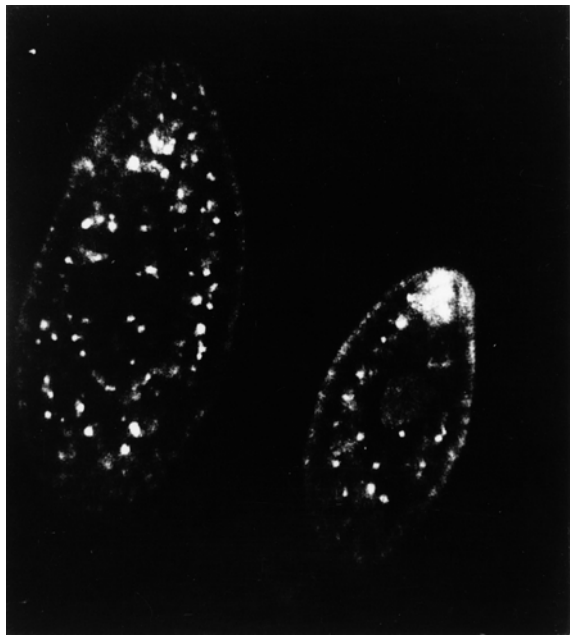

Fig. 6. Control (untreated) *Tetrahymena* cells studied with IL-6 exhausted anti-IL-6 antibody. 1100 × .

In the media of the IL-6-treated 24 h cells, a significantly higher quantity of IL-6 was present up to 1 h, which significantly decreased—related to the control—in the 120-h-old cultures.

Confocal microscopy

IL-6 antibody was bound intensively to the basal bodies and the oral apparatus of untreated *Tetrahymena* and the nuclear envelope also showed moderate labelling (Fig. 3). In fixed cells after treatment with IL-6 (1 ng/ml), the pattern of labelling was expressed more strongly, especially in the oral apparatus and nuclear envelope. The accessory filaments and kinetodesmal fibrils also show clear fluorescence (Fig. 4).

The IL-6 pretreated (imprinted) cells at 24 h after treatment showed a strong fluorescence pattern similar to the IL-6 treated cells after fixation (Fig. 5a). Addition of IL-6 after fixation of IL-6 imprinted cells resulted in a spectacular increase of fluorescence (Fig. 5b).

There was a significant decrease of fluorescence in the cells which was studied by using IL-6 exhausted primary antibody. The pattern of labelling was also different: only the food vacuoles showed fluorescence, the cortex of the cells being very weakly labelled (Fig. 6). There was no difference in the fluorescence between the control and IL-6-treated cells after 96 h.

DISCUSSION

In earlier experiments the presence of different hormone-like molecules (demonstrated by antibodies to mammalian hormones) was observed in *Tetrahymena* (LeRoith *et al.*, 1983; Roth *et al.*, 1982; Csaba *et al.*, 1998; Kõhidai and Csaba, 1995; Csaba and Kovács, 1999). Our experiments here clearly show that an immunoreactively IL-6-like molecule can also be found in this species. As yet we have no idea as to the possible function of IL-6 in *Tetrahymena*, however, we also do not know the function of other endogeneous hormones previously studied, though the exogeneously given hormones can specifically influence the physiology of *Tetrahymena* (Csaba 1994). The possible function of IL-6 in *Tetrahymena* has to be solved.

Tetrahymena can take up IL-6 very quickly and the level of the cytokine remains high for 1 h. However, after one day, the quantity of the cytokine is no more than in control cells. This means that *Tetrahymena* must degrade the cytokine taken up and that there is no induction of IL-6 production.

Imprinting by IL-6 enhanced the binding of IL-6 given subsequently (after 24 h) to the cells. This is in harmony with earlier experiments (Csaba *et al.*, 1995) when an enhanced growth response was provoked by IL-6, given 24 h after the first IL-6 treatment (imprinting).

Four days after IL-6 treatment, the IL-6 content was less than half of the control value in the cells and their media alike. These cultures were 120 h old (without changing the medium, which can lead to deterioration of the physiological functions of the cells). We suppose that the fall in IL-6 content is a part—and possibly a consequence—of this deterioration.

These experiments demonstrate the presence of an immunoreactively IL-6-like molecule, and its uptake and localization in *Tetrahymena*, an organism considered of very low level in phylogeny. This could mean that IL-6 is a very ancient molecule—ciliates are ~2 billion years old (Nanney, 1980)—and only the spectrum of its function has changed or extended with the progressing time and evolution.

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