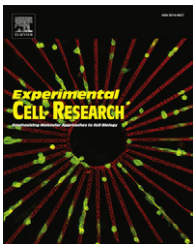
EXPERIMENTAL CELL RESEARCH 319 (2013) 56–67

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/yexcr



Research Article

The protein pheromone Er-1 of the ciliate *Euplotes raikovi* stimulates human T-cell activity: Involvement of interleukin-2 system

Davide Cervia[a,b,*], *Elisabetta Catalani*[a], *Maria Cristina Belardinelli*[a], *Cristiana Perrotta*[b], *Simona Picchietti*[a], *Claudio Alimenti*[c], *Giovanni Casini*[a], *Anna Maria Fausto*[a], *Adriana Vallesi*[c]

[a]*Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Viterbo, Italy*
[b]*Department of Biomedical and Clinical Sciences, "Luigi Sacco" University Hospital, University of Milan, Milano, Italy*
[c]*Department of Environmental and Natural Sciences, University of Camerino, Camerino (MC), Italy*

ARTICLE INFORMATION

Article Chronology:
Received 4 June 2012
Received in revised form
12 October 2012
Accepted 13 October 2012
Available online 24 October 2012

Keywords:
Ciliate pheromones
Jurkat cells
Cell growth
Cytokines
IL-2 receptor
ERK1/2

ABSTRACT

Water-soluble protein signals (pheromones) of the ciliate *Euplotes* have been supposed to be functional precursors of growth factors and cytokines that regulate cell–cell interaction in multi-cellular eukaryotes. This work provides evidence that native preparations of the *Euplotes raikovi* pheromone Er-1 (a helical protein of 40 amino acids) specifically increases viability, DNA synthesis, proliferation, and the production of interferon-γ, tumor necrosis factor-α, interleukin (IL)-1β, IL-2, and IL-13 in human Jurkat T-cells. Also, Er-1 significantly decreases the mRNA levels of the β and γ subunits of IL-2 receptor (IL-2R), while the mRNA levels of the α subunit appeared to be not affected. Jurkat T-cell treatments with Er-1 induced the down-regulation of the IL-2Rα subunit by a reversible and time-dependent endocytosis, and increased the levels of phosphorylation of the extracellular signal-regulated kinases (ERK). The cell-type specificity of these effects was supported by the finding that Er-1, although unable to directly influence the growth of human glioma U-373 cells, induced Jurkat cells to synthesize and release factors that, in turn, inhibited the U-373 cell proliferation. Overall, these findings imply that Er-1 coupling to IL-2R and ERK immuno-enhances T-cell activity, and that this effect likely translates to an inhibition of glioma cell growth.

Abbreviations: BrdU, 5-Bromo-2′deoxyuridine; CCL, chemokine (C–C motif) ligand; CFSE, carboxyfluorescein succinimidyl ester; CM, conditioned medium; DMEM, Dulbecco′s Modified Eagle′s Medium; DMSO, dimethyl sulfoxide; ERK1/2, extracellular signal-regulated kinase 1/2; FBS, foetal bovine serum; FITC, fluorescein isothiocianate; HRP, horseradish-peroxidase; IFN, interferon; IL, Interleukin; IL-2R, IL-2 receptor; MAPK, mitogen-activated protein kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; PI, propidium iodide; qPCR, quantitative real-time PCR; RFI, relative fluorescence intensity; SDS, sodium dodecyl sulphate; Th1/2, T helper type 1 or type 2; TNF, tumor necrosis factor

*Corresponding author at: Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, largo dell'Università snc, Blocco D, Viterbo 01100, Italy. Fax: +39 0761 357179.
E-mail address: d.cervia@unitus.it (D. Cervia).

0014-4827/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yexcr.2012.10.007

Introduction

In functional association with their mating type systems, ciliated protozoa synthesize cell type-specific chemical signals, usually designated as pheromones, that control the switching between the reproductive (mitotic growth) and mating (sexual) stages of their life cycles [1–3]. In species of *Euplotes*, pheromones have been isolated and characterized for their genetic determination and molecular structures either by direct chemical analysis of the purified molecules or molecular cloning of the coding genes [4,5]. They form homologous, species-specific families of water-borne proteins which are constitutively secreted throughout the cell life cycle into the environment, or incorporated into the plasma membrane as autocrine receptors of the soluble forms [1–3,6]. In *E. raikovi,* pheromones (denominated Er-1, Er-2, and so forth) contain 37–51 amino acid residues, of which only six cysteines forming three intra-chain disulfide bonds are highly conserved. Despite the variability in the amino acid sequence that diversifies pheromones from one another, the determination of the three-dimensional structures of several pheromones, carried out by nuclear magnetic resonance spectroscopy, showed that they share a molecular architecture based on a common core of three antiparallel α-helices tightly fastened together by the three conserved disulfide bonds [1,7–10]. Due to this structural homology, pheromones have been shown to bind in competition with one another to the receptors of the same cells from which they are secreted, as well as to the receptors of other cells [11]. While binding the own (self) pheromone promotes vegetative (mitotic) cell growth, binding of a foreign (non-self) pheromone induces cells to temporarily arrest their growth and develop competence for uniting in mating pairs [12]. It is essentially on the basis of this capacity of *Euplotes* pheromones to elicit varied and context-dependent cell responses that these signaling molecules have been proposed to represent potential prototypic cell growth factors [12].

There is general agreement that cell signaling mechanisms include fundamental components that cross-talk between uni- and multi-cellular organisms [13,14]. In line with this concept, it was earlier observed that the human cytokine interleukin (IL)-2 and epidermal growth factor act as effective competitors of pheromone/pheromone-receptor binding reactions [15]; then, it was shown that the *E. raikovi* pheromone Er-1 in particular binds with different affinities to the α and β chains of IL-2 receptor (IL-2R) on the surface of the mouse T lymphocyte cells CTLL-2, which are totally dependent on IL-2 binding for survival and proliferation [16]. In the light of these findings, we have now investigated the activity of the *E. raikovi* pheromone Er-1 on human lymphoid Jurkat T-cells, which express functional IL-2R and represent an useful T-cell model for immunological studies [17]. Cell growth, cytokine expression/release, IL-2R expression/trafficking, and the coupling to extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinases (MAPKs) have been analyzed in cells cultured in high- or low-serum media. Jurkat cells are leukemic cells in rapid cell division, and their proliferation rate and metabolic activity is enhanced in the presence of high-serum medium (standard culture conditions). On the other hand, low-serum media (restrictive culture conditions) are commonly used to unmask positive effects of chemical compounds on Jurkat cell functional activities [18]. Since cross-talking among IL-2 system, T-cells, and malignant cells plays an important role in brain tumors [19], it was also investigated the possible effects of Er-1 on the human glioma U-373 cells.

Materials and methods

Cells and pheromones

E. raikovi cells used as pheromone source were derived from an offspring clone 39 of the wild-type strain number 13 (deposited at the American Type Culture Collection, reference number "PRA-327"). They secrete only pheromone Er-1, consistently with a known homozygous combination at the genetic locus *mat*, and were grown on the green algae *Dunaliella tertiolecta* and maintained under controlled conditions at 22–24 °C. Homogeneous preparations of Er-1 were obtained through standard purification procedures [20,21].

Human leukemia Jurkat T-cells and U-373 astrocytoma cells were grown in RPMI 1640 medium (Jurkat cells) or Dulbecco's Modified Eagle's Medium (DMEM) (U-373 cells) supplemented with 10% heat inactivated foetal bovine serum (FBS), 2 mM glutamine, 100 UI/ml penicillin and 100 μg/ml streptomycin at 37 °C, 5% CO_2 in an humidified atmosphere. Cells were maintained in logarithmic growth phase by routine passages every three days. Experiments were performed on cells cultured in the presence of either high-serum (10% FBS, standard culture conditions) or low-serum (2% FBS, restrictive culture conditions) containing medium. In the latter case, cells were pre-treated with 2% FBS for 16 h (serum starvation) before experiments.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cell viability was assessed by the reduction of MTT, as previously described [22–25]. Jurkat or U-373 cells were seeded in 96-well plates at a density of 2.5×10^5 cells/ml, before treatments. Experiments were performed on cells cultured in standard or restrictive culture conditions for 24–48 h, either in the absence or in the presence of increasing concentrations, from 1 to −100 ng/ml, of Er-1. U-373 cells were also treated for 24–48 h with the medium of Jurkat cell cultures (conditioned medium, CM) (see below). Cells were then washed and rinsed with phosphate buffered saline (PBS) and 10 μl MTT (5 mg/ml stock solution). Plates were then incubated at 37 °C for 2–4 h. The assay was stopped by adding dimethyl sulfoxide (DMSO). Formazan salts were dissolved in DMSO by gentle shaking for 5 min at room temperature. Absorbance at 570 nm was quantified spectrophotometrically by a Tecan Infinite F50 microplate reader (Tecan Group, Mannedorf, Switzerland). Each experimental condition was replicated in eight wells.

Dual staining with Annexin V-fluorescein isothiocianate (FITC) and propidium iodide (PI)

Cell death was analyzed as described previously [26,27]. Jurkat cells were seeded in 6-well plates at a density of 2.5×10^5 cells/ml, before treatments. Experiments were performed on cells cultured in restrictive culture conditions for 48 h, either in the absence or in the presence of increasing Er-1 concentrations, collected and