Oxytocin Is a Growth Factor for Kaposi’s Sarcoma Cells: Evidence of Endocrine-Immunological Cross-Talk

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ABSTRACT

Oxytocin receptors (OTRs) are expressed in numerous tissues, including human normal endothelium. Here we investigated the expression and biological significance of OTRs in Kaposi’s sarcoma (KS), an intensely angioproliferative disease of possible vascular origin with a prominent inflammatory component. Immunohistochemistry and in situ hybridization studies showed OTR expression in tumor cells of cutaneous classic and AIDS-related KS lesions. OTR mRNA and protein were also detected on cultured KS-IMM spindle cells by reverse transcription-PCR and immunofluorescence procedures. In these cells, OTR expression was up-regulated by the supernatants of resting CD4+ and CD8+ lymphocytes through a still unidentified factor. Functionality of OTRs was demonstrated because OT treatment of KS-IMM cells led to a significant increase in cell proliferation, coupled to the increase of intracellular calcium, but did not effect cell migration in vitro or angiogenesis in vivo. In addition, we demonstrated that CD4+ and CD8+ cells produce OT themselves, thus constituting an intralesional source of peptide. These results indicate that: (a) functioning OTRs are expressed in KS cells and modulated by the inflammatory counterpart of KS lesions; (b) via OTRs, OT stimulates KS-IMM cell proliferation and could, therefore, be considered a new possible growth factor involved in KS progression; and finally (c) the evidence of OT synthesis by CD4+ and CD8+ lymphocytes strongly suggests the existence of local endocrine-immunological cross-talk in Kaposi’s sarcoma.

INTRODUCTION

The neurohypophyseal nonapeptide OT3 is involved in many biological functions, including lactation, parturition, sexual and maternal behavior (1), regulation of food intake (2), and regulation of cell proliferation under both physiological and neoplastic conditions (3–7). Its effects are mediated by specific membrane G-coupled receptors, OTRs (8).

Besides the “traditional” tissues (of uterine, mammary, nervous origin) expressing OTRs, many different cell types have recently been reported to harbor OTRs. Among them, human endothelial cells as well as rat vessels express OTRs (3, 9). Moreover, the rat vasculature (origin) expressing OTRs, many different cell types have recently been investigated for OT receptor presence and the OTR presence and the OTR expression in KS-IMM cells is up-regulated by the supernatants of CD4+ and CD8+ lymphocytes, the inflammatory counterpart of KS disease; (c) different cytokines have different effects on the modulation of OTR expression in KS-IMM cells: IL-1β, IL-3, and IL-4 down-regulate OTR, whereas IL-2, IL-6, IFN-γ, and TNF-α, as well as VEGF and Tat protein, do not affect OTR expression; (d) OT stimulates proliferation, but not motility, of KS-IMM cells in vitro and does not induce angiogenesis in vivo; (e) the mitogenic effect of OT is specifically coupled to an increase in intracellular calcium; and (f) CD4+ and CD8+ lymphocytes strongly suggests the existence of local endocrine-immunological cross-talk in Kaposi’s sarcoma.

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cytes actively produce and release OT in their supernatants and could, therefore, represent the local source of OT within the KS lesions.

MATERIALS AND METHODS

Reagents and Cell Cultures. The spontaneously immortalized KS cell line KS-IMM, derived from an immunosuppressed transplant patient (29), was routinely cultured in RPMI 1640 (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% FCS (Life Technologies, Inc.), in a 5% CO₂ humidified atmosphere at 37°C. OT and OTA [d(CH₂)₃ Tyr(Me)², Thr⁴, Tyr- NH₂]₁⁰₅VOT; Ref. 30] were kindly provided by Dr. Maurice Manning (University of Toledo, Toledo, Ohio). Human recombiant TNF-α, IL-1β, IL-2, IL-3, IL-4, IL-6, and IFN-γ were purchased from Sigma Chemical Co. (St. Louis, MO). Reombinant human VEGF164 was obtained from R&D Systems (Abingdon, United Kingdom). Purified Tat protein was obtained from Intraceel (London, United Kingdom). The human breast cancer cell line MCF7, which expresses OTR, was purchased from the American Type Culture Collection (Manassas, VA) and used as a positive control.

PBMCs, obtained from the local Blood Bank, were purified on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). CD4+ and CD8+ T lymphocytes were obtained from PBMCs by negative selection procedures, to avoid any subliminal activation triggered by mAbs binding to T-cell receptors. PBMC preparations underwent two cycles of incubation with a mixture (2 µg/10⁶ cells) containing anti-CD19, anti-CD20, anti-CD16, anti-CD11a II, anti-CD14 mAbs, and either anti-CD4 or anti-CD8. All of the mAbs were locally produced and purified. After removing the unbound mAbs, cells were incubated with a goat antimouse immunoglobulin Dynabeads (Dynal, Oslo, Norway). The resulting cells were >90% pure, as assessed by the expression of CD4+ or CD8+. After culturing for 24 h in a 5% CO₂ atmosphere, CD4+ and CD8+ lymphocytes were harvested along with the culture supernatants.

Detection of OTR in Cutaneous KS Lesions by ICC and ISH. Fifteen cutaneous KS lesions from AIDS-KS lesions were collected from the files of the Department of Pathology, University of Catania, Catania, Italy. The different cell types were characterized using either morphological criteria or ICC with Abs directed toward cell-specific antigens (such as CD34 for spindle cells or factor VIII for vessels). The presence of OTR in the CKS and AIDS-KS samples was studied by ICC and by ISH procedures.

For ICC, serial sections from representative paraffin-embedded blocks of human KS lesions were collected onto poly-L-lysine-coated slides and stained for OTR antigen. Endogenous peroxidase activity was blocked with 6% H₂O₂ for 5 min at room temperature. IF3 mAb, directed toward the NH₂-terminus of the OTR (31), was incubated at 60°C for 15 min at room temperature. Slides were washed with water and counterstained with hemalum for 3 min before mounting.

IF and Flow Cytometry for Detection of Expression and Modulation of OTR in KS-IMM Cells. For standard IF procedure, KS cells were grown on glass coverslips for 5 days. After washing in PBS, cells were fixed in 5% paraformaldehyde with 2% sucrose at pH 7.6 for 5 min (or in methanol for 10 min and acetone for 5 s at −20°C) and then incubated at room temperature for 30 min with antihuman OTR IF3 mAb (31) diluted 1:2 in PBS. Cells were then rinsed in PBS and finally incubated for 30 min at room temperature with the appropriate fluorescein-labeled secondary antisera (Serad-Lab Ltd, Sussex, England) diluted 1:2 in PBS. The reaction was evaluated with an Olympus Orthoplan fluorescence microscope equipped with Xenon lamp and epifluorescence apparatus. An unrelated primary mAb (common leukocytic antigen; Dako, Gølstrup, Denmark) as well as the omission of primary Ab were used as negative controls.

Surface expression of OTR on KS-IMM cells was also investigated by flow cytometry analysis (FACSort, Becton Dickinson, Milan, Italy). Briefly, 2 × 10⁵ cells were incubated with 1 µg of the IF3 mAb, and the secondary reagent was a FITC-conjugated F(ab′)² fragment of a goat antimouse immunoglobulin Ab (Caltag, Burlingame, CA). The intensity of fluorescence was recorded on a logarithmic scale, by scoring at least 10,000 cells/sample; background fluorescence intensity was obtained by incubating the cells with the goat antimouse immunoglobulin reagent alone.

The potential role of inflammatory cytokines in the modulation of OTR expression was tested by incubating (12 h) KS-IMM cells in standard medium added with ~50% (v/v) medium derived from cultured CD4+ and CD8+ T lymphocytes (see above). Furthermore, a panel of selected cytokines and growth factors were used alone or in combination to evaluate any influence on OTR expression. In detail, IL-1β (25–50 ng/ml), IL-2 (250, 500, and 1000 units/ml), IL-3 (10 ng/ml), IL-4 (1000 units/ml), IL-6 (100 units/ml), IFN-γ (250–500–1000–2000 units/ml), TNF-α (10 ng/ml), VEGF (10 ng/ml), and Tat protein (10 ng/ml) were used. Mixtures containing IFN-γ (1000 units/ml), TNF-α (10 ng/ml), and IL-2 (1000 units/ml) or IFN-γ (1000 units/ml), TNF-α (10 ng/ml), and Tat (10 ng/ml) were also assessed. OT (100 nm) and OTA (100 nm) effects on OTR expression were also studied. All of the treatments lasted 12 h.

Detection of OTR and OT mRNA by RT-PCR in KS-IMM Cells and CD4+ and CD8+ Lymphocytes. Total RNA was extracted from KS-IMM cells, CD4+ and CD8+ lymphocytes using the Tri-reagent extraction kit (Molecular Research Center Inc., Cincinnati, OH) according to the manufacturer’s recommendations. The concentration of RNA was estimated by spectrophotometry, and RNA degradation was assessed by 1% agarose gel electrophoresis. Total RNA (1 µg) was digested to avoid DNA contamination with 10 units of RNase-free DNase (Boehringer Mannheim, Mannheim, Germany) in a 10-µl solution containing 2 mM MgCl₂ at room temperature for 10 min, then heated for 5 min at 70°C to inactivate DNase. Forty pm of oligodeoxynucleotide primer [oligo(DT)$_n$] was then added, and the solution was heated at 70°C for 10 min, then was chilled on ice to allow primer hybridization. The final solution was reverse transcribed with 100 units of Superscript Reverse Transcriptase (Life Technologies, Inc.), and cDNA was generated in a 50-µl final reaction volume containing 50 mM Tris- HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 1 mM dNTPs, and 20 units of RNasin (Promega, Madison, WI). The solution was heated at 37°C for 90 min; then the enzymes were inactivated by heating at 70°C for 10 min. Negative control samples for further PCR amplification included omission of the reverse transcriptase enzyme.

RNA quality was assessed by amplification of β2-microglobulin mRNA. OTR primers were designed according to Takemura et al. (34). Primer sequences and location for OTR, OT, and β2-microglobulin PCR primers are listed in Table 1. PCR experiments were carried out in a final volume of 10 µl containing 1 µl of cDNA, 1 µM of sense and antisense primer, 200 µM dNTP, 1× PCR buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl; AmpliTaq, Perkin-Elmer, Roche, NJ], 1.5 mM MgCl₂, and 0.5 units of Taq polymerase (AmpliTaq Gold, Perkin-Elmer). Each reaction consisted of 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C (β2-microglobulin and OTR) and 61°C (OT) for 1 min, and extension at 72°C for 2 min. PCR products were then visualized under UV light in 1% agarose gels containing ethidium bromide. MCF7 human...
breast carcinoma cells and normal human hypothalamus (obtained from autopsies) were used as positive controls for OTR and OT, respectively. Negative control samples included omission of CDNA in the PCR mixture.

To further test RT-PCR product specificity, Southern Blot analysis was performed. Probe sequences are reported in Table 1. Membranes were hybridized at 42°C overnight with 25 pmol of digoxigenin-labeled OTR oligonucleotide probe. The membranes were then washed with 2× SSC-0.1% SDS for 10 min at room temperature and 0.5× SSC-0.1% SDS for 30 min at 42°C. Digoxigenin-labeled specific hybridization was visualized using an immunological detection system (Boehringer Mannheim) using antiglobulins conjugated with alkaline phosphatase. Detection was performed using the chemiluminescent substrate disodium 3-[4-methoxyspiro-1,2-dioxetane-3,2-5-(chloro)tricycl[3.3.1.1(6,8)]decan-4-yl] phenylphosphonate CSPD (Boehringer), according to the manufacturer’s instructions. All of the blots were exposed to X-ray films with intensifying screens at room temperature for 3 h.

**Measurement of Intracellular Calcium Levels in KS-IMM Cells.** OT is a growth factor for KS-IMM cells. Cell migration of quiescent, adherent KS-IMM cells (10^6 cells/well in RPMI plus 1% FCS) was studied over 4–6 h periods under a Nikon Diaphot inverted microscope with a ×10 phase-contrast objective as described previously (35). Cells were kept in an attached, hermetically sealed plealxiglass Nikon NP-2 incubator at 37°C. Cell migration was recorded with a JVC-1CCD video camera. Image analysis was performed with a MicroImage analysis system (Cast Imaging srl, Venice, Italy) and an IBM-compatible system equipped with a video card (Targa 2000; Truevision, Santa Clara, CA). Image analysis was performed by digital saving of images at 30-min time intervals. Migration tracks were generated by marking the position of the nucleus of individual cells on each image. The net migratory speed (“straight line velocity”) was calculated by the MicroImage software based on the straight-line distance between the starting and ending points divided by the time of observation. Migration of at least 30 cells was analyzed for each experimental condition. Values are expressed as the mean ± SD.

**Murine Matrigel Angiogenesis Assay.** To evaluate the possible role of OT in KS neovascularization, the direct angiogenic effect of OT was evaluated in the murine Matrigel angiogenesis assay. Briefly, Matrigel (0.5 ml) containing OT or the vehicle alone was s.c. injected into the abdominal tissue of female C57 mice along the peritoneal midline. After 6 days, mice (n = 6) were killed, and tissues were recovered, fixed in 10% buffered formalin, and embedded in paraffin and processed for histological analysis. Sections (3 μm) were cut and stained with H&E. Vessel structures were counted only if showing a patinated lumen with red globuli and/or leukocytes. The mean size of vessels was planimetrically assessed using the computing integral area calculation of the Lucia digital system (Nikon United Kingdom limited instrument division, Kingston, United Kingdom). Endothelial cells in the neoformed vessels were stained with fluorescein-labeled Griffonia simplicifolia tectin (Sigma Chemical Co.) and von Willebrand factor polyclonal Ab (Sigma Chemical Co.) by fluorescence.

### RESULTS

**OTR and OTR-mRNA Detection in Cutaneous KS Lesions.** OTR protein and mRNA were evaluated in 15 cutaneous KSs (Fig. 1a) and three cutaneous AIDS-KS lesions. OTR-immunoreactivity was similar in all of the lesions and was localized within the cell cytoplasm, with a membrane reinforcement, in the large majority of KS spindle cells, endothelial cells, and intraleional lymphocytes (Fig. 1b). No immunostaining was observed omitting the primary Ab (Fig. 1c). Using ISH procedure, we confirmed the presence of the specific mRNA for OTR in the cytoplasm of KS spindle cells, as well as in endothelial cells and intraleional lymphocytes (Fig. 1, d and e). The specificity of the signal was confirmed by the absence of hybridization stain using the sense probe (Fig. 1f).

**OTR Detection in KS-IMM.** The reactivity of the FITC anti-OT mAb was analyzed on KS-IMM cells by IF and FACS. By IF, all of the KS-IMM cells showed both bright spots outlining the cellular membrane and intracytoplasmic fluorescent granules (Fig. 2a). By flow cytometry, OTR positivity was evident in >80% of cells, albeit with variable epitope densities (Fig. 2b). No signal was detected when either the primary mAb was omitted or an unrelated mAb was used.

### Table 1

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<th>Primer</th>
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Fig. 1. OTR expression in primary KS cutaneous lesions by ICC and ISH. a, cutaneous CKS variant. Spindle cells are associated with lymphocytes and vascular structures (H&E staining). b, ICC reaction using IF3 anti-OTR-specific mAb. The expression of OTR is demonstrated in spindle cells, intralesional lymphocytes (arrowhead), as well as in plump endothelial cells of vessels (arrow). The cellular localization of OTR is into the cytoplasm with membrane reinforcement. c, ICC negative control of the same case was obtained by the omission of the primary Ab. d, ISH technique using a digoxigenin-labeled OTR riboprobe. The presence of specific OTR mRNA is demonstrated by the intense cytoplasmic brown granular staining in the spindle cells (right side) as well as in the inflammatory infiltrate (left side). e and f, at higher magnification, OTR mRNA is revealed by ISH reaction in the cytoplasm of the large majority of spindle cells, in the inflammatory cells (arrowhead in e indicate a cluster of lymphocytes) and in endothelial cells of intralesional vessels (arrow, e). ISH using the sense probe resulted negative (f).

Fig. 2. OTR expression in KS-IMM cells: IF and flow cytometry. a, by IF and using a specific anti-OTR mAb, the large majority of KS-IMM cells showed many bright spots all along the cell surface as well as some fluorescent intracytoplasmic granules. b, flow cytometry analysis of KS-IMM cells reacted with IF3 mAb (white profile) or with an isotype-matched irrelevant mAb (dark profile). X axis, fluorescence intensity/cells; Y axis, number of cells registered/channel.
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Modulation of OTR Expression. The observation that the majority of KS lesions display T-lymphocyte infiltrate prompted us to test the hypothesis that the surface expression of OTR could be regulated by locally released soluble factors. This hypothesis was confirmed by a 12-h exposure of KS-IMM cells to CD4+ and CD8+ T-lymphocyte supernatants, which caused a marked increase in terms of mean fluorescence intensity of the OTR. The increase was a monomorphic characteristic, being detectable after incubation with supernatants from all of the lymphocyte donors analyzed (Fig. 3).

The factor(s) responsible for this phenomenon remains to be identified. In fact, VEGF, Tat, IL-6, TNF-α, and IFN-γ were ineffective (not shown). Moreover, the mixture of IFN-γ, TNF-α, and IL-2, or of IFN-γ, TNF-α, and Tat was also unable to modulate OTR expression (not shown). On the contrary, IL-1β, IL-3, and IL-4 exerted a negative regulation. Similarly, both OT and OTA (100 nM) down-regulated OTR expression within the first 2 h of cell incubation.

Evaluation of OTR and OT mRNA in KS-IMM Cells and in CD4+ and CD8+ Lymphocytes by RT-PCR. Southern blot after RT-PCR procedure for OTR mRNA detection showed a specific 391-bp intense signal in KS-IMM cells (Fig. 4, Lane 2) and in CD4+ and CD8+ lymphocytes (Lanes 3 and 4) as well as in MCF7 cells (Lane 1) that have been previously described as containing OTR mRNA (4). Southern blot of OTR PCR products was negative for KS-IMM cells and positive for both CD4+ and CD8+ lymphocytes.

OT Concentration in the CD4+ and CD8+ Supernatants. To demonstrate that OT was effectively produced by CD4+ and CD8+ lymphocytes (both containing OT mRNA as demonstrated by RT-PCR) the peptide concentrations were determined in the lymphocyte supernatants by an enzymatic immunoassay. OT concentration was 70 pg/ml in the CD4 supernatant and 47 pg/ml in the CD8 supernatant. No OT was detected in the culture medium (RPMI+5% FCS) alone, which indicated a real OT production and release by CD4+ and CD8+ lymphocytes.

OT Effects on KS-IMM Cell Proliferation. The KS-IMM cell line responded to OT treatment with a significant increase in cell proliferation, which was evident at 48 h and later. OT (100 nM) produced a maximal (40%) increase of KS-IMM cell number; 10 nM OT still significantly increased cell proliferation, although to a lower extent, whereas 1 nM OT gave a slight, not significant, stimulation of cell growth. The OT effect was dose dependent (100 nM OT versus control: ***, P < 0.001; OT 10 nM versus control: **, P < 0.01) and fully abolished by incubation with a selective OTR antagonist, 100 nM OTA, which inhibited cell proliferation when used alone (OTA versus control: *, P < 0.01; Fig. 5).

Effect of OT Treatment in Intracellular Ca2+ Levels in KS-IMM Cells. Application of 1 μM OT to KS-IMM cells induced a slow and persistent increase in cytosolic-free [Ca2+]i (60 cells total; Fig. 6a). A similar effect was evident after application of 100 nM and 10 μM OT, whereas 10 nM OT induced a weaker calcium response and 1 nM OT was ineffective (not shown). These results indicated a dose dependency of the OT effect on intracellular calcium levels in KS-IMM cells.

The addition of 10 nM EGTA in the bath during the response to OT induced a rapid and complete decline of [Ca2+]i, to the resting levels, showing that a calcium entry from extracellular side was involved in the response (Fig. 6b). EGTA application failed to induce any effect on [Ca2+]i, in resting conditions (Fig. 6b, first application of EGTA). A release from intracellular calcium stores could be detected in only 2 out of 60 cells tested (not shown). The selective antagonist of OTR,
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Effect of OT on Cell Migration and Angiogenesis. Several mitogenic factors are also able to induce the migration of KS cells. We therefore investigated the possible motogenic ability of OT on quiescent adherent KS-IMM cells using a time-lapse migration assay. Baseline spontaneous cell motility was minimal, never exceeding 1 μm/h. Stimulation with OT (100 nM) did not increase the scattered cell motility over a 4-h period (Fig. 7A). As positive control, cells were exposed to 10 ng/ml VEGF. A relevant enhancement of cell proliferation was evident after OT stimulation (Fig. 7A inset, after 6-h exposure) but not during the control experiments. Moreover, incubation of KS-IMM cells with OT induced shape changes, leading to more elongated, spindle-like cells (Fig. 7B, inset).

The effect of OT on cell migration was also tested on human endothelial cells derived from the umbilical vein. Endothelial cell motility did not increase after stimulation with OT (100 nM). We further investigated whether OT could participate in the KS growth by promoting neoangiogenesis. Using a murine model of angiogenesis in Matrigel, we failed to detect any effect of OT (100 nM) on in vivo angiogenesis.

DISCUSSION

In this study, we demonstrated that OTRs are present in KS spindle cells, and that they mediate the mitogenic effect of OT through the increase of intracellular calcium. We further report that CD4+ and CD8+ supernatants promote OTR expression in KS-IMM cells, and that different cytokines exert specific effects in modulating OTR expression. Moreover, we demonstrated for the first time that CD4+ and CD8+ lymphocytes produce OT and could, therefore, represent a local OT source within KS lesions. Together, these observations suggest the existence of a paracrine loop in KS lesions, in which inflammatory cells could both provide OT and modulate OTR expression in spindle cells, which would increase the number of binding sites available for OT to exert its mitogenic effect.

The presence of OTRs in normal human endothelial cells has been recently reported (3). In these cells, OT stimulation increased cell proliferation through a calcium and protein kinase C-dependent pathway.

In the present study, we provide evidence of a wide OTR expression in both CKS- and AIDS-related KS cutaneous lesions. The presence of OTRs was demonstrated at mRNA and protein levels in spindle cells, in endothelial cells, and in the majority of inflammatory intrallesional cells. Similarly, in our experimental model, KS-IMM spindle cells, which could be considered modified/activated endothelial cells, express OTRs and are stimulated by OT as is their normal counterpart. In fact, OT treatment significantly increased KS-IMM cell proliferation through intracellular calcium increase. This biological effect and the signaling pathways were both blocked by the addition of OTA, a specific OTR antagonist, which indicates the specificity of the OT/OTR binding and excludes the possibility of an OT effect through vasopressin receptors. Moreover, OTA itself was able to counteract basal KS-IMM cell proliferation, which suggests a possible therapeutic role for the OT antagonists in contrasting KS progression.

In contrast to the recent report on rat vasculature (9), we failed to detect OT-mRNA in KS-IMM cells. However, the data on rat vasculature were obtained on homogenized tissues (9), and it was, therefore, impossible to define the effective source of OT among the different components of the vessel wall. An alternative hypothesis is that, in modified endothelium as KS-IMM cells, the OTR expression is still preserved, whereas the OT synthesis is lost.

Among the growth factors known to be involved in the pathogen-
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Fig. 7. Effect of OT on cell motility. Micrographs represent a time-lapse analysis of KS-IMM cell motility, performed by digital saving at 15-min intervals. Migration tracks (×120) were generated by marking the position of the nucleus of individual cells of each image. A, (positive control), KS-IMM cell motility after 4-h incubation with 10 ng/ml VEGF. In B, OT (100-ns) treatment was not affecting cell motility after the same time interval. Conversely, an increased number of elongated cells became evident after OT incubation (inset), as well as an increased number of mitoses (inset, arrow.)

esis and evolution of KS, VEGF exerts a role on KS-IMM cell proliferation that is almost identical to that here reported for OT (28). However, VEGF also stimulates KS cell migration, whereas OT does not affect KS-IMM cell motility. In fact, we did not observe any motogenic effect of OT, although an effect on cell contractility was deduced by the enhanced spindle phenotype of KS-IMM cells after OT treatment.

KS is a complex disease in which the inflammatory component plays a major role, mostly in the early stages. The most represented lymphocytes of this infiltrate are CD8+ and CD4+ cells (in AIDS-related and classical lesions, respectively; Ref. 28), which produce a variety of cytokines that have been reported to induce normal endothelial cells to acquire the KS phenotype (36). In this study, we demonstrated that supernatants from both CD4+ and CD8+ lymphocytes up-regulate the expression of OTR on the KS-IMM cell surface. This effect of CD4+ and CD8+ supernatants in KS-IMM cells indicates that lymphocytes should produce one or more factors able to up-regulate the OTR expression: as a consequence, KS-IMM cell sensitivity to OT could be augmented. Therefore, the OT effect either on KS cell morphology (enhancement of the spindle phenotype after contraction) or on cell proliferation could be amplified.

Examining different cytokines, we observed that the large majority of the tested lymphokines, including TNF-α, IFN-γ, IL-2, and IL-6, were ineffective on OTR expression, either used alone or variably associated. On the contrary, IL-1β, IL-3, and IL-4 down-regulated the OTR expression. This latter observation is in agreement with the one recently observed in uterine smooth muscle cells, in which IL-1β down-regulated OTR expression (37, 38).

Neither HIV-1 Tat protein nor VEGF (both known to be involved in the proliferation of KS lesions; Refs. 39, 40) modified OTR expression in KS-IMM. Incubation of KS-IMM cells with OT and OTA down-regulated OTR expression, consistently with the previously reported effect of OT in reducing its own receptor density in other cell types (41). It has been reported that a cytokine mixture instead of a treatment using single cytokines may be necessary to influence KS cell proliferation (42). However, we failed to demonstrate any effect of even “mixed” cytokine treatment on OTR expression in KS-IMM cells. The factor determining the increased OTR expression in KS-IMM cell after incubation with CD4+ and CD8+ supernatants remains, therefore, to be defined.

Conversely, here we presented the first evidence of OT production by CD4+ and CD8+ lymphocytes. Although previous evidence demonstrated that lymphocytes might express OTRs (43), as we also observed in CD4+ and CD8+ cells, no indications were available on the possible OT synthesis by lymphatic cells. This observation opens new perspectives on the possible role of OT as a neuro-endocrine-immuno-modulator in a large variety of pathologies. Focusing on KS, our data indicate that the inflammatory counterpart of KS may play an active role in KS growth, directly providing the mitogenic factor OT to spindle cells.

In conclusion, these data suggest that OT should be considered a novel growth factor for KS. Moreover, the modulation of OTR expression on KS spindle cell surface by lymphocyte supernatants as well as the lymphocyte production of OT lead us to hypothesize the existence of endocrine-immunological cross-talk within the KS lesions. Finally, the expression of OTR in KS spindle cells makes KS eligible for the imaging and therapeutic prospects opened by the recently described radioligands derived from OT analogues, which could directly transfer radioactive or other pharmacological agents to neoplastic cells (44).

REFERENCES


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