Brain cytokine mRNAs in anorectic rats bearing prostate adenocarcinoma tumor cells

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Plata-Salamán, Carlos R., Sergey E. Ilyin, and Dave Gayle. Brain cytokine mRNAs in anorectic rats bearing prostate adenocarcinoma tumor cells. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R566–R573, 1998.—Cancer is consistently associated with anorexia. The Lobund-Wistar rat model of prostate cancer exhibits clinical manifestations (including anorexia) that resemble many aspects of the human disease. Cytokines are proposed to be involved in cancer-associated anorexia. Here we investigated mRNA profiles of feeding-modulatory cytokines and neuropeptides in specific brain regions of anorectic Lobund-Wistar rats bearing prostate adenocarcinoma tumor cells. Interleukin (IL)-1β system components (ligand, signaling receptor, receptor accessory proteins, receptor antagonist), tumor necrosis factor-α, transforming growth factor-β, glycoprotein 130 (IL-6 receptor signal transducer), proopiomelanocortin (POMC, opioid peptide precursor), and neuropeptide Y (NPY) mRNAs were analyzed with sensitive and specific RNase protection assays. The same brain region sample was assayed for all components. The data show that early anorexia in tumor-bearing rats was associated with an upregulation of IL-1β mRNA in the brain regions examined (cerebellum, cortex, and hypothalamus). IL-1 receptor antagonist (IL-1Ra) mRNA and IL-1 receptor type I mRNA levels were also significantly increased in the cortex and hypothalamus. All other cytokine components, POMC, or NPY mRNA levels were not significantly different between tumor-bearing and pair-fed (control) rats. IL-1β mRNA and IL-1Ra mRNA were also significantly upregulated in the spleen of tumor-bearing rats. These data suggest that 1) IL-1β mRNA upregulation in the brain may be relevant to the anorexia exhibited by the tumor-bearing Lobund-Wistar rat and 2) in vivo characterization of cytokine components in discrete brain regions during cancer is necessary to understand underlying molecular mechanisms responsible for cancer-associated neurological manifestations.

interleukin; tumor necrosis factor; growth factor; nervous system; neuroimmunology; hypothalamus; cortex; cerebellum; cancer; food intake; feeding; anorexia

PROSTATE ADENOCARCINOMA is the most common cancer in men (10). The Lobund-Wistar rat model of autochthonous prostate cancer exhibits clinical manifestations (including anorexia) that resemble many aspects of the human disease (24, 25). Lobund-Wistar rats can be inoculated with prostate adenocarcinoma tumor cells derived from prostate adenocarcinomas that develop spontaneously in aged pathogen-free Lobund-Wistar rats (25). The transplanted prostate adenocarcinoma cells produce a local subcutaneous tumor. These tumor cells are transplantable only to the Lobund-Wistar strain (26). Thus the Lobund-Wistar tumor-bearing rat model allows investigation of tumor-associated processes from initiation to promotion to progression stages.

Cancer progression is multifactorial and involves complex chemical cascades and cell-to-cell interactions. These can be mediated by chemical factors produced by the tumor and/or the host, including cytokines, which are proposed to play a role in various aspects of tumor biology. Constitutive production of cytokines and growth factors has been reported in prostate gland cancer (9, 10). Studies in humans and animals support the involvement of cytokines in the induction of clinical manifestations (including anorexia) during cancer (18). Several studies have reported increased circulating levels of cytokines in a number of patients with various types of cancer, including prostate adenocarcinoma, but not all types (14, 18, 32). Thus no conclusive evidence has been obtained on a requirement of increased cytokine concentrations in the circulation to demonstrate cytokine involvement in the induction of cancer-associated neurological manifestations (15, 18). Cytokines have a short half-life and act not only in an endocrine fashion but also via paracrine, autocrine, and intracrine manners, activities that cannot be detected in the circulation. In fact, paracrine interactions represent a predominant mode of cytokine action. This suggests that cytokines may be involved in cancer-related clinical manifestations due to local synthesis of cytokines in an organ, e.g., the brain. In the present study we tested this possibility.

Lobund-Wistar rats bearing prostate adenocarcinoma tumor cells were continuously monitored for the initiation and progression of anorexia. After a definitive establishment of early anorexia, the control (pair fed) and tumor-bearing rats were killed, and their brains and peripheral organs were dissected. Various cytokine and neuropeptide component mRNAs were determined in the brain (cerebellum, cerebral cortex, and hypothalamus) and periphery (spleen, liver, and tumor) using sensitive and specific RNase protection assays. We focused on the interleukin (IL)-1β system and tumor necrosis factor-α (TNF-α) because of their relevance as anorexigenic cytokines (18) and their proposed involvement in malignant processes (e.g., Refs. 4, 13, 18, and 31). The IL-1β system includes IL-1β [predominant released form of IL-1 (3)], IL-1 receptor type I [IL-1RI; responsible for IL-1β signaling (27)], modulation of the in vivo acute phase response (16), and induction of neurological manifestations (12, 28)]. IL-1 receptor accessory protein [IL-1R AcP; a protein that increases binding affinity of IL-1β for IL-1RI when the 2 proteins
are coexpressed (5, 33); IL-1R AcP expression also correlates with IL-1 responsiveness (33), and IL-1 receptor antagonist [IL-1Ra; an endogenous inhibitor that antagonizes, by competitive inhibition, IL-1β-induced central nervous system actions (3, 17) and binds to IL-1RI with nearly the same affinity as that for IL-1β]. The simultaneous investigation of various components of a cytokine system (i.e., the ligand, signaling receptor, receptor accessory protein, and endogenous inhibitor) can provide information on the feedback regulation and contribution of each cytokine system component.

We also examined transforming growth factor-β1 (TGF-β1), which is proposed to stimulate tumor progression (6) and inhibit IL-1β and TNF-α activity (21). Messenger RNA levels of the following components were also assayed: glycprotein 130 (gp130), a common signal transducer among receptors for members of the anorexigenic IL-6 subfamily (19) that is homologous to the leptin receptor (1); proopiomelanocortin (POMC), the precursor of β-endorphin and melanocyte-stimulating hormone that can modulate feeding; and neuropeptide Y (NPY), a potent feeding-inducing peptide (30). Thus the concomitant analysis of cytokine and neuropeptide systems can provide information on potential endogenous chemical interactions (22).

**MATERIALS AND METHODS**

Subjects and maintenance. Male Lobund-Wistar rats (control and tumor bearing) were purchased from Dr. Morris Pollard (Lobund Laboratory, University of Notre Dame, Notre Dame, IN). The prostate gland adenocarcinoma tumor cells were inoculated subcutaneously into the outer side of the right leg of ~12-wk-old rats in Dr. Pollard’s laboratory; control Lobund-Wistar rats received the subcutaneous administration of vehicle solution. Shortly afterward, the rats were received at the University of Delaware, placed individually, and maintained ad libitum on powdered rat food (Labdiet, PMI Feeds, St. Louis, MO) and tap water as previously described (23). The behavior of the rats and development of the tumor were monitored daily. Artificial light illumination was from 0600 to 1800, and room temperature was kept at 21 ± 2°C.

Powdered food consumption. The measurement of powdered food intake was the same as in previous studies (23). In all cases, food intake was measured to within 0.1 g. Rats were fed ad libitum daily, except between 1730 and 1800 when food was removed to be measured and replaced. Premeasured food was presented at 1800. Food intake was measured at 1730 (total daily consumption from 1800 to 1730).

Dissection of brain regions. After the monitoring period, rats were decapitated and their brains were quickly removed (in ~30 s from time of decapitation) and immediately placed in oxygenated PBS solution at 2–4°C. The brain was rinsed several times, and the cerebellum (vermis), parietofrontal cortex, and the complete hypothalamus were dissected with a tissue-slicer blade. Samples from peripheral organs (spleen and liver) and the tumor were also taken. In all cases, the same investigator performed the procedure. Each tissue sample was immediately homogenized with guanidiethiocyanate-pheno1 solution (see RNA isolation and analysis of IL-1β, IL-1Ra, IL-1RI, IL-1R AcP I and II, TNF-α, TGF-β1, gp130, POMC, NPY, β-actin, and GAPDH mRNAs). Total cell RNA was isolated from the tissue samples after homogenization of the samples in guanidine thiocyanate-pheno1 solution (Tr Reagent, Molecular Research Center, Cincinnati, OH) using a microtissue grinder. Each sample was homogenized and processed individually. RNA concentration was determined by spectrophotometry at an absorbance of 260 nm. RNA integrity was assessed by agarose gel electrophoresis with ethidium bromide staining. The levels of rat β-actin and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were determined by RNase protection to confirm that an assay was consistent and that an equal amount of total cell RNA was used for each assay.

Riboprobes were prepared as previously described (7, 22). Probe synthesis was conducted with 1 mM each of CTP, ATP, and UTP, 9.38 mM of [32P]GTP (800 Ci/mmol), and 25 μM of unlabeled GTP, [32P]GTP that was not incorporated into the probe was removed by two ethanol precipitations in the presence of 2.5 M ammonium acetate. RNase protection assays were used to detect the IL-1β, IL-1RI, IL-1R AcPs, IL-1Ra, TNF-α, TGF-β1, gp130, POMC, NPY, β-actin, and GAPDH mRNAs.

Hybridization reactions containing 20.0 μg of total cell RNA and 2.5 × 10^4 counts/min each of IL-1β, IL-1RI, IL-1R AcP, IL-1Ra, TGF-β1, and 1.5 × 10^4 counts/min of TNF-α antisense probe; 6.0 μg of total cell RNA and 2.5 × 10^4 counts/min each of gp130, POMC, and NPY antisense probes, or 3.0 μg of total cell RNA and 2.0 × 10^4 counts/min each of β-actin and GAPDH antisense probes in 30 μl of hybridization buffer (80% formamide, 0.4 M NaCl, 1 mM EDTA, and 40 mM PIPES (pH 6.4)) were heated to 85°C for 5 min and then incubated at 48°C for 12–18 h. After hybridization, 280 μl of RNase digestion buffer [50 mM sodium acetate (pH 4.5) and 2 mM EDTA] was added with 30 U of T1 RNase (Sigma, St. Louis, MO) for all assays followed by incubation at 30°C for 30 min. RNase digestion was terminated by the addition of 10% SDS and 50 μg of proteinase K and incubation for 30 min at 37°C. RNA was extracted with phenol–chloroform and precipitated with 70 μg of yeast transfer RNA by the addition of 100 μl of ethanol. RNA was dissolved in loading buffer [80% formamide, 2 mM EDTA (pH 7.4) containing 0.05% bromophenol blue and 0.05% xylene cyanol], denatured at 85°C for 5 min, and resolved on 5% acrylamide–8 M urea gels using TBE buffer (89 mM Tris pH 8.0, 89 mM boric acid, and 2.7 mM EDTA). Gels were autoradiographed, and results were quantified with an image analyzer (Image Quant; Molecular Dynamics, Sunnyvale, CA). Densitometric values for each mRNA analyzed were converted to percentage values of the total values for a particular mRNA.

In control experiments on hybridization specificity, an appropriate amount of yeast transfer RNA was hybridized and processed as described above. No signal corresponding to IL-1β, IL-1RI, IL-1R AcPs, IL-1Ra, TNF-α, TGF-β1, gp130, POMC, or NPY was detected.

Riboprobe templates. Rat IL-1β expression plasmid containing the entire mature peptide coding sequence of rat IL-1β with one extra methionine codon at the 5'-end donor into plasmid pET-21d (Novagen, Madison, WI) between Eco RI and Nco I sites was used. Plasmid rat riboprobe IL-1β (RFL-1β) was generated by cloning 150 bp containing an Xba I-Eco RI fragment of the rat IL-1β expression plasmid into pGEM-2 between the Xba I and Eco RI sites. Plasmid pRFL-1β was linearized with Hind III and transcribed with SP6 RNA polymerase to generate an ~570 nt-long antisense probe that protects 492 nt in the rat IL-1β mRNA.
Plasmid rat IL-1RI 3'-clone containing a 3'-fragment (nt 473–1,826 of accession no. M95578) of the rat IL-1RI cDNA cloned into the Smal site of pBluescript SK (Stratagene) was used. Linearization of this plasmid with Hind III and transcription with T3 RNA polymerase produced an ~520-nt-long antisense probe that protects 436 nt in the rat IL-1RI mRNA.

Rat IL-1Ra cDNA (nt 874–1,212 of accession no. U48592) cloned into EcoRV site of pBluescript SK was used. Linearization of this plasmid by Hind III and transcription by T3 RNA polymerase resulted in an ~420-nt-long antisense probe that protects 339 nt in the membrane-bound form of rat IL-1Ra.

Plasmid pBS-RA containing a fragment (nt 3–451 of accession no. M63101) of the rat IL-1Ra cDNA cloned into the Sma I site of pBluescript SK was linearized with Xmn I and transcribed with T3 RNA polymerase to produce an ~250-nt-long antisense probe that protects 207 nt in the rat IL-1Ra mRNA.

Plasmid pTRI-GAPDH (Ambion, which contains a 126-bp cDNA fragment of the rat GAPDH gene) was used. Linearization of this plasmid with Hind III and transcription by T3 RNA polymerase produced an ~320-nt-long antisense probe that protects 244 nt in the rat TNF-α mRNA.

Rat IL-1R AcP cDNA (nt 874–1,212 of accession no. M63101) of the rat IL-1Ra cDNA cloned into the Smal I site of pBluescript SK was linearized with Xmn I and transcribed with T3 RNA polymerase to produce an ~250-nt-long antisense probe that protects 207 nt in the rat IL-1Ra mRNA.

Plasmid pBS-RA containing a fragment (nt 3–451 of accession no. M63101) of the rat IL-1Ra cDNA cloned into the Sma I site of pBluescript SK was linearized with Xmn I and transcribed with T3 RNA polymerase to produce an ~250-nt-long antisense probe that protects 207 nt in the rat IL-1Ra mRNA.

Plasmid pBS-RA containing a fragment (nt 3–451 of accession no. M63101) of the rat IL-1Ra cDNA cloned into the Sma I site of pBluescript SK was linearized with Xmn I and transcribed with T3 RNA polymerase to produce an ~250-nt-long antisense probe that protects 207 nt in the rat IL-1Ra mRNA.

Plasmid pBS-RA containing a fragment (nt 3–451 of accession no. M63101) of the rat IL-1Ra cDNA cloned into the Sma I site of pBluescript SK was linearized with Xmn I and transcribed with T3 RNA polymerase to produce an ~250-nt-long antisense probe that protects 207 nt in the rat IL-1Ra mRNA.

Plasmid pBS-RA containing a fragment (nt 3–451 of accession no. M63101) of the rat IL-1Ra cDNA cloned into the Sma I site of pBluescript SK was linearized with Xmn I and transcribed with T3 RNA polymerase to produce an ~250-nt-long antisense probe that protects 207 nt in the rat IL-1Ra mRNA.

Plasmid pBS-RA containing a fragment (nt 3–451 of accession no. M63101) of the rat IL-1Ra cDNA cloned into the Sma I site of pBluescript SK was linearized with Xmn I and transcribed with T3 RNA polymerase to produce an ~250-nt-long antisense probe that protects 207 nt in the rat IL-1Ra mRNA.

Plasmid pBS-RA containing a fragment (nt 3–451 of accession no. M63101) of the rat IL-1Ra cDNA cloned into the Sma I site of pBluescript SK was linearized with Xmn I and transcribed with T3 RNA polymerase to produce an ~250-nt-long antisense probe that protects 207 nt in the rat IL-1Ra mRNA.
amples of the RNase protection assays used. As shown, samples from both control and tumor-bearing groups and the three brain regions were analyzed concomitantly. Consistency was verified by analyzing all samples individually, from which all means ± SE were generated. It should also be noted that each individual sample was obtained from a different rat, that the same rat provided all three brain regions, and that the same samples were analyzed with all of the antisense probes as described in MATERIALS AND METHODS. The levels of rat β-actin mRNA (Fig. 2) and GAPDH mRNA (Fig. 3) were relatively constant between treatments within a brain region; this indicates consistency of processing and that an equal amount of total cell RNA was used for each assay. Moreover, the specificity of the probes used has been demonstrated previously by various procedures (7, 21), including control experiments on hybridization specificity in which no signal corresponding to IL-1β, IL-1Ra, IL-1RI, IL-1R AcP, TNF-α, TGF-β1, gp130, POMC, or NPY mRNA was detected.

IL-1β mRNA in brain regions from control and tumor-bearing rats. The profile of IL-1β mRNA in the cerebellum, cortex, and hypothalamus is shown in Fig. 4.

ANOVA showed that groups differed significantly in IL-1β mRNA \( F(5, 36) = 23.0, P < 0.0001 \), power of performed test \( \text{ppt} = 1.0 \). All three brain regions examined had robustly significant differences between the control and tumor-bearing groups: cerebellum, \( P = 0.0008 \), \( \text{ppt} = 0.98 \); cortex, \( P < 0.0001 \), \( \text{ppt} = 1.0 \); and hypothalamus, \( P < 0.0005 \), \( \text{ppt} = 0.99 \).

IL-1Ra mRNA in brain regions from control and tumor-bearing rats. The data are summarized in Fig. 5. No significant difference in IL-1Ra mRNA levels between groups was observed in the cerebellum \( (P = 0.5) \). The cortex, on the other hand, exhibited a significant increase in IL-1Ra mRNA levels in the tumor-bearing group relative to the control group \( (P < 0.0001, \text{ppt} = 1.0) \). The difference in the hypothalamus was also significant \( (P < 0.05) \).

Fig. 2. RNase protection assay of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-1 receptor type I (IL-1RI), IL-1 receptor accessory protein I and II (IL-1R AcP I and II), transforming growth factor-β1 (TGF-β1), IL-1 receptor antagonist (IL-1Ra), and β-actin mRNA levels in the cerebellum (Cer), parietofrontal cortex (Cor), and hypothalamus (Hyp) from male Lobund-Wistar rats bearing prostate adenocarcinoma tumor (T) cells or from control (C) rats. Brain tissue samples were collected 36 days after tumor cell inoculation or vehicle administration. Figure shows that samples from each treatment and brain region were processed concomitantly. Each individual sample was obtained from a different rat, and the same rat provided all 3 brain regions; the same samples were analyzed with all antisense probes as described in MATERIALS AND METHODS.

Fig. 3. RNase protection assay of glycoprotein 130 (gp130), proopiomelanocortin (POMC), neuropeptide Y (NPY), and GAPDH mRNA levels. Other explanations as for Fig. 2.

Fig. 4. IL-1β mRNA levels in brain regions from control and tumor-bearing rats. Values (means ± SE; \( n = 7 \) for each group) were standardized to arbitrary units. *\( P < 0.001 \) compared with controls.
IL-1RI mRNA in brain regions from control and tumor-bearing rats. The profile of the IL-1 signaling receptor mRNA is summarized in Fig. 6. The difference in IL-1RI mRNA levels in the cerebellum was not significant (P = 0.1). On the other hand, IL-1RI mRNA levels were significantly increased in the cortex (P = 0.001) and hypothalamus (P = 0.0006) obtained from tumor-bearing rats.

![Graph showing IL-1Ra mRNA levels in brain regions from control and tumor-bearing rats. *P < 0.001 compared with controls; †P < 0.05 compared with controls.](image)

**Fig. 5.** IL-1Ra mRNA levels in brain regions from control and tumor-bearing rats. *P < 0.001 compared with controls; †P < 0.05 compared with controls.

IL-1R AcPs in brain regions from control and tumor-bearing rats. The profiles of IL-1R AcP I or membrane-bound IL-1R AcP and IL-1R AcP II or soluble form of IL-1R AcP mRNA levels did not change significantly in all three brain regions examined (data not shown).

TGF-β1 mRNA in brain regions from control and tumor-bearing rats. The data are summarized in Table 1. TGF-β1 mRNA levels were not significantly different in the three brain regions examined.

TNF-α mRNA in brain regions from control and tumor-bearing rats. The data are also summarized in Table 1. The levels of cerebellar, cortical, and hypothalamic TGF-β1 mRNA did not differ significantly between control and tumor-bearing rats.

gp130 mRNA, POMC mRNA, and NPY mRNA in brain regions from control and tumor-bearing rats. As shown in Table 2, no significant differences in gp130, POMC, or NPY mRNA levels in the brain regions examined were observed.

IL-1β mRNA and IL-1Ra mRNA in peripheral organs from control and tumor-bearing rats. We also examined IL-1β mRNA and IL-1Ra mRNA levels in two peripheral organs, the spleen and liver. Peripheral tissue samples were obtained from the same rats that provided the brain regions. The data obtained in the spleen are summarized in Fig. 7. Both IL-1β mRNA and IL-1Ra mRNA levels were significantly increased in the Lobund-Wistar rats bearing prostate adenocarcinoma tumor cells relative to the controls. The liver also exhibited significantly higher levels of IL-1Ra mRNA (32.6 ± 1.7 arbitrary units in tumor-bearing rats vs. 18.1 ± 2.2 arbitrary units in controls; t = 5.26, P = 0.0004, ppt = 1.0).

IL-1β mRNA in the tumor. The prostate gland from control Lobund-Wistar rats had undetectable levels of IL-1β mRNA. On the other hand, the signal for IL-1β mRNA was robust in all tumor samples taken from the tumor-bearing rats (n = 7).

**DISCUSSION**

The data show that anorectic tumor-bearing Lobund-Wistar rats exhibit an upregulation of IL-1β mRNA in discrete brain regions (cerebellum, cortex, and hypothalamus). IL-1RA mRNA and IL-1RI mRNA levels are also significantly increased in the cortex and hypothalamus. All other cytokine components, POMC, or NPY were

![Graph showing IL-1RI mRNA levels in brain regions from control and tumor-bearing rats. *P < 0.001, †P < 0.002 compared with controls.](image)

**Fig. 6.** IL-1RI mRNA levels in brain regions from control and tumor-bearing rats. *P < 0.001, †P < 0.002 compared with controls.

**Table 1.** TNF-α and TGF-β1 mRNA levels in brain regions from Lobund-Wistar rats

<table>
<thead>
<tr>
<th></th>
<th>Cerebellum</th>
<th>Cortex</th>
<th>Hypothalamus</th>
</tr>
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<tbody>
<tr>
<td>TNF-α mRNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.3±0.9</td>
<td>7.5±1.0</td>
<td>10.0±0.5</td>
</tr>
<tr>
<td>Tumor bearing</td>
<td>8.8±1.1</td>
<td>7.1±0.9</td>
<td>9.1±1.4</td>
</tr>
<tr>
<td>TGF-β1 mRNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.8±0.6</td>
<td>9.5±0.4</td>
<td>8.9±0.5</td>
</tr>
<tr>
<td>Tumor bearing</td>
<td>5.8±0.4</td>
<td>9.6±0.5</td>
<td>9.4±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE (standardized to arbitrary units) from 7 individual samples examined for each case. Tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β1 mRNAs were examined in same brain region samples used to determine all other mRNA components.
mRNA levels were not significantly different between tumor-bearing and control rats. These data suggest that IL-1β mRNA upregulation in the brain may be relevant to the early anorexia exhibited by the Lobund-Wistar rat bearing prostate adenocarcinoma tumor cells. Previous studies proposed that IL-1 can participate in the induction and progression of cachexia (4, 18, 31). Using a colon tumor model, Strassmann et al. (31) showed that intratumoral administration of IL-1Ra significantly reduced the cachexia associated with the tumor. Gelin et al. (4) also reported that treatment of rodents bearing methylcholanthrene-induced sarcoma with monoclonal antibodies against the IL-1R inhibited tumor growth and improved food intake. Moreover, the growth of Morris hepatoma in rodents was also associated with increased levels of IL-1 in the spleen (13).

The present study is the first to report upregulation of IL-1β mRNA in peripheral organs and discrete brain regions of the same rat responding to a peripheral tumor. The consistent increase in levels of IL-1β mRNA in the tumor, spleen, and discrete brain regions suggests that the tumor induces a series of events that result in local organ production of IL-1β. This is supported by our data because we used IL-1β mRNA as an index of local production of the cytokine. Thus the evidence suggests that local production of IL-1β in the brain (e.g., hypothalamus, an important feeding-regulatory brain site) could be involved in the induction of early anorexia or exacerbation of early anorexia in the Lobund-Wistar model of prostate cancer. This is consistent with the fact that IL-1β induces anorexia by direct action in the hypothalamus (18) and the report that intrahypothalamic administration of IL-1Ra improves feeding in anorectic rats bearing methylcholanthrene-induced sarcoma cells (11).

IL-1Ra mRNA and IL-1RI mRNA levels were also upregulated in brain regions. IL-1Ra mRNA upregulation accompanies IL-1β mRNA upregulation in various models (8, 21, 22). A balance between IL-1β and IL-1Ra appears critical for an appropriate modulation of IL-1β-associated cellular responses in the brain (8, 21). However, a significant excess of IL-1Ra is required to modulate IL-1β-induced cellular responses because the IL-1 system exhibits spare receptor effects, with maximal biological response observed with occupancy of only 1–10% of IL-1 receptors. We have discussed this modulation in detail (21). Upregulation of IL-1RI mRNA may also participate in the modulation of cellular responses to IL-1β (8, 21).

The brain region cytokine profile obtained in Lobund-Wistar rats bearing adenocarcinoma tumor cells is significantly different from the brain cytokine profile observed in all other rodent models we have investigated. In various models, for example, using specific bacterial products (22), viral glycoproteins (7), or cytokines (8, 21), TNF-α and TGF-β1 mRNAs are also significantly modulated. This indicates that distinct cytokine profiles and cytokine-cytokine interactions occur in brain regions depending on the underlying pathophysiological process or challenge.

### Table 2. gp130, POMC, and NPY mRNA levels in brain regions from Lobund-Wistar rats

<table>
<thead>
<tr>
<th>Region</th>
<th>Cerebellum</th>
<th>Cortex</th>
<th>Hypothalamus</th>
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<tbody>
<tr>
<td>gp130 mRNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.8 ± 0.3</td>
<td>3.4 ± 0.3</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>Tumor bearing</td>
<td>5.4 ± 0.5</td>
<td>3.6 ± 0.4</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>POMC mRNA</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>11.8 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor bearing</td>
<td>13.3 ± 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPY mRNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.1 ± 0.2</td>
<td>8.2 ± 0.6</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Tumor bearing</td>
<td>2.1 ± 0.2</td>
<td>8.8 ± 0.7</td>
<td>4.1 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE (standardized to arbitrary units) from 7 individual samples examined for each case. Levels of proopiomelanocortin (POMC) in cerebellum and cortex were below sensitivity of detection. Glycoprotein 130 (gp130), POMC, and neuropeptide Y (NPY) mRNAs were examined in the same brain region samples used to determine all other mRNA components.

Fig. 7. IL-1β mRNA and IL-1Ra mRNA levels in spleen from control and tumor-bearing rats. *P < 0.001 compared with controls.
Our previous studies also showed that cytokine-cytokine (29) and cytokine-neuropeptide (30) interactions are important in cytokine-induced anorexia, depending on the model. The present study examined other components associated with feeding: gp130, POMC, and NPY mRNAs. These did not differ significantly in any brain region between control and tumor-bearing rats. This suggests that early anorexia in the Lobund-Wistar rat model of prostate cancer is not associated with changes in hypothalamic mRNA for gp130 [a transducer among receptors for IL-6 subfamily receptor members that have been shown to induce anorexia (19)], POMC (opioid peptide precursor that generates various feeding-modulatory opioid peptides), or NPY (a feeding-enhancer peptide proposed to participate in the maintenance of normal feeding). A previous study reported that hypothalamic concentration and release of NPY was reduced in anorectic rats bearing methylcholanthrene-induced sarcoma (2). Thus tumor type and clinical stage of the malignant process could be associated with differential neuropeptide profiles.

Perspectives

The data show that in vivo characterization of cytokine components in discrete brain regions during cancer induction and progression is essential to understand the underlying molecular mechanisms [including cytokine-neurotransmitter-neuropeptide interactions (20)] responsible for cancer-associated neurological manifestations. For example, cytokine upregulation in the hypothalamus is relevant to cancer anorexia, whereas in the cerebral cortex it could be involved in the induction of neuropsychiatric manifestations (e.g., depression, anxiety, and delirium) that commonly occur during cancer. Cytokine profile characterization in other organs may also be relevant to the understanding of the mechanisms involved in the host’s biochemical and physiological response to tumor progression.

We thank Dr. Morris Pollard (Lobund Laboratory, University of Notre Dame) for providing the Lobund-Wistar rats. We also thank Dr. Ronald P. Hart (Department of Biological Sciences, Rutgers University) for providing the rat IL-1β, IL-1Ra, IL-1RI, and IL-1R AcP cDNAs; Dr. Karl Decke (Biochemisches Institut der Albert Ludwigs Universität) for providing the rat TNF-α cDNA; Dr. David Danielpour (National Cancer Institute) for providing the rat TGF-β1 cDNA; Dr. Gerald M. Fuller (Department of Cell Biology and Anatomy, University of Alabama at Birmingham) for providing the rat gp130 cDNA; Dr. Andrea Gore (Center for Neurobiology, The Mount Sinai Medical Center) for providing the rat POMC cDNA; and Dr. Steven L. Sabol (Laboratory of Biochemical Genetics, National Heart, Lung, and Blood Institute) for providing the rat NPY cDNA.

Research was supported by funds from the University of Delaware and the National Institutes of Health (C. R. Plata-Salaman).

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Received February 5, 1998; accepted in final form May 8, 1998.

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