Mechanisms of Experimental Cancer Cachexia

Local Involvement of IL-1 in Colon-26 Tumor

Gideon Strassmann,† Yoshihiro Masui,‡ Richard Chizzonite,§ and Miranda Fong*

†Department of Immunology, Otsuka America Pharmaceutical Inc., Rockville, MD 20850; ‡the Cellular Technology Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan 771-01; and the §Department of Molecular Genetics, Hoffman-LaRoche Inc., Nutley, NJ 07110

ABSTRACT. In the colon-26 (C-26) tumor model, the cytokine IL-6 is an important factor involved in experimental cancer cachexia. Recent in vitro data indicated that IL-1 plays a role in the interaction between host macrophages and C-26 cells that express IL-1R, resulting in the amplification of tumor IL-6 production. To investigate the role of IL-1 on the development of C-26 cachexia in vivo, the effect of specific blockade of the action of IL-1 with reagents against IL-1R was evaluated. Both IL-1R antagonist (IL-1RA) and the mAb 35F5 directed against IL-1R type I, prevented binding of radioactive IL-1, and inhibited IL-1-induced IL-6 synthesis by the C-26 cell line. Whereas a systemic administration of these reagents did not reverse weight loss in C-26-bearing mice, intratumoral injections of IL-1RA significantly reduced cachexia. Furthermore, body composition analysis confirmed that this treatment improved lean tissue and fat, as well as hypoglycemia and serum IL-6 level. The fact that the treatment did not change the tumor burden suggests that it affected the host directly. These results support the hypothesis that, at the microenvironment of the C-26 tumor, IL-1 is involved in the cachexia endured by the host. Journal of Immunology, 1993, 150: 2341.

Neoplastic diseases are frequently associated with metabolic changes collectively known as cancer cachexia. These abnormalities include wasting of both fat and muscle tissues, anorexia, asthenia, hypoglycemia, and anemia (1, 2). Cachexia long has been recognized as an important cause of death in cancer patients (3), and patients who exhibit weight loss have a reduced response to chemotherapy (4). Understanding the mechanisms that lead to cachexia therefore is important.

TNF has been suggested as a mediator of cancer cachexia because it suppresses key metabolic enzymes and induces anorexia and weight loss in animals (5–8). Recently, however, an experimental cachexia model has been identified that appears to involve another cytokine. The model uses a cell line derived from C-26,2 which retains the transplantability of the original tumor in syngeneic mice, and fulfills the criteria of early onset wasting without apparent anorexia. It also involves a relatively small tumor burden (9). In at least this model, IL-6 appears to have a more significant role than TNF in mediating the myriad parameters of cachexia (9). In culture, the C-26.IVX cell line expresses high affinity IL-1R type I, and fM concentrations of IL-1 but not TNF, upregulates IL-6 production (10). In addition, significant potentiation of tumor IL-6 secretion can be seen when the line is co-cultured with syngeneic mononuclear phagocytes. In this cellular interaction, the mAb 35F5, directed against murine IL-1R type I, blocks IL-6 synthesis (10).

IL-1RA is a naturally occurring protein (11), which is useful in blocking several IL-1-mediated pathologies in vivo (12). Accordingly, utilizing IL-1R blocking reagents, the role of IL-1 in the development of C-26-mediated cachexia was investigated.

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† Address correspondence and reprint requests to Dr. Gideon Strassmann, Department of Immunology, Otsuka America Pharmaceutical Inc., 9900 Medical Center Drive, Rockville, Maryland, 20850.

2 Abbreviations used in this paper: C-26, colon-26 adenocarcinoma; IL-1RA, IL-1R antagonist; (CD)Fl, BALB/c × DBA/2F1.
Materials and Methods

Mice

Virus-free, male BALB/C × DBA/2 (CD)F1, mice were purchased from Charles River Breeding Laboratories (Wilmington, MA). Mice were housed under conventional conditions and used at 10 to 12 wk of age.

Reagents

Human rIL-1β was a gift from Dr. Y. Hirai (Otsuka Pharmaceutical, Tokushima, Japan). [125I]IL-1α (sp.act. 2000 Ci/mmol.) was from Amersham (Arlington Heights, IL). Purified IL-1RA was obtained after the cloning, expression, and purification procedures described previously (11, 13). The sterile material had a purity of greater than 99% by SDS-PAGE, and endotoxin content was determined to be less than 0.5 ng/mg of protein. For in vivo experiments, IL-1RA was diluted in sterile Dulbecco's Ca++- and Mg++-free PBS (GIBCO, Grand Island, NY). This diluent also served as a vehicle for control injections. The rat mAb 35F5 (14) (IgG1) was obtained from Dr. R. Chizzonite (Hoffman-LaRoche, Nutley, NJ). Purified rat IgG was obtained from Sigma (St. Louis, MO).

Assays

The IL-1 radioreceptor assay on C-26.IVX (obtained without trypsinization) and on EL-4.6.1 cells was performed as previously described (10). The presence of IL-6 in serum and culture-conditioned medium was performed by utilizing the B-9 cell line assay (10) and ELISA (Endogen, Boston, MA). The addition of mAb against murine IL-6 and a murine IL-6R completely abrogated IL-6-dependent proliferation by test samples.

Measurement of cachexia markers

Mice were inoculated with 0.5 × 10⁶ C-26.IVX cells s.c. to the right flank as described (9, 10). Treatments were performed as indicated in the tables. Mice were weighed between 9 a.m. and 11 a.m. three times per week. The length and width of their tumors were measured by using an engineering caliper, and estimation of tumor weight was calculated, as described, for the same tumor (15). Significant weight loss in C-26-bearing mice occurred between 12 and 14 days after tumor inoculation. Host weight was calculated by subtracting tumor weight (obtained by resection) from total weight. Blood was obtained by cardiac puncture (approximately 0.8 ml), and serum was harvested after the clotting of blood at room temperature for 1 h. Serum was kept frozen (−45°C) until analysis. Measurements of serum glucose were performed using an Ektachem DT-60 analyzer (Eastman Kodak Co., Rochester, NY). Dry weight was determined (after removal of the tumor, blood, and right epididymal fat) by oven drying for 3 days at 85°C.

Table I

<table>
<thead>
<tr>
<th>Addition to Radioreceptor Assay</th>
<th>[125I]IL-1α Bound (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-26.IVX</td>
</tr>
<tr>
<td>Medium</td>
<td>2237 ± 10</td>
</tr>
<tr>
<td>Human IL-1β (100 ng/ml)</td>
<td>400 ± 10</td>
</tr>
<tr>
<td>Rat IgG (2 μg/ml)</td>
<td>2257 ± 80</td>
</tr>
<tr>
<td>Anti-IL-1R (2 μg/ml)</td>
<td>495 ± 5</td>
</tr>
<tr>
<td>IL-1RA (30 ng/ml)</td>
<td>437 ± 20</td>
</tr>
</tbody>
</table>

* Radioassay was performed as described in Materials and Methods.  

Statistical analysis

Results are presented as mean ± SD. Differences in cachexia markers were calculated using computerized analysis of variance.

Results and Discussion

First we determined whether reagents capable of recognizing IL-1R would block binding of radioactive IL-1 to the C-26.IVX cell line. As shown in Table I, the mAb 35F5 and IL-1RA completely blocked binding of [125I]IL-1α to both the C-26 cell line and to EL-4.6.1 cells in a radioreceptor assay. In a dose-dependent manner, both the mAb and the IL-1RA inhibited IL-1 induced IL-6 production by the tumor line (Table II). On a molar basis, IL-1RA was approximately 240-fold more active than the 35F5 mAb in inhibiting IL-6 synthesis. Also in the table, IL-1RA at millimolar concentration had no agonist activity in inducing IL-6 production by the tumor line.

Next we examined the role of IL-1 in C-26 mediated cachexia. Mice were inoculated with the tumor line and treated with the mAb and IL-1RA, as indicated in Table III. No significant or sustained improvement in tumor-mediated weight loss could be seen when these reagents were administered systemically. Of note, the amount of IL-1RA used in this experiment exceeded the amount used to protect mice from graft-vs-host disease mortality (16) and to block IL-1 induction of IL-6 in vivo (17). The amount of 35F5 mAb used was greater than that shown to increase mortality of irradiated mice (18) and to attenuate turpentine-induced weight loss (19). Together with our previously published data (9), these results suggest that IL-1 does not act in concert with IL-6 in the circulation of C-26 bearing mice as they were losing weight. Nevertheless, the possibility still exists that the amount of blocking reagents used was insufficient to reach the tumor and effectively exert their action. Thus, we attempted to prevent cachexia by the direct administration of IL-1RA to the tumor. As shown in Tables IV and V, intratumoral injections of IL-1RA significantly improved the deleterious systemic effects of the tumor. Typical cachectic parameters, including
Table II
IL-1RA and anti-IL-1R type I mAb inhibit IL-1-induced IL-6 production by C-26.IVX cells

<table>
<thead>
<tr>
<th>Addition to Culture</th>
<th>IL-6 Production</th>
</tr>
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<tbody>
<tr>
<td>IL-1</td>
<td>IL-1RA</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>

* C-26 IVX (2 x 10^5/well) cells were cultured with IL-1 (10 pg/ml) and increasing amounts of IL-1 RA or anti IL-1 R type I mAb (indicated in the table as nanograms per milliliter and micrograms per milliliter, respectively). At 24 and 48 h, culture supernatants were collected and subjected to IL-6 bioassay (in units per milliliter) or ELISA (in picograms per milliliter). Variation of triplicate determinations did not exceed 10%.

Table III
Systemic administration of IL-1 RA and anti IL-1 R type I mAb does not improve weight loss

<table>
<thead>
<tr>
<th>Tumor Bearing</th>
<th>Treatment</th>
<th>No.</th>
<th>Host weight (g) on Day 10</th>
<th>Tumor weight (g) on Day 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ PBS</td>
<td>6</td>
<td>25.0 ± 0.5</td>
<td>20.6 ± 1.0</td>
<td>19.0 ± 0.8</td>
</tr>
<tr>
<td>+ IL-1 RA</td>
<td>6</td>
<td>25.5 ± 0.7</td>
<td>23.0 ± 0.9</td>
<td>21.0 ± 1.0</td>
</tr>
<tr>
<td>+ Anti IL-1R</td>
<td>6</td>
<td>25.9 ± 0.6</td>
<td>20.1 ± 0.8</td>
<td>19.1 ± 0.8</td>
</tr>
<tr>
<td>+ Rat IgG</td>
<td>6</td>
<td>25.6 ± 0.3</td>
<td>20.4 ± 0.5</td>
<td>18.9 ± 0.5</td>
</tr>
<tr>
<td>- PBS</td>
<td>5</td>
<td>25.3 ± 0.3</td>
<td>25.3 ± 0.5</td>
<td>25.4 ± 0.5</td>
</tr>
</tbody>
</table>

* CD2Fl male mice were injected with C-26.IVX cells (day 0). On day 7 mice were randomized to receive PBS (0.25 ml, s.c. daily), IL-1 RA (10 mg/kg, s.c. daily), anti-IL-1R type I mAb and polyclonal rat IgG (12.5 mg/kg, i.p. on days 7, 10, and 13).

Table IV
Inhibition of Cachexia by Intratumoral Administration of IL-1RA is Dose Dependent

<table>
<thead>
<tr>
<th>Tumor Bearing</th>
<th>Treatment</th>
<th>Initial Weight (g)</th>
<th>Final Body Weight (g)</th>
<th>% Weight Loss</th>
<th>Tumor Weight (g)</th>
<th>Serum Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- PBS</td>
<td>25.1 ± 0.9</td>
<td>25.8 ± 0.4</td>
<td>-</td>
<td>-</td>
<td>126 ± 3</td>
<td></td>
</tr>
<tr>
<td>+ PBS</td>
<td>25.1 ± 1.3</td>
<td>20.2 ± 0.3</td>
<td>19.5</td>
<td>1.1 ± 0.3</td>
<td>32 ± 6</td>
<td></td>
</tr>
<tr>
<td>+ IL-1RA (20)</td>
<td>24.3 ± 0.9</td>
<td>20.2 ± 0.8</td>
<td>17.5</td>
<td>1.1 ± 0.5</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>+ IL-1RA (50)</td>
<td>24.6 ± 0.8</td>
<td>22.6 ± 1.2</td>
<td>8.2</td>
<td>1.0 ± 0.4</td>
<td>64 ± 17</td>
<td></td>
</tr>
<tr>
<td>+ IL-1RA (100)</td>
<td>25.2 ± 1.5</td>
<td>23.7 ± 1.7</td>
<td>5.9</td>
<td>1.1 ± 0.4</td>
<td>72 ± 14</td>
<td></td>
</tr>
</tbody>
</table>

* CD2F1 male mice were injected with C26.IVX cells (day 0). On day 7 mice were randomized. On day 9, 11, 13, and 14 tumors were injected with 0.1 ml PBS or with increasing amounts (shown in parentheses in micrograms) of IL-1RA. Mice were killed on day 15 and final body weight was determined after resection of the tumor. Results are expressed as mean ± SD of five mice per group.

* Not tested.
* Probability value of at least 0.03 from the group which received intratumoral injection of PBS.

The intratumoral administration of IL-1RA resulted in a least two orders of magnitude more potent than the mAb in blocking IL-1 activities on the C-26 line in culture (Table II). It is also conceivable that the significantly lower m.w. of the IL-1RA—as compared with the mAb—allowed it to diffuse more easily and thus affect more cells when injected into the tumor.

The intratumoral administration of IL-1RA resulted in a
relatively modest reduction (−40%) of serum IL-6, at a
time when the protection of the host’s weight was more
pronounced (Table V). IL-1RA is a competitive antagonist
to the action of IL-1 on the C-26 cell line (Table II). Its
effect, therefore, is not permanent and may not be sufficient
to inhibit IL-6 production by the additional tumor cells
generated between the last administration of IL-1RA and
the completion of the experiment. Alternatively, our still
incomplete understanding of the regulation of IL-6R in the
C-26 model, and the fact that serum IL-6 level may rep-
resent the unused portion of the cytokine in vivo, make it
difficult to evaluate accurately the importance of serum
IL-6 level. In addition, we cannot exclude the possibility
that the level of IL-6 in the serum is of limited importance,
as was recently demonstrated by May et al. (20)

To the best of our knowledge, the results presented here
are the first to suggest that events occurring in the tumor
mass contribute significantly to wasting, and that IL-1 plays
an important role locally at the level of the tumor to initiate
and/or exacerbate the wasting syndrome. IL-1 is a potent
stimulator of IL-6 synthesis by the C-26 tumor line (10).
The results shown here, although supporting the stimulator
role of IL-1 in vivo, do not exclude the possibility that the
cytokine may also amplify the production of a still unde-
termined factor/mediator that participates in C-26 cachexia,
in addition to IL-6. These factors may also affect the phys-
iology of tumor infiltrating macrophages present in C-26
tumors (10).

IL-6 is a multifunctional cytokine. In addition to its in-
volved in other tumor-associated weight loss (9), it has been
implicated in several unrelated chronic inflammatory con-
ditions (21). Thus, an important question in the IL-6 field
is how this pleiotropic cytokine contributes to such a wide
variety of diseases and whether its involvement in vivo
requires other factors that cooperate or synergize with its
action.

The fact that intratumoral injection of IL-1RA did not
influence tumor burden is important. A previous study at-
tempted to assess the effect of IL-1 on the development of
cachexia in the MCG-101 tumor model (22). In that study,
however, it was difficult to document a direct effect of IL-1
on wasting because the antibody used inhibited the tumor,
thus implicating a role for the cytokine in promoting tumor
growth.

The observation that systemically administered IL-1RA
and 35F5 mAb could not affect cachexia is not surprising.
Many conventional drugs and biologics, including cytok-
ines and mAb, only minimally affect solid tumors, and sev-
eral physiologic factors have been identified as responsible
for the poor delivery of macromolecules to solid tumors
(23). Identifying these factors in the C-26 model may fur-
ther the design of therapeutics that inhibit cachexia.

Acknowledgments

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