Experimental Cancer Cachexia: The Role of Host-derived Cytokines Interleukin (IL)-6, IL-12, Interferon-γ, and Tumor Necrosis Factor α Evaluated in Gene Knockout, Tumor-bearing Mice on C57 Bl Background and Eicosanoid-dependent Cachexia


Surgical Metabolic Research Laboratory and Lundberg Laboratory for Cancer Research, Department of Surgery, Sahlgrenska University Hospital, Göteborg University, S-413 45 Göteborg, Sweden

ABSTRACT

MCG 101 tumors were implanted sc. on wild-type C57 Bl and gene knockout mice to evaluate the role of host-produced cytokines [interleukin (IL)-6, IL-12, IFNγ, tumor necrosis factor (TNF) receptor 1, and TNF receptor 2] to explain local tumor growth, anorexia, and carcass weight loss in a well-defined model with experimental cachexia. Indomethacin was provided in the drinking water to explore interactions between host and tumor-derived prostaglandins and proinflammatory cytokines for tumor growth. Wild-type tumor-bearing mice developed cachexia because of rapid tumor growth, which were both attenuated in IL-6 gene knockouts. Similar findings were observed after provision of anti-IL-6 to wild-type tumor-bearing mice. Alterations in food intake were not directly related to systemic IL-6 but rather secondarily to IL-6-dependent tumor growth. The absence of host-derived IL-12, IFN-γ, or the TNF receptor 1 or receptor 2 gene did not attenuate tumor growth or improve subsequent cachexia. Thus, carcass weight loss was not improved by the omission of host cytokine (TNF-α, IL-12, or IFN-γ) except for IL-6. Systemic indomethacin provision decreased plasma prostaglandin E2 in five of six groups of gene knockout tumor-bearing mice, which was associated with improved carcass weight in these groups. Indomethacin seemed to improve food intake to a similar extent in both wild-type and gene knockouts, which agree with the speculation that eicosanoids are more important to explain anorexia than host cytokines. Our results demonstrate that host- and tumor-derived cytokines and prostaglandins interact with tumor growth and promote cachexia in a more complex fashion than usually presented based on previous information in studies on either anti-cytokine experiments in vivo or on gene knockouts with respect to a “single cytokine model.” Overall, host cytokines were quantitatively less important than tumor-derived cytokines to explain net tumor growth, which indirectly explains subsequent cachexia and anorexia.

INTRODUCTION

It is well established that proinflammatory cytokines are important to induce and promote development of experimental cancer cachexia (1, 2), although a corresponding role has not been confirmed in clinical studies (3, 4) except for a potential role of IL-6 (5, 6). A quantitative discrepancy in effects on host metabolism and tumor growth among species may be manifold, but it cannot be excluded that prolonged artificial transplantation of tumors on inbred mice and rats would select tumor clones with a particular dependency for certain growth factors and cytokines. Accordingly, it has been observed repeatedly that monospecific neutralizing antibodies to certain cytokines improve anorexia, attenuate cachexia, and reduce tumor growth in tumor-bearing mice (7–11). Such experiments with whole-body provision of neutralizing antibodies imply that several cytokines are quantitatively important to explain cachexia. It is, however, unclear to what extent large-sized antibodies, with limited tissue penetration, can effectively extinguish paracrine-acting cytokines for integrated organ functions, such as the hepatic acute phase response, central nervous system-related anorexia and sympathetic/parasympathetic circulatory alterations. Also, systemic provision of antibodies do not distinguish between effects from host versus tumor-produced cytokines. The availability of gene knockout mice bearing implanted tumors may therefore improve the possibility to evaluate the role of host versus tumor-derived cytokines for tumor progression (12–14). The aim of the present study was therefore to evaluate to what extent host-derived cytokines of IL-6, IL-12, IFN-γ, and TNF-α contribute to progressive cachexia in MCG 101-bearing mice on a C57 Bl genetic background and eicosanoid-dependent cachexia.

MATERIALS AND METHODS

Animals and Experimental Design. Mice with C57 Bl genetic background (18–22 g) were used in all experiments. The animals were housed prior to experimentation in a temperature-controlled room with a diurnal 12 h light cycle and provided with tap water and standard rodent chow ad libitum (B & K Universal, Stockholm, Sweden) for 2 weeks. The animals were then transferred to plastic cages with screen floors that permitted collection and quantification of spilled food. Under anesthesia with i.p. injection of 0.10 ml from a 1-ml stock solution of 0.4 ml of ketamine (100 μg/ml; Parke-Davis), 0.05 ml xylocain (5 μg/ml), and 0.55 ml saline, 3 mm3 of viable methylcholantrene-induced sarcoma (MCG 101) was implanted s.c. on each side of the midline. MCG 101 is a nonmetastasizing, undifferentiated, epithelial-like solid tumor that has been extensively used in our laboratory for study of cancer cachexia, and it has been grown continuously in vivo since 1975 (15). The tumors grow locally with a reproducible growth pattern when implanted s.c. The animals die because of cancer cachexia 12–15 days after implantation (16–18). The tumor produces IL-1α, IL-1β, TNF-α, and IL-6 (8, 19) but not IFN-γ and IL-12.4 Prostaglandins, particularly PGE2, is also produced both in vitro and in vivo, leading to elevated host plasma concentrations (20, 21). Cyclooxygenase inhibition by indomethacin, which normalizes systemic levels of PGE2, has been reported to improve nutritional state and food intake, reduce tumor growth, and prolong survival in wild-type C57 Bl mice (22).

Mice with genetic knockout of IL-6 (23, 24) were provided by Professor Manfred Kopf (Basil Institute for Immunology, Basel, Switzerland) and Professor Andreij Tarkowski (Department of Immunology, Göteborg University, Göteborg, Sweden); IL-12 (Roche, Nutley, NJ), IFN-γ (Jackson Laboratories, Bar Harbor, ME), and TNF R1 and TNF R2 (The Jackson Laboratory, Bar Harbor, ME) were used in parallel to wild-type C57 Bl mice from Boltmols Gård (Ry, Denmark).

Received 12/28/99; accepted 4/28/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by Grants 2014-B98-12XAC and 01PAA from the Swedish Cancer Society, Grants K99-72X-08712-11A and 13159 from the Medical Research Council, and grants from the Tore Nilsson Foundation, Assar Gabrielsson Foundation (AB Volvo), Jubileumsklinikten Foundation, IngaBritt and Arne Lundberg Research Foundation, Axel and Margaret Axson Johnson Foundation, Knut and Alice Wallenberg Foundation, Swedish and Göteborg Medical Societies, and the Medical Faculty, University of Göteborg.

2 To whom requests for reprints should be addressed, at Department of Surgery, Sahlgrenska University Hospital, Göteborg University, S-413 45 Göteborg, Sweden.

3 The abbreviations used are: IL, interleukin; TNF, tumor necrosis factor; R1, receptor 1; R2, receptor 2; PGE2, prostaglandin E2; SAP, serum amyloid P.

4 Unpublished observations.
Surgery, University of Florida, Gainesville, FL) was provided as i.p. injections (300 µg every third day). This experiment was performed to follow up on previous experiments with anti-IL-1 and TNF-α (7, 21). The hybridoma-grown monoclonal antibody, directed against murine IL-6, completely blocked the SAP, serum amyloid A protein, and orosomucoid response in absence of models (25). Preimmune sera (IgG; Sigma Chemical Co., St. Louis, MO) served as control. Indomethacin (5 mg/ml; Dumex) was provided in the drinking water at a final concentration of 6.5 µg/ml. Experiments with indomethacin were performed to evaluate whether cyclooxygenase-related tumor growth inhibition involves host cytokines. Murine IL-12 (0.2 µg/day i.p.) was obtained from Labkemi (Västra Frölunda, Sweden). These experiments were performed to demonstrate the paradox of attenuated tumor growth and improved nutritional state, because presently used batches of gene knockout mice and corresponding carcass weight are presented as carcass weight changes during tumor progression and drying to constant weight as described previously (28). Alterations in weight was registered, and body composition was evaluated by lipid extraction and lipid/g oil content. The animals were killed by cervical dislocation 10–12 days after tumor implantation. Ethics at the University of Gothenburg, Sweden.

RESULTS

Tumor Growth. Tumor growth was similar among different cohorts of wild-type mice when compared, although our experiments on various groups were carried out at different occasions. All of these control mice were pooled to represent one reference group in comparison to the smaller groups with gene knockout mice (Table 1). Tumor growth on IL-6 knockout mice (IL-6−/−) displayed a significant reduction (48 ± 14%; P < 0.01), whereas tumor growth was not significantly changed on IL-12 (−/−), IFN-γ (−/−), TNF R1 (−/−), and TNF R2 (−/−) knockout mice compared with wild-type controls.

Provision of neutralizing antibodies toward IL-6 in wild-type tumor-bearing mice reduced tumor growth (40 ± 10%; P < 0.01) by an order of magnitude observed for tumor growth in gene knockout IL-6 (−/−) tumor-bearing mice. This growth inhibition was associated with reduction in the hepatic acute phase response demonstrated by reduced plasma levels of SAP protein from 109 ± 24 to 27 ± 4 ng/ml (P < 0.01) and plasma IL-6 close to detection limits. However, suramin, an assumed functional IL-6 receptor antagonist, had no effect to reduce tumor growth in wild-type tumor-bearing mice (results not shown).

Indomethacin reduced tumor growth by 32 ± 8% (P < 0.03) in wild-type C57 Bl mice. This effect was also observed in IL-12 (−/−) knockout mice (36 ± 9%; P < 0.05), whereas indomethacin had no clear cut effect to reduce tumor growth in groups of IL-6 (−/−), IFN-γ (−/−), TNF R1 (−/−), or TNF R2 (−/−) deficient mice (Table 2).

Daily i.p. provision of recombiant IL-12 to wild-type tumor-bearing mice reduced tumor growth by 75 ± 13% (P < 0.001), whereas the same provision of IL-12 to IFN-γ (−/−) knockouts had no effect on tumor growth (Table 3).

Carcass Weight Change. Tumor growth in wild-type mice caused reduction of carcass weight, which was significantly attenuated in IL-6 (−/−) knockouts, in agreement with the effects observed in wild type mice when provided i.p. with antibodies toward IL-6 (not shown). However, carcass weight change in IL-12 (−/−) and IFN-γ (−/−) gene knockout mice were not significantly changed compared with wild type. By contrast, carcass weight losses in TNF R1 or R2 (−/−) mice were even significantly more pronounced compared with either wild-type mice or to the other groups of gene knockout mice (Table 1). Indomethacin attenuated carcass weight loss in IFN-γ (−/−), TNF R1, and TNF R2 (−/−) tumor-bearing mice and even increased carcass weight in IL-6 (−/−) and IL-12 (−/−) tumor-bearing gene knockout mice (Table 2). IL-12 treatment to wild-type tumor-bearing mice was also associated with significantly increased carcass weight, whereas this effect was entirely absent after IL-12 treatment of IFN-γ (−/−) tumor-bearing mice (Table 3).

Food Intake. Food intake was stable in nontumor-bearing wild-type and knockout mice during a 2-week period, indicating steady-state conditions in our experimental environment (not shown). Progressve tumor growth was associated with the appearance of anorexia in all groups of tumor-bearing animals, i.e., in both wild-type and

Table 1 Tumor and carcass weight changes in relation to plasma PGE2 and IL-6 concentrations in wild-type (+/+ ) and gene knockout (−/−) tumor-bearing mice 12 days after tumor implantation

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Tumor weighta (g)</th>
<th>Carcass weight changeb (g)</th>
<th>Plasma PGE2a (pg/ml)</th>
<th>Plasma IL-6a (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57 Bl; wild-type</td>
<td>19</td>
<td>1.690 ± 0.117</td>
<td>-1.923 ± 0.190</td>
<td>1574 ± 309</td>
<td>413 ± 75</td>
</tr>
<tr>
<td>C57 Bl; IL-6 (−/−)</td>
<td>16</td>
<td>0.895 ± 0.118</td>
<td>-0.230 ± 0.290</td>
<td>2413 ± 504</td>
<td>104 ± 33a</td>
</tr>
<tr>
<td>C57 Bl; IL-12 (−/−)</td>
<td>10</td>
<td>1.744 ± 0.207</td>
<td>-1.330 ± 0.37</td>
<td>2408 ± 942</td>
<td></td>
</tr>
<tr>
<td>C57 Bl; IFN-γ (−/−)</td>
<td>10</td>
<td>1.445 ± 0.168</td>
<td>-2.343 ± 0.348</td>
<td>767 ± 78b</td>
<td>1579 ± 814</td>
</tr>
<tr>
<td>C57 Bl; TNF R1 (−/−)</td>
<td>10</td>
<td>1.722 ± 0.241</td>
<td>-3.329 ± 0.625</td>
<td>1247 ± 165</td>
<td></td>
</tr>
<tr>
<td>C57 Bl; TNF R2 (−/−)</td>
<td>10</td>
<td>2.039 ± 0.198</td>
<td>-3.061 ± 0.463</td>
<td>1687 ± 75</td>
<td></td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Mean ± SE.

a: freely fed, non-tumor-bearing wild-type C57 Bl had 65 ± 18 g/ml PGE2 and 4 ± 2 pg/ml IL-6 concentrations.

b: P < 0.05 vs C57 Bl wild type.

c: n = 34.

d: n = 19.

**ROLE OF IL-6, IL-12, IFNγ, AND TNFα IN CANCER CACHEXIA**

Downloaded from cancerres.aacrjournals.org on September 13, 2017. © 2000 American Association for Cancer Research.
ROLE OF IL-6, IL-12, IFN-γ, AND TNFα IN CANCER CACHEXIA

Table 2 Effect of indomethacin treatment on tumor weight, carcass weight change, plasma PGE2, and IL-6 concentrations in wild-type and gene knockout (−/−) tumor-bearing mice 12 days after tumor implantation

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Tumor weighta (g)</th>
<th>Carcass weight changea (g)</th>
<th>Plasma PGE2b (pg/ml)</th>
<th>Plasma IL-6b (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57 Bl wt</td>
<td>15</td>
<td>1.752 ± 0.141</td>
<td>−1.86 ± 0.28</td>
<td>1574 ± 309</td>
<td>413 ± 75</td>
</tr>
<tr>
<td>+ indomethacin</td>
<td>9</td>
<td>1.199 ± 0.128</td>
<td>−0.230 ± 0.290</td>
<td>404 ± 48</td>
<td>1528 ± 139</td>
</tr>
<tr>
<td>C57 Bl (IL-6 /− /−)</td>
<td>16</td>
<td>0.895 ± 0.118</td>
<td>−0.230 ± 0.290</td>
<td>2413 ± 504</td>
<td>104 ± 33</td>
</tr>
<tr>
<td>+ indomethacin</td>
<td>9</td>
<td>0.819 ± 0.178</td>
<td>0.290 ± 0.310</td>
<td>765 ± 237</td>
<td>77 ± 20</td>
</tr>
<tr>
<td>C57 Bl (IL-12 /− /−)</td>
<td>10</td>
<td>1.744 ± 0.207</td>
<td>−1.330 ± 0.370</td>
<td>2408 ± 942</td>
<td></td>
</tr>
<tr>
<td>+ indomethacin</td>
<td>9</td>
<td>1.100 ± 0.200</td>
<td>0.00 ± 0.200</td>
<td>788 ± 440</td>
<td></td>
</tr>
<tr>
<td>+ indomethacin</td>
<td>10</td>
<td>1.353 ± 0.270</td>
<td>−1.992 ± 0.235</td>
<td>767 ± 78</td>
<td></td>
</tr>
<tr>
<td>C57 Bl (IFN-γ /− /−)</td>
<td>9</td>
<td>1.577 ± 0.180</td>
<td>−1.073 ± 0.292</td>
<td>134 ± 33</td>
<td></td>
</tr>
<tr>
<td>+ indomethacin</td>
<td>9</td>
<td>1.722 ± 0.241</td>
<td>−3.329 ± 0.625</td>
<td>1247 ± 165</td>
<td></td>
</tr>
<tr>
<td>C57 Bl (TNF R1 /− /−)</td>
<td>10</td>
<td>1.315 ± 0.174</td>
<td>−0.653 ± 0.361</td>
<td>277 ± 137</td>
<td></td>
</tr>
<tr>
<td>+ indomethacin</td>
<td>9</td>
<td>2.039 ± 0.198</td>
<td>−3.061 ± 0.463</td>
<td>1687 ± 75</td>
<td></td>
</tr>
<tr>
<td>+ indomethacin</td>
<td>10</td>
<td>1.578 ± 0.287</td>
<td>−1.455 ± 0.646</td>
<td>93 ± 21</td>
<td></td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SE.
* These animals are included in C57 wt Table 1.
* P < 0.05 versus the study group without indomethacin.
* The same animals as presented in Table 1.

Table 3 Effect of IL-12 treatment (1 μg/day, i.p.) on tumor weight, carcass weight change, plasma PGE2, and IL-6 concentrations in wild-type (+/+ ) and gene knockout (−/−) tumor-bearing mice 12 days after tumor implantation

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Tumor weighta (g)</th>
<th>Carcass weight changea (g)</th>
<th>Plasma PGE2b (pg/ml)</th>
<th>Plasma IL-6b (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57 Bl wt</td>
<td>10</td>
<td>1.609 ± 0.181</td>
<td>−1.139 ± 0.361</td>
<td>834 ± 62</td>
<td>1071 ± 64</td>
</tr>
<tr>
<td>+ IL-12 treatment</td>
<td>10</td>
<td>0.408 ± 0.041b</td>
<td>0.519 ± 0.01b</td>
<td>6750 ± 250b</td>
<td>3180 ± 638b</td>
</tr>
<tr>
<td>C57 Bl IFN-γ /− /−</td>
<td>10</td>
<td>1.353 ± 0.270</td>
<td>−1.999 ± 0.235</td>
<td>767 ± 78</td>
<td>1579 ± 814</td>
</tr>
<tr>
<td>+ IL-12 treatment</td>
<td>10</td>
<td>1.533 ± 0.131</td>
<td>−2.064 ± 0.342</td>
<td>775 ± 40</td>
<td>1219 ± 237</td>
</tr>
</tbody>
</table>

* Mean ± SE.
* P < 0.01 wild-type tumor-bearing mice.
* The same animals as given in Table 1.

gene knockout mice (P < 0.001). Indomethacin improved food intake in wild-type tumor-bearing mice when evaluated over the entire experimental period (days 1–10; P < 0.01; Fig. 1). Evaluations of food intake between study and control mice were limited to the period between day 6 and 10 after tumor implantation to primarily focus on the tumor-induced anorexia, because days 4–6 were the period when food intake was substituted in all animal groups subsequent to the initial anorexia associated with anesthesia and tumor implantation. All groups of tumor-bearing knockouts had statistically significant anorexia when compared with wild-type non-tumor-bearing controls (P < 0.01), but no significant changes were observed between wild-type and gene knockout mice (not shown). i.p. provision of neutralizing antibodies toward IL-6 in wild-type tumor-bearing mice did not improve anorexia (not shown), which was also true for indomethacin treatment of IL-6 knockout mice (Fig. 2). However, indomethacin improved food intake when evaluated in pooled groups of knockout tumor-bearing mice by the same magnitude as observed for indomethacin in wild-type tumor-bearing mice (Fig. 3).

Plasma PGE2 and IL-6. Plasma PGE2 levels were 65 ± 18 pg/ml in C57 Bl wild-type non-tumor-bearing mice. These levels were increased to 1574 ± 309 pg/ml in wild-type tumor-bearing mice (Table 1), whereas indomethacin treatment reduced PGE2 concentrations to 404 ± 48 pg/ml in wild-type tumor-bearing mice (Table 2). Tumor-bearing IFN-γ (−/−) mice had significantly reduced plasma PGE2 levels comparable with wild-type tumor-bearing mice, whereas all other tumor-bearing gene knockout mice showed plasma PGE2 concentrations that were not significantly different from wild-type concentrations. IL-12 provision to wild-type tumor-bearing mice increased plasma PGE2 8-fold in wild-type mice (P < 0.01) but not in IFN-γ (−/−) tumor-bearing mice (Table 3).

IL-12 provision to wild-type tumor-bearing mice also increased plasma IL-6 3-fold (P < 0.005) but had no stimulatory effect on plasma IL-6 in IFN-γ (−/−) knockout mice (Table 3). IL-12 provision to wild-type mice reduced plasma SAP by 50 ± 8% (not shown), despite elevation of plasma IL-6 in these mice (Table 3).

Measurable concentrations of plasma IL-6 occurred in IL-6 (−/−) knockouts, which represents tumor-derived IL-6 (Table 1), because IL-6 knockouts lack IL-6 mRNA expression in host tissues, even in response to lipopolysaccharide stimulation (24). Indomethacin treatment did not reduce tumor-derived IL-6 in IL-6 (−/−) knockouts further but increased plasma IL-6 in wild-type tumor-bearing mice (Table 2), which confirms our previous findings on circulatory bioactive hepatocyte growth factor/IL-6 activity (19).

DISCUSSION

The aim of this study was to estimate the quantitative role of host-derived cytokines (IL-6, IL-12, IFN-γ, and TNF-α) to explain progressive cachexia associated with tumor growth in a defined mouse model with eicosanoid-dependent cachexia. Possible mechanisms behind cancer cachexia have earlier been explored using neutralizing monospecific antibodies toward cytokines (7, 8, 10, 11). This is a straightforward technique but with some theoretical limitations. Drawbacks may be time- and distribution-related restrictions for penetration of large antibodies throughout tissue compartments at the cellular level, but paradoxical effects may also occur (25). Therefore, we considered it valuable to extend previous observations on tumor growth and cachexia in a well-characterized tumor model on gene knockout mice as applied by others (12, 14) to specify potential contributions of host-derived cytokines versus some tumor-derived cytokines. For this purpose, we were restricted to the use of gene knockout mice with a genetic background similar to that of a well-characterized tumor with the production of IL-6, and TNF-α, whereas IFN-γ and IL-12 were only produced in host tissues. The MCG 101 tumor kills the host because of negative energy balance by inducing anorexia, carcass weight loss, and altered body composition without...
Accordingly, tumor growth in IL-6 (2) knockout mice was associated with a corresponding decline in tumor growth and improved carcass weight but without direct effects on food intake. Thus, IL-6 experiments imply that antibodies neutralize plasma IL-6, originating from either tumor or host tissues attenuating tumor growth, which secondarily leads to improved nutritional state. IL-6 continued to appear in the circulation of IL-6 (−/−) knockouts, because the tumor cells were IL-6 (+/+)-competent, whereas the host completely lacks IL-6 expression, even in response to bacterial antigens (24). Thus, our results suggest that IL-6, derived from both tumor and host tissues, is a significant tumor growth factor with similar effects as IL-1 and TNF-α on MCG 101 cells (8). Therefore, it seems tempting to conclude that increasing plasma IL-6 would deteriorate appetite and nutritional state (2, 14), but our results did not reveal improved anorexia in IL-6 knockout mice or in anti-IL-6-treated tumor bearers, findings that agree with prolonged anorexia following sepsis in IL-6 knockout mice (32), pronounced anorexia in IL-6-deficient mice (33), as well as a lack of anorexia after intracerebroventricular infusions of IL-6 (34). In addition, IL-6 content in brain areas of importance for food regulation did not support IL-6 as a directly acting central cytokine at a time (1, 13, 14).

In agreement with previous results, i.p. provision of monospecific antibodies to IL-6 in wild-type tumor-bearing mice caused a decrease in systemic inflammation, demonstrated by lower plasma concentrations of SAP and IL-6 associated with decreased tumor growth but unexpectedly without improved food intake (14). Thus, anti-IL-6 had to some extent similar effects as anti-IL-1 and anti-TNF-α (9, 10, 31). Accordingly, tumor growth in IL-6 (−/−) knockout mice was associated with a corresponding decline in tumor growth and improved carcass weight but without direct effects on food intake. Thus, IL-6 experiments imply that antibodies neutralize plasma IL-6, originating from either tumor or host tissues attenuating tumor growth, which secondarily leads to improved nutritional state. IL-6 continued to appear in the circulation of IL-6 (−/−) knockouts, because the tumor cells were IL-6 (+/+)-competent, whereas the host completely lacks IL-6 expression, even in response to bacterial antigens (24). Thus, our results suggest that IL-6, derived from both tumor and host tissues, is a significant tumor growth factor with similar effects as IL-1 and TNF-α on MCG 101 cells (8). Therefore, it seems tempting to conclude that increasing plasma IL-6 would deteriorate appetite and nutritional state (2, 14), but our results did not reveal improved anorexia in IL-6 knockout mice or in anti-IL-6-treated tumor bearers, findings that agree with prolonged anorexia following sepsis in IL-6 knockout mice (32), pronounced anorexia in IL-6-deficient mice (33), as well as a lack of anorexia after intracerebroventricular infusions of IL-6 (34). In addition, IL-6 content in brain areas of importance for food regulation did not support IL-6 as a directly acting central cytokine in MCG 101-bearing mice.4 Also, our experiments with i.p. provision of recombinant IL-12 to wild-type tumor-bearing mice caused a dramatic reduction in tumor growth and improved nutritional state and food intake as observed by others (35, 36), despite a pronounced increase in both plasma IL-6 and PGE2. These seemingly discrepant results may indicate that IL-6 regulation of tumor growth is more predictive for outcome of cachexia than even substantially elevated plasma levels of cytokines (IL-6) and PGE2. Also, reduced tumor growth did override the effect of increased plasma IL-6 on circulating acute phase reactants in IL-12-treated tumor bearers, which was unexpected in the light of previous conclusions on the role of cytokine regulation of acute phase reactants (33, 34). Similarly, reversion of cachexia, despite high plasma concentrations of IL-6, has been reported by others after systemic elevation of IL-10 in colon 26-bearing mice (37). Thus, IL-6 seems to be necessary for tumor induction of cachexia but not sufficient (38), as concluded after lipopolysaccharide induction of cachexia in IL-6-deficient, non-tumor-bearing mice (32).

In contrast to our previous findings that provision of anti-TNF-α attenuated cachexia (7, 8), we could not demonstrate that the lack of host receptors of either TNF R1 or R2 had any positive effect on tumor growth, carcass weight, or food intake. These discrepancies compared with previous results may either indicate that subtypes of TNF receptors can substitute for each other, or that TNF binding to host receptors is of less importance. Therefore, TNF and IL-1 receptors on tumor cells must be more important than corresponding TNF receptors in host tissues, a conclusion in agreement with our previous report that both TNF-α and IL-1 represent growth factors for MCG 101 cells (8), now also demonstrated for IL-6. Thus, our present and previous results suggest that tumor production of cytokines is more important to indirectly explain cachexia than a concept with tumor-induced production of host cytokines (TNF-α, IL-12, and IFN-γ), whereas IL-6 seems to have a dual role, as also supported by clinical studies (5, 6). Thus, we may either imply additional effector molecules in host tissues (39) or simply conclude that substrate flows among tumor and host compartments remain a major factor behind experimental cachexia (40–42). This old and conservative concept agrees with our earlier observations that glucose consumption in MCG 101 tumors explains the major part of carcass weight loss in combination with anorexia, and death occurs when tumor glucose consumption exceeds the capacity for liver gluconeogenesis (17).

Our present results demonstrate that the lack of either IFN-γ or limiting survival because of metastatic spread (15–18, 20, 22, 27, 28, 30). Our present results demonstrate that development of experimental cachexia seems to be more complex than emphasized previously in reports on the role of anti-IL-1, anti-TNF-α, anti-IL-6, and anti-IFN-γ to explain cachexia (1, 7) experiments that focused on only one cytokine at a time (1, 13, 14).

\[ P < 0.01 \]

\[ a, b, g, n \]

\[ SE. \]

\[ g \]

\[ 5491 \]

\[ a, AND TNF \]

\[ g \]

\[ 5 \]

\[ n \]

\[ 5 \]

\[ n \]
**ROLE OF IL-6, IL-12, IFNγ, AND TNFα IN CANCER CACHEXIA**

Fig. 3. Food intake of wild-type tumor-bearing mice (wt tb, df = 9; 23 mice in each group) compared with tumor-bearing knockouts with (ko tb indo, df = 12; 60 mice) and without (ko tb, df = 12; 60 mice) indomethacin treatment as described in "Materials and Methods." The decline in food intake was statistically significant over time in all three groups (P < 0.01). Indomethacin-treated knockout tumor-bearing mice had significantly improved food intake compared with the other groups evaluated by factorial ANOVA for repeated measures (P < 0.04). Statistical degrees of freedom (df) within parentheses refer to number of metabolic cages where food intake was registered as the mean intake/mouse in groups of five animals/cage. Bars, SE.

**IL-12 genes in host tissues did not influence tumor growth and carcass weight, despite the fact that provision of exogenous IL-12 decreased tumor growth, a phenomenon again suggesting that host production of these cytokines is of less importance for tumor growth and related cachexia. The effect of exogenous IL-12 on tumor growth was completely absent in IFN-γ (−/−) knockout mice, suggesting that IFN-γ was the effector molecule as described by others (35). Confusingly, published results suggest IFN-γ as a potent trigger for development of experimental cancer cachexia based on either experiments with neutralizing antibodies toward IFN-γ or in experiments with excess production of IFN-γ in vivo by engineered tumor cells (11, 43), but clinical studies do not support such a concept (5). Although the release of IFN from either tumor or host cells after IL-12 provision was not quantified, it is likely that inhibition of tumor growth by IL-12 was explained by increased host production of IFN-γ in response to IL-12 (44, 45), because tumor cells were IFN-γ gene competent also in host gene knockout experiments. All of these results support the conclusion that host production of IFN-γ or IL-12 was not involved in the systemic development of cancer cachexia in this model, which does not exclude the apparent observation that high concentration of IL-12 and IFN-γ can trigger reactions that restrict tumor growth, which secondarily improves cachexia. Therefore, the most simple explanation to our present and previous results is that a number of cytokines control local tumor growth, which is the primary and overall determinant of experimental cancer cachexia.

We have repeatedly reported that provision of indomethacin to MCG-bearing mice retards tumor growth, as confirmed in the present study (20, 22). This effect may be regarded as related to either host production of cytokines or to host and tumor production of prostaglandins, particularly PGE₂ (21, 46). Tumor inhibition by indomethacin in the MCG 101 model is not related to any particular vascular bed or organ (20), but we have repeatedly assumed that it is partly dependent on decreased host production of systemic proinflammatory cytokines. Our present results indicate that indomethacin clearly retarded tumor growth in wild-type tumor-bearing mice but had less clear cut effects on tumor growth in gene knockout mice. However, the improved effect by indomethacin on carcass weight was clear cut in all groups of host gene knockout mice, directionally correlated to declines in PGE₂ but not to IL-6 (Table 2). Thus, we are left with the impression that indomethacin improves carcass weight by either decreased tumor or systemic production of PGE₂, which however led to minor and variable effects on tumor growth. This conclusion agrees with our previous observations in survival experiments with indomethacin, where treatment of wild-type mice allows tumor-bearing mice to die with larger tumors but less depleted carcasses compared with tumor bearers without indomethacin. Similar observations have also been made in cancer patients (47). The observations in the present study also agree with recent observations in our laboratory that brain cytokines do not explain tumor-induced anorexia, whereas prostaglandins (PGE₂) may do so. If so, it may explain why food intake was significantly improved in pooled groups of indomethacin-treated knockouts (Fig. 3).

In conclusion, present experiments in gene knockout tumor-bearing mice demonstrate that evaluations behind systemic effects of tumor growth may be extended when performed in combined experiments with neutralizing antibodies and gene knockout mice, because tumor cells were wild-type and host cells were knockout for cytokines that control tumor growth. This study confirms that host production of IL-6 contributes to the development of cachexia, whereas host production of TNF-α, IL-12, and IFN-γ was insignificant. Therefore, a role of TNF-α in host wasting is related to tumor production of TNF, whereas neither host nor tumor production of IL-12 and IFN-γ explains cachexia. However, large amounts or high tissue concentrations of IL-12 and IFN-γ can obviously override the cachetic effects of both IL-6 and PGE₂ by restriction of tumor growth. Thus, tumor mass is the outstanding predictor of cachexia. This conclusion has support in older observations that tumor-bearing mice die when tumor energy consumption exceeds counter regulatory mechanisms such as gluconeogenesis (17), and that s.c. expansion of an inert mass can induce similar metabolic alterations in such animals as observed in tumor bearers (42). How indomethacin improves the metabolic balance between a growing tumor and its host remains to be determined.

**REFERENCES**

27. Lundholm, K., Karlberg, I., Ekman, L., Edstrom, S., and Schersten, T. Evaluation of
24. Kopf, M., Baumann, H., Freer, G., Freudenberg, M., Lamers, M., Kishimoto, T.,
22. Gelin, J., Andersson, C., and Lundholm, K. Effects of indomethacin, cytokines, and
15. Lundholm, K., Edstrom, S., Ekman, L., Karlberg, I., Bylund, A. C., and Schersten, T.
14. Molotkov, A., Satoh, M., and Togahama, C. Tumor growth and food intake in
7. Molotkov, A., Satoh, M., and Togahama, C. Tumor growth and food intake in
Experimental Cancer Cachexia: The Role of Host-derived Cytokines Interleukin (IL)-6, IL-12, Interferon-γ, and Tumor Necrosis Factor α Evaluated in Gene Knockout, Tumor-bearing Mice on C57 Bl Background and Eicosanoid-dependent Cachexia
