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


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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, IFN-γ, 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-m1 stock solution of 0.4 ml of ketamine (100 μg/g; Parke-Davis), 0.05 ml xylazine (5 μg/g), 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, 9) 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 Tarkowskij (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 Bomholt Gård (Ry, Denmark).

Anti-IL-6 (kindly obtained by Professor L. L. Moldawer, Department of
Analyses of serum concentrations of prostaglandins. The serum was acidified and added to blood samples to prevent further breakdown of arachidonic acid in carcass weight are presented as carcass weight changes during tumor progression and drying to constant weight as described previously (28). Alterations in analyses. The tumors were extirpated, weighed, and dried. Body carcass weight loss in colon 26-bearing mice (26).

Functional deficiency compared with the situation with all genes being intact. Based on the assumption that any loss of a host cytokine gene could imply a functional deficiency compared with the situation with all genes being intact. These experiments were performed to demonstrate the paradox of attenuated tumor growth and improved nutritional state in combination, despite increased plasma PGE2 and IL-6 (Table 3). Suramin (Calbiochem), a functional IL-6 receptor antagonist, was provided on days 2, 5, and 8 i.p. at 100 μg/g, which has been demonstrated to decrease IL-6-related weight loss in colon 26-bearing mice (26).

Food intake and body weight were measured daily as described (22, 24, 27). The animals were killed by cervical dislocation 10–12 days after tumor implantation. Blood was drawn into heparinized syringes and frozen until analyses. The tumors were extirpated, weighed, and dried. Body carcass weight was registered, and body composition was evaluated by lipid extraction and drying to constant weight as described previously (28). Alterations in carcass weight are presented as carcass weight changes during tumor progression, because presently used batches of gene knockout mice and corresponding controls had a comparatively large variation in pre-experimental body weight.

Biochemical Analyses. Indomethacin (final concentration, 10 μg/ml) was added to blood samples to prevent further breakdown of arachidonic acid in analyses of serum concentrations of prostaglandins. The serum was acidified and ethanol precipitated after centrifugation. PGE2 was extracted on AmPrep C18 mini columns (Amersham; RPN 1900) and quantified by RIA (Amersham) within 6 days. SAP was quantified by rocket immunoelectrophoresis as described (29). Plasma IL-6 was measured by ELISA from Amersham (Buckinghamshire, United Kingdom).

Statistics. Results are expressed as mean ± SE. Multiple group comparisons were performed by one-way ANOVA. Time course changes between study and control mice were evaluated by ANOVA for repeated measures. P < 0.05 was considered statistically significant. Scheffe-F test was used post hoc.

Primarily, tumor-bearing gene knockout mice were compared statistically to randomly selected wild-type tumor-bearing mice (Tables 1–3). For evaluation of anorexia, statistical computations were also performed on pooled groups of wild-type and gene knockouts (Fig. 3) to increase the number of statistical degrees of freedom in measurements on food intake, where each cage contained the average intake of five animals. Thus, the total number of cages was 33 containing 143 mice. The biological justification of this procedure was based on the assumption that any loss of a host cytokine gene could imply a functional deficiency compared with the situation with all genes being intact.

The experimental protocol was approved by the Committee for Animal Ethics at the University of Göteborg, 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 (IL-−/−), IFN-γ (IFN−/−), TNF R1 (IL-10−/−), and TNF R2 (IL-2−/−) 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 (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 (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 (IL-6−/−), IFN-γ (IFN−/−), TNF R1 (IL-10−/−), or TNF R2 (IL-2−/−) deficient mice (Table 2).

Daily i.p. provision of recombinant 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 (IFN−/−) 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 (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 (IL-12−/−) and IFN-γ (IFN−/−) gene knockout mice were not significantly changed compared with wild type. By contrast, carcass weight losses in TNF R1 or R2 (IL-1−/−) 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 (IFN−/−), TNF R1, and TNF R2 (IL-2−/−) tumor-bearing mice and even increased carcass weight in IL-6 (IL-6−/−) and IL-12 (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 (IFN−/−) 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). Progressive 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 PGE2c (pg/ml)</th>
<th>Plasma IL-6d (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57 Bl; wild-type</td>
<td>19</td>
<td>1.690 ± 0.117</td>
<td>−0.192 ± 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>2143 ± 504</td>
<td>104 ± 33</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>767 ± 784</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>1247 ± 165</td>
<td>1579 ± 814</td>
</tr>
<tr>
<td>C57 Bl; TNF R1 (−/−)</td>
<td>10</td>
<td>1.722 ± 0.241</td>
<td>−3.532 ± 0.625</td>
<td>1687 ± 75</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>1627 ± 85</td>
<td></td>
</tr>
</tbody>
</table>

a Mean ± SE.

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

c P < 0.05 vs C57 Bl wild type.

d n = 19.
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 restored 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 administration to wild-type tumor-bearing mice (Fig. 3).

** Plasma PGE$_2$ and IL-6. ** Plasma PGE$_2$ levels were 65 ± 18pg/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 PGE$_2$ concentrations to 404 ± 48 pg/ml in wild-type tumor-bearing mice (Table 2). Tumor-bearing IFN-γ (−/−) mice had significantly reduced plasma PGE$_2$ levels comparable with wild-type tumor-bearing mice, whereas all other tumor-bearing gene knockout mice showed plasma PGE$_2$ concentrations that were not significantly different from wild-type concentrations. IL-12 provision to wild-type tumor-bearing mice increased plasma PGE$_2$ 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).

**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 straight-forward 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.

**Table 3** | Effect of IL-12 treatment (1 μg/day, i.p.) on tumor weight, carcass weight change, plasma PGE$_2$, 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 weight$^a$ (g)</th>
<th>Carcass weight change$^a$ (g)</th>
<th>Plasma PGE$_2$ (pg/ml)</th>
<th>Plasma IL-6 (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 ± 0.041$^b$</td>
<td>0.519 ± 0.01$^b$</td>
<td>6750 ± 250$^b$</td>
<td>3180 ± 638$^b$</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>1.333 ± 0.131</td>
<td>−2.064 ± 0.342</td>
<td>775 ± 40</td>
<td>1219 ± 257</td>
</tr>
</tbody>
</table>

$^a$ Mean ± SE.

$^b$ $P < 0.01$ wild-type tumor-bearing mice.

$^c$ The same animals as given in Table 1.
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).

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 bears, 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 nervous cytokine in MCG 101-bearing mice. 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, nontumor-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

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**Fig. 1.** Time course changes of food intake in wild-type tumor-bearing mice with and without indomethacin treatment from days 1–10 as described in “Materials and Methods.” The difference over time in food intake was statistically different between the two groups evaluated by ANOVA for repeated measures (P < 0.01). Bars, SE.

**Fig. 2.** Food intake in tumor-bearing IL-6 (−/−) knockout mice with (n = 17) and without (n = 17) indomethacin treatment from day 1 as described in “Materials and Methods.” The decline in food intake was statistically significant in both groups (P < 0.01), without any difference between the two groups. 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 by 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 prosta
glandins, 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, whereas neither host nor tumor production of 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

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