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Role of Endogenous Tumor Necrosis Factor α and Interleukin 1 for Experimental Tumor Growth and the Development of Cancer Cachexia[1]

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ABSTRACT

The aim of this study was to evaluate to what extent tumor necrosis factor α (TNF-α) and interleukin 1 may explain the development of experimental cancer cachexia. For this purpose, C57BL/6J mice bearing a transplantable low differentiated rapidly growing tumor were passively immunized every other day with rabbit or rat neutralizing immunoglobulins against either TNF-α (anti-TNF) or against an interleukin 1 receptor (anti-IL-1r). Anti-IL-1r in itself had no agonistic effect to the type I, T-cell/fibroblast IL-receptor. Tumor-bearing mice receiving either preimmune antiserum or nonimmune rat hybridoma IgG served as controls.

Anti-TNF and anti-IL-1r inhibited tumor growth significantly, as measured by a lower wet and dry tumor weight at the end of 11 days of antiserum treatment ($P < 0.05$). The acute phase response in tumor-bearing animals, measured as an increase in liver weight, hepatic RNA content, and increases in plasma concentrations of circulating IL-6, serum amyloid P, transferrin, complement (C3), and a decrease in plasma albumin, were unaffected by the specific antiserum treatments. Food intake, which declined significantly in pre/nonimmune injected tumor-bearing controls, was significantly improved in tumor-bearing animals immunized against TNF-α or the IL-1r. Whole body lipid content showed a trend to improvement in specifically immunized animals ($P < 0.07$). The effects on whole body fat-free dry weight were insignificant, although numerically higher in specifically immunized tumor-bearing animals. The combination of anti-TNF and anti-IL-1r antiserum had no additive effects compared to single antiserum treatment suggesting that the two antibody treatments acted through a common mechanism. Cultured tumor cells, established from growing tumors, were sensitive to anti-TNF and anti-IL-1r, which both reduced tumor growth *in vitro*. This inhibitory effect by the antiserum could in part be reversed by the addition of recombinant IL-1α and TNFα.

We conclude that both TNF and IL-1 are involved in tumor growth and thus the progression of cancer cachexia. It seems as if the role of TNF and IL-1 was to promote tumor growth rather than restrict tumor growth in the present model. In this sense both TNF and IL-1 may act as tumor growth factors.

INTRODUCTION

The mechanisms underlying cancer cachexia are not fully clarified, although we have suggested that increased production and release of cytokines are contributing factors (1). Recently we described the administration of TNF-α[3] antibodies to tumor-bearing mice with the purpose of evaluating the extent to which endogenous TNF release contributed to tumor-host wasting in two murine tumor models (2). The results demonstrated that neutralizing antibodies against TNF attenuated anorexia, tumor growth, and improved body composition in mice bearing a rapidly growing tumor. However, those results also confirmed that TNF was not the only or even a major component behind tumor host wasting. The limited effect of TNF may in part be explained by the fact that several cytokines interact *in vivo*, since it is well known that the effect of one cytokine may potentiate the effects of other cytokines (3, 4). Therefore, we have now evaluated to what extent neutralizing antibodies to TNF and to the interleukin 1 receptor exert additive effects and thus explain significant parts of host wasting in mice bearing a low differentiated and rapidly growing tumor originally induced by methylcholanthrene. This tumor produces measurable amounts of both IL-1 and TNF (5), which may induce catabolism and promote anorexia.

MATERIALS AND METHODS

Experimental Procedures. Adult, weight-stable, female C57BL/6J mice (Alab, Stockholm, Sweden) were lightly anesthetized with pentobarbital (60 mg/kg body weight) and implanted s.c. with 3–5 mm^3 of a transplantable methylcholanthrene-induced sarcoma (MCG 101). This sarcoma-bearing mouse model has been studied in the past as a model for human cancer cachexia (6, 7). Animals were studied 11 days later when the tumor was 10–15% of body weight to agree with our previous reports. Previous studies from this laboratory have shown that tumor-bearing mice uniformly die 14–17 days following tumor implantation (8). Anorexia, host wasting, and tumor growth are gradual and progressive. All control mice received anesthesia and a sham implantation.

For the next 11 days tumor-bearing mice received i.p. injections every other day of 100 mg/kg body weight of a rabbit polyclonal immunoglobulin fraction raised against murine TNF or 10 mg/kg bw of a rat anti-mouse IL-1 receptor monoclonal antibody. Sham injections consisted of rabbit preimmune IgG and nonimmune hybridoma IgG in equivalent amounts to the specific antisera. Food intake was recorded daily. At the end of the study, the animals were killed by cervical dislocation and heparinized whole blood was obtained by cardiac puncture. Tumors were removed and dried at 80°C. Carcasses were analyzed for composition as described elsewhere (8). Fat-free dry weight was used as the estimate of lean tissue. Liver samples were also taken for measurements of RNA content as described elsewhere (9). Plasma samples were analyzed by rocket immunoelectrophoresis (10) for concentrations of albumin, transferrin, serum amyloid P protein, and complement (C3). Interleukin 6 activity was measured by the B-9 cell proliferation assay (11). Specificity of the assay was confirmed by the ability of polyclonal rabbit anti-murine IL-6 antisera to neutralize plasma bioactivity (12).

Production of TNF-α antibodies was performed as described elsewhere (13). The quantities of immunoglobulin required to neutralize *in vivo* TNF production were determined in preliminary studies by administering various doses of anti-TNF immunoglobulin fraction to mice receiving a lethal endotoxin challenge and by assessing survival, as previously described by Beutler *et al.* (13). Anti-TNF immunoglobulin

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[3] The abbreviations used are: TNF, tumor necrosis factor; IL, interleukin; IL-1r, interleukin 1 receptor.

fraction (100 or 200 mg/kg body weight) administered 6 h before a 500-μg endotoxin challenge (*Salmonella enteritidis*; Difco) improved survival to potentially lethal endotoxemia, and the serum of such antibody-treated mice did not contain detectable TNF bioactivity as determined by the L-929 cytotoxicity assay (14). Lower doses did not improve survival or completely neutralize the TNF-dependent L-929 cytotoxicity in serum after challenge. For this reason, a 100-mg/kg body weight dose of anti-TNF immunoglobulin fraction was selected for i.p. administration to tumor-bearing mice every other day. Initial experiments revealed that injections every day gave the same results. Two sets of experiments were done. First was one experiment in which tumor-bearing and non-tumor-bearing animals received anti-TNF. An additional group of tumor-bearing mice received preimmune IgG. In the next experiment, tumor-bearing and non-tumor-bearing animals received either anti-TNF, anti-IL-1r, or the combination of anti-TNF plus anti-IL-1r. An additional group of tumor-bearing mice received rat hybridoma nonimmune IgG. The results from the two experiments were pooled for statistical evaluation.

The anti-IL-1r monoclonal antibody was obtained by fusing NSO myeloma cells with splenocytes from a rat passively immunized with highly purified murine T-cell/fibroblast type I IL-1 receptor (gp80) (15). Antibodies were purified from ascites fluid from plasmacytoma-bearing nude mice (*nu/nu*) by affinity chromatography or protein G-agarose. Previous studies have demonstrated that a single injection of 10 mg/kg body weight of anti IL-1r monoclonal antibodies prevented the acute phase changes to 5 μg/kg body weight of recombinant mouse IL-1α (12). Tumor-bearing animals were passively immunized i.p. with 200 μl of 0.9 NaCl containing either affinity-purified monoclonal rat anti-murine IL-1r IgG or nonimmune rat hybridoma IgG every other day as defined in previous experiments on C57BL/6 mice (12).

Tumor Cell Cultures. Fibroblast-free tumor-cell cultures were established from *in vivo* growing tumors by standard techniques. Tumor cells were cultured at 37°C in McCoy's 5a medium (Flow Laboratories) in the presence of ordinary (10%) or low (2%) concentrations of heat-inactivated fetal calf serum. Rabbit anti-murine IL-1α, anti-IL-1r, and rabbit anti-murine TNF-α, recombinant human IL-1α, and murine TNF-α (Genzyme, Boston, MA) rabbit preimmune serum and nonimmune rat hybridoma IgG were added separately, in combinations and subsequently to each other in order to evaluate the potential role of IL-1 and TNF for tumor cell growth *in vitro*. Cell proliferation was documented by [^{3}H]thymidine incorporation into DNA and by photographic illustrations of cell cultures.

Statistical Analysis. Differences among various groups of animals were determined by one factor factorial analysis of variance. Differences in the longitudinal changes in food intake within and between groups of animals were tested by analysis of variance for repeated measures (16).

RESULTS

Tumor-bearing and non-tumor-bearing mice were divided into groups with the same initial body weight. Carcass weight plus tumor weight at the end of the experiments were approximately equal indicating that the tumor grew at the expense of host tissues, in agreement with our previous studies (6–9). Body compositional data revealed that both fat and lean tissues were lost during tumor growth (Table 1). Tumor-bearing mice receiving either anti-TNF, anti-IL-r, or the combination of both had a trend to more preserved body fat stores ($P < 0.07$) evaluated by analysis of variance. However, when individual groups of antiserum-treated animals were tested against each other by a multiple range test, anti-IL-1r animals had a clearcut significantly preserved body fat compared to untreated tumor-bearing animals ($P < 0.03$).

Tumor growth was associated with a significant acute phase response as determined by significantly increased liver weight, increased hepatic content of cellular RNA, increased circulating plasma concentrations of transferrin, serum amyloid P protein, complement C3, interleukin 6, and decreased concentrations of the negative acute phase protein albumin. This acute phase response was not attenuated by the presence of circulating antibodies to TNF or IL-1r. Actually, the hepatic concentration of RNA was significantly higher in tumor-bearing animals treated with either anti-IL-1r or the combination of anti-TNF plus anti-IL-1r ($P < 0.05$; Table 2; Fig. 1).

Tumor wet weight and dry weight were significantly lower in groups of animals treated with either anti-TNF, anti-IL-1r or the combination of both (Table 3). Food intake declined in tumor-bearing animals ($P < 0.001$) in agreement with our previous experiments. Antiserum treatment attenuated anorexia significantly towards the end of the experiments (days 8–11), without any additive effects of the combined anti-TNF and anti-IL-1r treatments ($P < 0.05$), (Fig 2).

Evidence for interaction/binding and tumor growth regulation of IL-1 and TNF-α were obtained in cell culture experiments. These experiments demonstrated that addition to the cell culture medium of preimmune rabbit serum or nonimmune hybridoma IgG did not influence on tumor cell growth (Fig. 3). However, the addition of rabbit anti-murine IL-1α, anti-IL-1r, or anti-murine TNF-α reduced tumor cell proliferation by around 60–70% compared to baseline incubates (Fig. 4*A versus* 4*B* and Fig. 5*A*). This inhibitory effect could be partially restored by the addition of recombinant human IL-1α or TNFα in excess to the antibodies (results not shown). The combination of anti-TNF and anti-IL-1 in cell culture medium did not inhibit cell growth significantly more than found by one type of antibodies. The addition of either recombinant human IL-1α or murine TNFα to tumor cells cultured in the presence of low concentrations of fetal calf serum (0.5–2%) stimulated cell growth by 20–30% compared to baseline incubates (Fig. 4*A versus* Fig. 4*C* and Fig. 5*B*).

DISCUSSION

The results in the present study agree with our previous report demonstrating that endogenous TNF-α release can significantly explain a minor part of tumor-induced host wasting in at least some experimental tumor models (2). In the present study we have extended this information to include interleukin 1 by administration of neutralizing antibodies against the high affinity interleukin 1 receptor. The findings demonstrate that combined TNF and IL-1 receptor blockade caused no additive effects. Although negative, this is an interesting observation, since our own experiments and reports by others demonstrate that the combination of recombinant TNF and IL-1 may result in a variety of potentiating effects in experimental animals (3, 4). Our findings may thus suggest that anti-TNF and anti-IL-1 may reduce tumor growth both *in vivo* and *in vitro* and slow development of cancer cachexia through a common mechanism.

Receptors for both interleukin 1 and TNF occur on many cell types. We have not characterized receptor expression in our tumor cells by conventional ligand/displacement experiments. Instead, we chose a more simplistic and functional approach. Our previous experiments have demonstrated the production of both IL-1 and TNF-α in our tumor cells when cultured at standard conditions, particularly in the presence of low concentrations of fetal calf serum. Our recent results have also demonstrated the presence of IL-1 and TNF-α mRNA within the tumor *in vivo*. The growth-inhibitory effect by anti-IL-1 and anti-TNF to cultured cells in the present study sup-

Table 1 *Body compositional data in tumor-bearing animals (TB) treated with anti-TNF-α to anti-IL-1r*

Group	N	Initial body wt (g)	Final body wt (g)	Carcass composition (g)			
				Body wt	Dry wt	Fat	Fat free dry wt
TB control	15	21.0 ± 0.3	21.2 ± 0.7	17.0 ± 0.5[a]	5.4 ± 0.2[a]	1.4 ± 0.2[a]	4.2 ± 0.1
TB + anti-TNF-α	18	21.1 ± 0.3	22.1 ± 0.5	18.1 ± 0.4	6.0 ± 0.2	1.8 ± 0.2	4.4 ± 0.1
TB + anti-IL-1r	8	20.9 ± 0.2	22.6 ± 0.3	19.0 ± 0.3	6.2 ± 0.1	2.0 ± 0.1	4.5 ± 0.1
TB + anti-TNF-α + anti-IL-1r	8	20.9 ± 0.3	22.0 ± 0.5	18.4 ± 0.5	6.0 ± 0.2	2.0 ± 0.2	4.3 ± 0.1
TB + pre/nonimmune	16	20.9 ± 0.3	20.3 ± 0.7	16.4 ± 0.6[a]	5.4 ± 0.3[a]	1.4 ± 0.2[a]	4.2 ± 0.1
Non-tumor-bearing control	10	21.2 ± 0.3	21.1 ± 0.3	21.1 ± 0.3[b]	6.7 ± 0.3[b]	2.2 ± 0.1[b]	4.5 ± 0.1
ANOVA[c]		NS	NS	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

[a] A trend ($P < 0.07$) to a significant difference compared to specifically immunized tumor-bearing animals.
[b] Significantly higher compared to the other groups.
[c] ANOVA, analysis of variance; NS, not significant.

Table 2 *Liver composition and acute phase proteins in tumor-bearing animals (TB) treated with anti-TNF-α or to anti-IL-1r*

Samples from preimmune injected animals were not analyzed, since the specific antisera had no significant effect. There were no significant differences between sham-injected and specifically immunized tumor-bearing animals. All tumor animal groups were significantly different from the non-tumor-bearing group.

	N	Liver wt (g)	Liver RNA (mg/g liver)	Albumin (g/liter)	Transferrin (g/liter)	SAP[a] (μg/liter)	C3 (g/liter)
TB control	15	1.01 ± 0.04	13.0 ± 0.3	17.7 ± 1.6	5.16 ± 0.52	179 ± 16	0.73 ± 0.03
TB + anti-TNF-α	18	1.08 ± 0.02	13.0 ± 0.1	17.5 ± 1.4	4.87 ± 0.40	181 ± 28	0.67 ± 0.07
TB + anti-IL-1r	8	0.99 ± 0.02	13.7 ± 0.2	20.4 ± 1.7	5.58 ± 0.65	208 ± 37	0.81 ± 0.11
TB + anti-TNF-α + anti-IL-1r	8	0.98 ± 0.05	14.2 ± 0.3	18.1 ± 0.4	6.05 ± 0.45	255 ± 21	0.64 ± 0.05
Non-tumor-bearing control	10	0.90 ± 0.02	8.5 ± 0.2	41.1 ± 1.1	3.35 ± 0.07	7 ± 1	0.49 ± 0.03
ANOVA		$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

[a] SAP, serum amyloid P protein; ANOVA, analysis of variance.

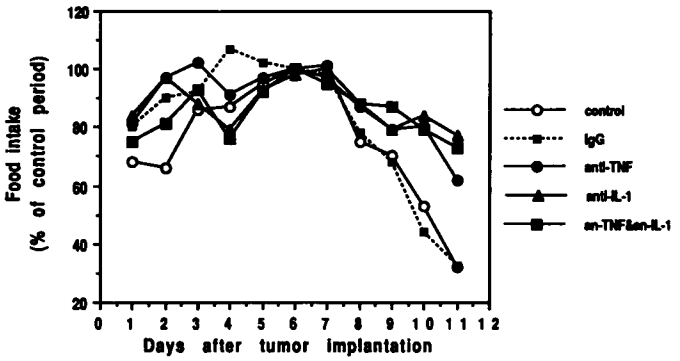

Fig. 1. Time course in food intake in tumor-bearing animals treated with anti-TNF-α and to anti-IL-1r, the combination of both (*an-TNF&an-IL-1*) as compared to sham-injected (controls) and pre/nonimmune injected (IgG) tumor-bearing mice. The decline in food intake from day 6 and on was statistically significant ($P < 0.001$) evaluated by analysis of variance for repeated measures ($P < 0.05$). Antiserum-treated animals had significantly higher food intake between days 8 and 11 compared to sham-injected and preimmune-injected tumor-bearing controls ($P < 0.006$). The daily variation in food intake was 5–8% of mean values. Results for IgG mice were pooled from tumor-bearing mice given injections of either rabbit preimmune serum ($n = 8$) or rat nonimmune hybridoma IgG ($n = 8$).

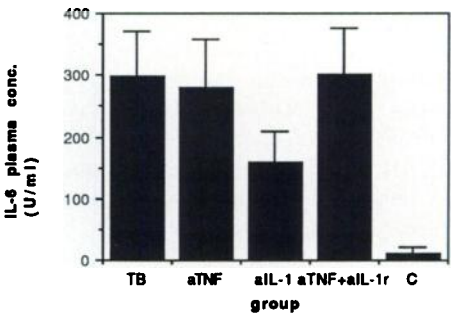

Fig. 2. Plasma concentration of IL-6 activity in untreated tumor-bearing animals (*TB*) and in tumor-bearing animals treated with antibodies against TNFα (*aTNF*), the IL-1 receptor (*aIL-1*), and the combination of antibodies (*aTNF + aIL-1r*) as compared to non-tumor-bearing control animals (*C*). No significant difference was found among the treated groups with one factor analysis of variance. Plasma from pre/nonimmune injected controls was not analyzed, since the specific antibodies had no significant effects on IL-6 plasma levels.

Table 3 *Tumor weight at the end of the experiments*

	N	Tumor wet wt (g)	Tumor dry wt (g)
TB control	15	2.51 ± 0.11	0.46 ± 0.02
TB + anti-TNF-α	18	2.09 ± 0.10[a]	0.38 ± 0.02[a]
TB + anti-IL-1r	8	1.73 ± 0.15[a]	0.32 ± 0.03[a]
TB + anti-TNF-α + anti-IL-1r	8	1.78 ± 0.21[a]	0.33 ± 0.04[a]
TB + pre/nonimmune	16	2.56 ± 0.12	0.47 ± 0.03
ANOVA[b]		$P < 0.01$	$P < 0.01$

[a] Significantly different from tumor-bearing (TB) controls and pre/nonimmune injected animals.
[b] ANOVA, analysis of variance.

ports the suggestion that both IL-1 and TNF may actually act as growth factors, perhaps through an autocrine loop. It is thus far unknown whether such effects are mediated by classic ligand/receptor interactions. However, the finding that anti-IL-1r also had inhibitory effects *in vitro* supports this idea. Therefore, it is possible that the *in vivo* inhibition of tumor growth by anti-IL-1r and anti-TNF-α was in part explained by a direct tumor interaction effect.

We have previously proposed that tumor host wasting is mainly explained by anorexia in both experimental (17) and clinical cancer (18), although elevated energy expenditure explains a significant part of the negative energy balance (19), mediated by an increased adrenergic state and increased sensitivity to adrenergic agonists in the tumor-bearing host (20, 21). Thus, the mechanism behind the declining food intake in tumor disease, stress, and infection is intriguing and remains unclear. Many authors, including ourselves, have speculated that cytokines may be one such mechanism, since recombinant human and murine cytokines acutely elicit anorexia in normal healthy animals (22–24). It is, however, unclear to what extent the doses and hence the circulating concentrations of cytokines really can be compared to tissue levels occurring following endogenous production. There is no doubt that many of the physiological effects attributed to cytokines and identified by

Fig. 3. Appearance of tumor cells when cultured to confluent state in McCoy's 5a medium containing 2% heat-inactivated fetal calf serum in the presence of either preimmune serum or nonimmune rat hybridoma IgG at 40 μg/well. The results confirm that preimmune IgG did not influence on tumor growth *in vitro* compared to cells without pre/nonimmune IgG.

exogenous administration are due to unphysiological concentrations of these potent cellular regulators. In this context, we have been unable to demonstrate elevated circulating levels of either TNF or IL-1 in tumor-bearing animals, but IL-6 circulated at high levels in both tumor-bearing and antigen-stimulated non-tumor-bearing animals (11, 25). Only in severely septic animals in a state of toxic shock syndrome and in severely infected humans have circulating TNF been reported as measured by the same assay as we have used in the present model. Our animal experiments in this regard agree with our findings in cancer patients, although patient results are not consistent throughout the literature (26–28). It has been demonstrated that insertion of the TNF gene into tumor-genomic DNA resulted in the development of experimental cachexia (29), but the TNF gene was purposely located to an active promoter in order to increase the TNF expression. This resulted in high circulating levels of TNF. Although experimentally elegant, those results are necessarily not principally different from those when recombinant or biologically derived TNF is injected into animals (24, 30).

The inability to detect circulating TNF and IL-1 in our present animal model is probably not a question of insensitive methods. Hence, it is more likely that IL-1 and TNF act as paracrinic/autocrinic mediators rather than being circulating messengers in experimental cancer. In the previous experiments we were, however, unable to demonstrate a clear increase in the overall expression of mRNA for these cytokines in most tumor host tissues, although the gene expression of TNF was elevated in spleen tissues from tumor-bearing animals (5). We have also found that the expression of the IL-1 gene was down-regulated in peritoneal macrophages during progression of the tumor disease, an effect that was reversed by prostaglandin inhibitors (31). However, the present study confirms that both IL-1 and TNF are qualitatively involved to explain cancer cachexia, at least in this particular model. Whether tumor production or host production(s) of cytokines in some compartment(s) explain the major effect remains to be evaluated. In this context it is interesting to note that TNF blockade by antibodies *in vivo* make sublethal infections fatal, an overall effect which seems to be quite opposite to that found in tumor disease (32).

In the light of our recent findings that tumor growth is extremely sensitive to alterations in food intake, *i.e.*, reduced food intake inhibits tumor growth and *vice versa*, it was interesting to note that antiserum blockade to TNF and IL-1r improved the spontaneous appetite in tumor-bearing animals with progressive anorexia. These findings may fit with the previous suggestion that TNF and IL-1 may directly regulate anorexia in tumor disease (23, 24, 33), although we have not been able to confirm a clearcut elevated intrabrain production of TNF or IL-1 in tumor-bearing animals (5). IL-1 receptors have, however, been visualized in brain tissue (34). At present, we find it more likely that the attenuated anorexia in the antiserum-treated animals was rather secondary to decreased net tumor growth than being the result of a primary effect on the brain. The role of IL-6 behind tumor-induced anorexia may be important, since IL-6 in recombinant and natural form has been reported by others to depress food intake (35) and is circulated at increased levels in our tumor-bearing mice.

It is well established that recombinant IL-1 and TNF result in a classic hepatic acute phase response when injected even at low physiological concentrations to normal healthy animals (24). This effect has been related to either a direct liver cellular effect (36, 37) or being mediated by an indirect effect via IL-6, which is now regarded as an important factor behind the hepatic derived acute phase response (38). Therefore, it was interesting to note that both TNF and IL-1r blockade had no influence at all on the hepatic acute phase response including the net production of both negative and positive acute phase plasma proteins and IL-6 concentrations. These results support the suggestion that IL-6 may be the most important factor to explain

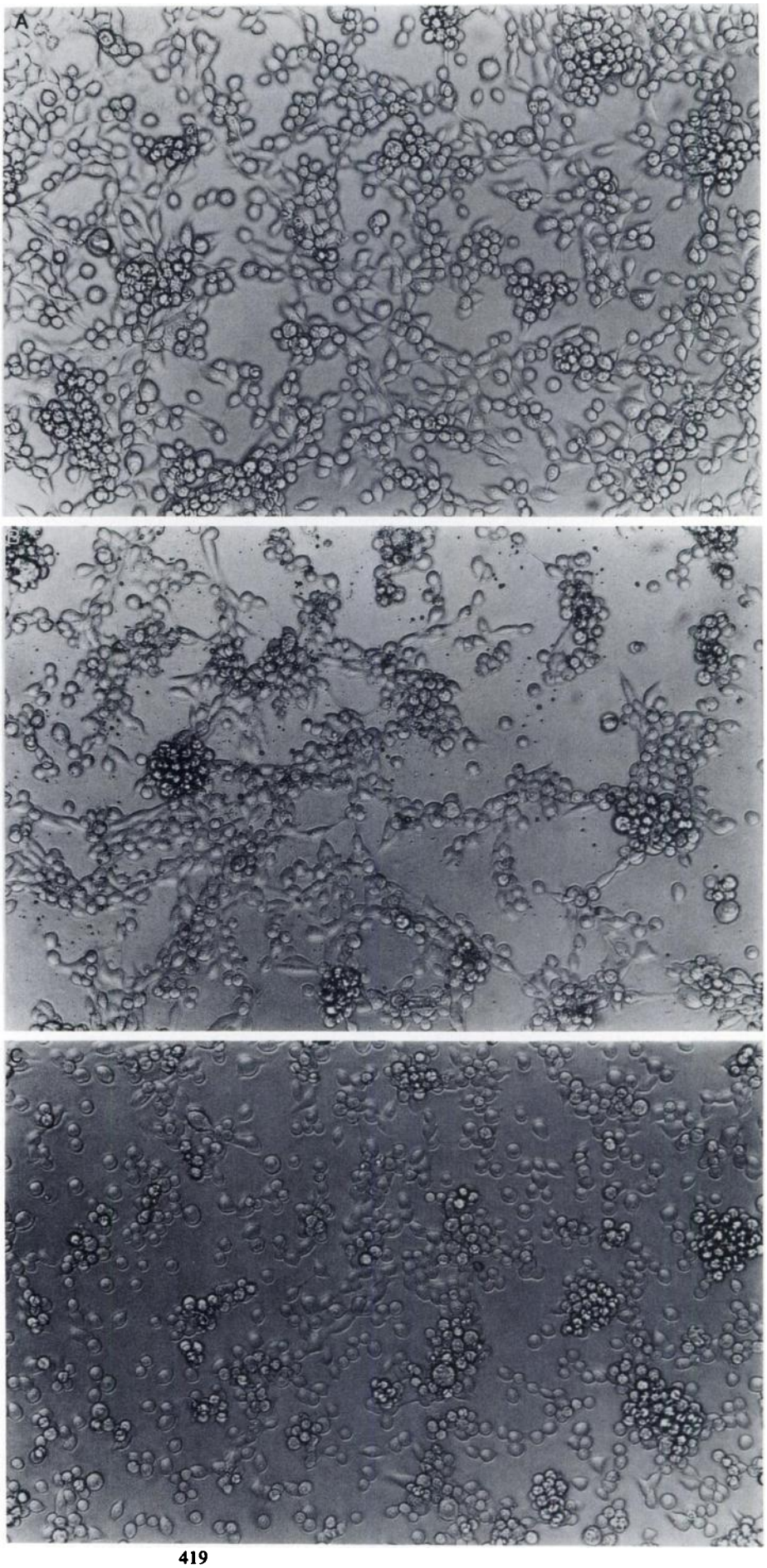

Fig. 4. Confluent tumor cells, as shown in Fig. 3, were transferred to new incubation vessels with standard medium and 2% fetal calf serum in the presence of either rabbit anti-murine IL-1α or anti-IL-1r antibodies (40 μg/well) during 48 h incubation. Tumor cell growth was evaluated by [^{3}H]thymidine incorporation and by photographs after 48 h incubation. *A*, control cells that were incubated without any anti-IL-1 antibodies, thus representing baseline incubates. *B*, cells cultured in the presence of anti-IL-1α (Similar results were obtained by anti-IL-1r). When recombinant human IL-1α (250–800 units) was added to baseline cultures (*A*), it was seen that tumor cells growth was stimulated by 20–30% evaluated by [^{3}H]thymidine incorporation to DNA (*C*). The results support that blockade of the endogenous production of IL-1α in the tumor cells, as reported in Ref. 5, influence tumor growth and that IL-1α can modulate tumor cell growth *in vitro* in analogy to the results found *in vivo* in the present study.

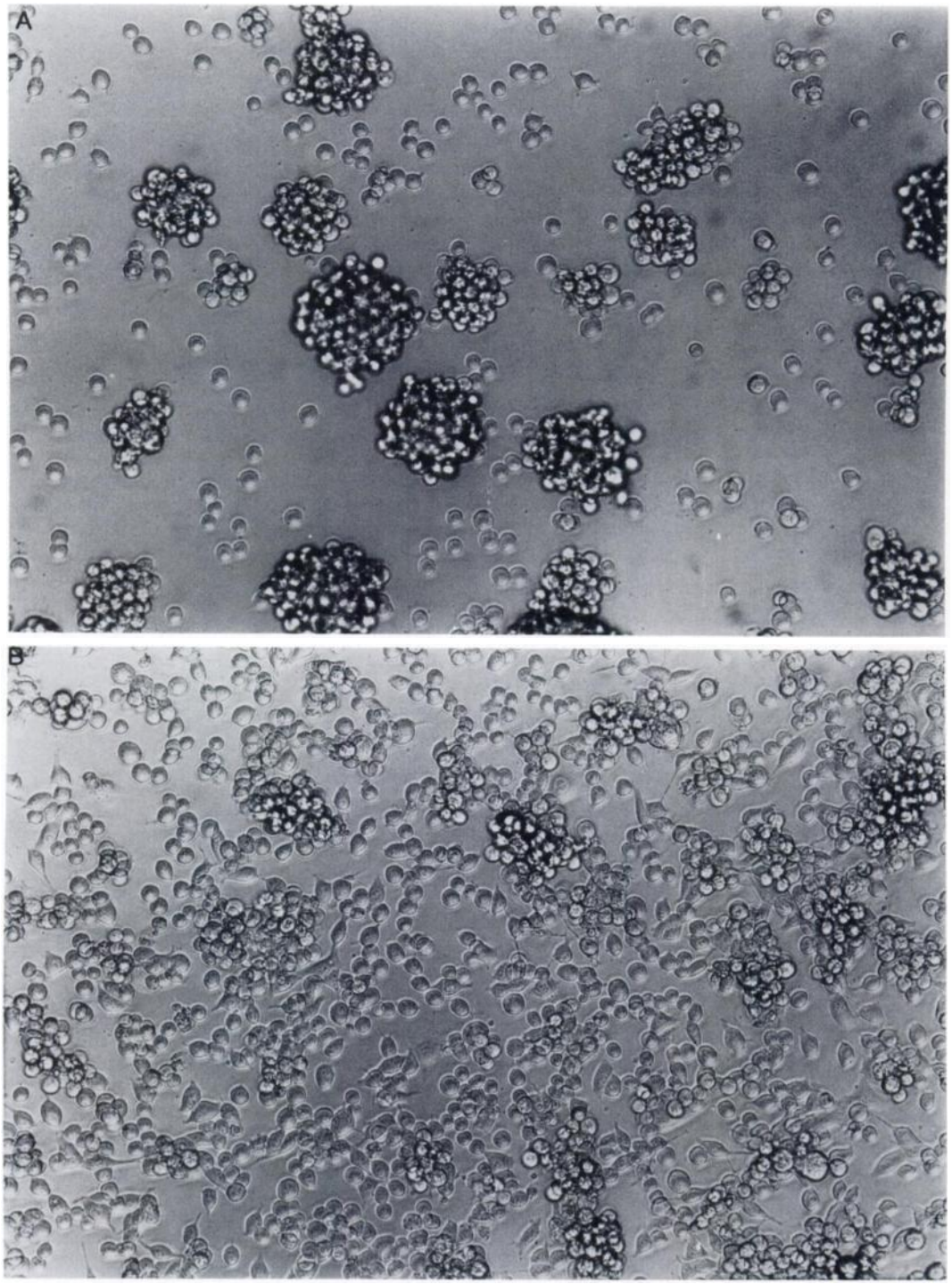

Fig. 5. Confluent tumor cells, as shown in Fig. 3, were transferred to new incubation vessels with standard medium and 2% fetal calf serum in the presence of rabbit anti-murine TNF-α (40 μg/ml) during 48 h incubation. Tumor cell growth was evaluated by [^{3}H]thymidine incorporation and by photographs after 48 h incubation. Fig. 4*A* shows control cells that were incubated without any anti-TNFα and thus represent baseline incubates. Fig. 5*A* shows cells cultured in the presence of anti-TNF-α. When recombinant murine TNF-α (800 units) was added to baseline cultures (Fig. 4*A*), it was seen that tumor cells growth was stimulated by 25–30% evaluated by [^{3}H]thymidine incorporation (Fig. 5*B*). The results support that blockade of the endogenous production of TNF-α in the tumor cells, as reported in Ref. 5, influence tumor growth and that TNFα can modulate tumor cell growth *in vitro* in analogy to the results found *in vivo* in the present study.

the hepatic acute phase response in tumor disease. In a recent study the host responses that occur during inflammation following s.c. injections of turpentine requiring IL-1 were studied. In that particular model anorexia, weight loss, carcass protein and fat loss, and plasma IL-6 and amyloid P responses were dependent upon endogenous IL-1 activity, while hypoalbuminemia, leukopenia, and hypercorticosteronemia were not influenced by blockade of the T-cell/fibroblast type IL-1 receptor. TNF-α blockade had no effect on the acute inflammatory response in turpentine-injected animals, while the same antibodies confer survival against endotoxinemia (13) and inhibit tumor growth (2) as confirmed in the present study. Hence, it appears that cytokine responses vary and may be more or less important for the inflammatory response among various models or conditions such as tumor disease, infection with living microbes and endotoxinemia. The failure of anti-IL-1r monoclo-

nal antibody to block the hepatic acute phase response in the present study presumably mediated by IL-1 may be partly explained by the presence of at least one additional class of IL-1 receptor that is immunologically distinct from the EL-4 T-cell receptor (15).

In conclusion, this study demonstrates that both IL-1 and TNF are involved in the complicated interplay of cytokines and growth factors required to explain the development of cancer cachexia. Our cell culture experiments provide evidence to support both host and direct tumor effects *in vivo* by the antibodies to IL-1r and TNF-α. However, the magnitude of the effect may be less than initially speculated of. It is possible that the major effects of TNF and IL-1 are within tumor cells or close to the tumor, perhaps creating a positive growth environment for the tumor. We were unable to demonstrate that IL-6 production in a tumor-bearing host was directly dependent on the tumor/host production of TNF and IL-1. IL-6, which is a circulating messenger, may explain the hepatic acute phase response and perhaps additional parts of the anorexia and host wasting in tumor-bearing animals.

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