Interleukin 7 Induces CD4+ T Cell–dependent Tumor Rejection

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Summary

The potential of interleukin 7 (IL-7) to induce an antitumor response in vivo was analyzed. Therefore, the IL-7 gene was expressed in the plasmacytoma cell line J558L. Although the growth of IL-7-producing cells was not retarded in vitro, the IL-7-producing cells were completely rejected upon injection into mice. Tumor rejection was observed only in syngeneic but not in nude mice. The tumor-suppressive effect could be abolished by the parallel injection of an anti-IL-7 monoclonal antibody. Immunohistochemical analysis revealed IL-7-dependent infiltration of the tumor tissue by $CD4^+$ and $CD8^+$ T lymphocytes, and also type 3 complement receptor–positive ($CR3^+$) cells, predominantly macrophages. Depletion of T cell subsets in tumor-bearing mice showed the absolute dependence of the antitumor response on $CD4^+$ cells, whereas tumor rejection was unaffected by depletion of $CD8^+$ cells. In addition to $CD4^+$ cells, $CR3^+$ cells were also needed for tumor rejection. The antitumor effect of IL-7 was confirmed by expression of the IL-7 gene in a second tumor cell line of different cellular origin. Together, our results demonstrate that a high local IL-7 concentration at the tumor site obtained by tumor cell–targeted gene transfer leads to tumor rejection involving a cellular mechanism that seems to be different from the ones observed in analogous experiments with other cytokines.

IL-7 has originally been described as a growth factor for B cell progenitors (1, 2) and subsequently shown to be a pleiotropic cytokine that has both proliferative and differentiative effects on cells of various lineages in vitro. In particular, IL-7 supports proliferation of mature $CD4^+$ and $CD8^+$ cells (3–5), and induces differentiation of cytotoxic T cells, LAK cell activity (6–8), and tumoricidal activity of macrophages (9). We have investigated the possibility as to whether any of the IL-7-induced cellular functions alone or in concert could induce an antitumor reaction in vivo. For this purpose, tumor cells were genetically engineered to produce IL-7 in order to provide a locally increased cytokine concentration upon injection into mice. This experimental approach has been successfully used for the analysis of the antitumor effects of IL-2 (10–12), IL-4 (13, 14), IFN-γ (15, 16), TNF (17–19), and granulocyte CSF (G-CSF)[1] (20). In search of antitumor activities in vivo, tumor cell–targeted cytokine gene transfer not only circumvents some of the practical problems of systemical application (short half-life, effective doses at the tumor site, toxicity) but it also offers the advantage of a more physiological mode of action if an immune response is considered at least in part as a locally directed process (21). The analysis of the antitumor responses induced by different cytokines has revealed characteristical differences in the effector mechanisms. The mechanism induced by some cytokines merely reflected their known function. For instance, the roles of cytotoxic $CD8^+$ T cells during the IL-2-induced immune response (10) or the activation of granulocytes by G-CSF-producing tumor cells (20) were consistent with known activities of these cytokines. In contrast, the possible involvement of macrophages and eosinophiles in the IL-4-induced antitumor response was rather unexpected (13). However, not all cytokines are capable of inducing an antitumor response when produced by the tumor cells. Notably, the parallel expression of IL-6 and TNF genes in the same tumor cell line showed that IL-6 did not cause tumor suppression, whereas TNF was rather effective (17). In contrast to the above-mentioned immunostimulatory antitumor effects of some cytokines, transfer of the TGF-β gene into an immunogeneic tumor cell line led to the inhibition of a tumor-specific immune response (22). Due to these opposite effects of cytokines on tumor growth (reviewed in reference 23), it seems to be necessary to systematically study cytokines with respect to their potential antitumor activity and to elaborate the different cytokine-induced immunological responses.

[1] *Abbreviations used in this paper:* G-CSF, granulocyte CSF; LTR, long terminal repeat; MoMuLV, Moloney murine leukemia virus.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/91/12/1291/08 $2.00
Volume 174 December 1991 1291–1298

Materials and Methods

Cell Lines. J558L is a heavy chain loss variant of the BALB/c plasmacytoma J558 (24) and was cultured as described (14). TS/A is a nonimmunogeneic mammary adenocarcinoma cell line derived from BALB/c AnN mice (25). TS/A cells were cultured in RPMI 1640, 10% FCS, 2 mM glutamine, and antibiotics.

Construction of IL-7 Gene Expression Vectors and Gene Transfer Experiments. The mouse IL-7 cDNA was isolated by PCR using primers containing the translation start signal (5' GAC TGG ATC CAC CTC CCG CAG ACC ATG TTC CAT G 3') and the translation stop signal (5' TAC TAC GGA TCC TGT TTA TAT ACT GCC CTT CAA AAT TTT 3') (2). For PCR, total RNA was extracted from mouse thymocytes and 1 μg was reverse transcribed into cDNA using random primers and Moloney murine leukemia virus (MoMuLV) reverse transcriptase (Gibco Laboratories, Grand Island, NY; Bethesda Research Laboratories, Bethesda, MD) by standard procedures (26). Amplification of the equivalent of 250 ng RNA occurred in the presence of 200 μM dNTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 1 mg/ml gelatin, 200 nM of each sense and antisense primers, and 0.5 U Taq polymerase (Perkin-Elmer Corp., Norwalk, CT) in a 50-μl volume. The PCR was performed for 40 cycles (1 min at 94°C, 1.5 min at 60°C, and 2 min at 72°C). Cloning of the PCR product was facilitated by the presence of BamHI restriction sites located in the primers. The purified BamHI fragment was cloned into the plasmid pBAneo (27) by replacing the neomycin gene and into the plasmid pLTR. For the generation of pLTR, plasmid pAPtag-1 (28) was digested with BspMII and HpaI in order to get rid of the alkaline phosphatase gene. The vector fragment was blunt-ended with Klenow polymerase, religated, and, subsequently, the mouse IL7 cDNA fragment of pBA-IL7 was subcloned as BamHI fragment into the BglII site of pLTR (pLTR-IL-7). The sequence identity of the IL-7 fragment with the published mouse IL-7 cDNA was confirmed by sequence analysis. The two plasmids pBA-IL-7 and pLTR-IL-7, which contain the IL-7 gene under β-actin or MoMuLV long terminal repeat (LTR) promotor control, were transfected into J558L and TS/A cells by electroporation using a Gene-Pulser (Bio-Rad Laboratories, Richmond, CA). 5 × 10^6 cells in 0.5 ml D-PBS (without Ca^{2+} and Mg^{2+}) were pulsed in a 0.4-cm cuvette with 200 V, 960 μ Farad (J558L) or 350 V, 250 μ Farad (TS/A cells). The transfection solutions contained a 10:1 molar ratio between the IL-7 constructs and the neomycin gene containing plasmid pWL neo (Stratagene, La Jolla, CA). Transfected cells were selected for 2 wk in medium containing 1 mg/ml G418 (Gibco Laboratories; Bethesda Research Laboratories) and were subsequently cloned.

Detection of IL-7 Activity. IL-7 was measured by its ability to directly stimulate thymocytes (29). Briefly, thymocytes of 4-wk-old C57 BL/6 mice were cultured for 72 h at a density of 5 × 10^6 cells/ml in a 0.2-ml volume in a 96-well flat-bottomed microtiter plate in RPMI 1640 containing 10% FCS, 50 μM 2-ME, 2 mM glutamine, and serial dilutions of standard recombinant mIL7 (British Biotechnology, Oxford, UK) or conditioned medium of transfectants. Cells were pulse labeled during the last 6 h of culturing with 0.5 μCi [^{3}H]thymidine (5 Ci/mmol sp act). Finally, cells were harvested onto glass fibre plates, and [^{3}H]thymidine incorporation was quantified by scintillation counting. 1 U of IL-7 was defined as the amount of IL-7 required to obtain half-maximal proliferation (0.3 ng/ml rIL-7). Maximal proliferation resulted in 12,000 cpm on the average; background was <500 cpm. Medium of transfectants was conditioned during a 48-h culture period at a concentration of 10^6 cells/ml.

Analysis of Tumor Cell Growth. Cells of exponentially growing cultures were harvested (in case of TS/A cells by trypsinisation), washed three times in D-PBS, and injected in a volume of 0.2 ml subcutaneously in the neck of 6-wk-old female BALB/c or BALB/c *nu/nu* (Bomholtgard, Ry, Denmark) or BALB/cAnN mice (Charles River Breeding Laboratories, Sulzfeld, FRG). In some experiments, mice were injected subcutaneously at a shaved abdominal location in order to better monitor the early phase of tumor growth. Mice bearing a tumor >0.5 cm in diameter were considered as positive. Tumor size was determined as the mean of the largest diameter and the diameter at right angle.

Antibody Treatment In Vivo. 4 × 10^6 cells were injected subcutaneously simultaneously with various mAbs. A rat anti-mouse IL-7 mAb (IgG2b) (no. 1689-01; Genzyme, Cambridge, MA) was injected intravenously in doses of 200 μg/0.2 ml at days 0 (the day of tumor cell injection), 2, 4, 6, and 8 after tumor cell injection. This mAb was shown to completely neutralize 1 U of IL-7 activity in the thymocyte proliferation assay at a concentration of 0.1 μg/ml. Depletion of T cells in vivo was accomplished by administration of the following rat mAbs: 30-H12 (αThy-1.2; IgG2b) (30), GK1.5 (αCD4; IgG2b) (31), and 2.43 (αCD8; IgG2b) (32). These antibodies were used as ascites fluids, diluted in D-PBS to a concentration of ~1 mg/ml. 0.5 ml of the respective antibodies were injected intraperitoneally at days −1 and 7. This treatment led to the virtual absence of the respective cell population from the spleen until at least day 14 after tumor cell injection, as shown by flow fluorocytometric analysis (data not shown). The rat anti-mouse CR3 mAb 5C6 (IgG2b) (33) was injected at days 0, 2, 4, 6, 8, and 10 intraperitoneally at doses of 0.5 mg in 0.5 ml D-PBS. The rat mAb 23-378 (IgG2b) (unpublished) served as an isotype-matched control mAb in all experiments.

Immunohistochemistry. Tumors were removed at different time points, frozen immediately in liquid nitrogen, and stored at −80°C. 6-μm frozen sections were cut from the tissue, fixed in acetone (30 min) and chloroform (30 min), and stained with the following rat mAbs (in TBS + 10% BSA): anti-CR3 mAb Mac-1 (IgG2b) (34), anti-CD4 mAb RM 4-5 (IgG2a; Paesel and Lorei, Frankfurt, FRG), anti-CD8 mAb 53-6.7 (IgG2a; Becton Dickinson & Co., Heidelberg, FRG), and the control mAbs 23-7 (IgG2a) and 23-378 (IgG2b), followed by incubation with alkaline phosphatase-conjugated secondary (goat anti-rat IgG[H+L]; Dianova Hamburg, FRG) and tertiary antibodies (rabbit anti-goat IgG[H+L]; Dianova). Incubation was performed for 30 min, and the sections were washed three times in TBS between different incubation steps. Alkaline phosphatase activity was visualized by New Fuchsin as described (35), and sections were counterstained by hemalaun.

Results

IL-7 Gene Transfer and Expression in Tumor Cell Line J558L. To generate IL-7-producing tumor cell lines, two plasmids were constructed that allow expression of IL-7 under the control of either the β-actin promotor (pBA-IL-7) or the MoMuLV-LTR (pLTR-IL-7) (Fig. 1). The plasmacytoma cell line J558L was transfected with plasmid pBA-IL7 by electroporation together with the neomycin gene containing plasmid pWLneo. Transfectants were selected for G418 resistance, cloned, and analyzed for IL-7 activity in the supernatant. Finally, three clones (J558-IL-7, J558-IL-7A, and J558-IL-7^{low}) with levels of IL-7 activity between 4 and 65 U were selected for further analysis (Table 1). In a parallel experiment, J558L cells were mock-transfected with plasmid

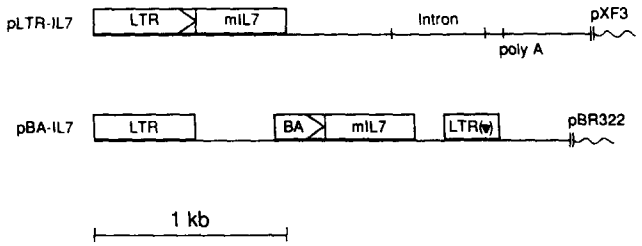

Figure 1. Structure of IL-7 expression vectors. In plasmid pLTR-IL-7, the mouse IL-7 (mIL-7) is expressed by the MoMuLV LTR promoter as indicated by the arrow. In plasmid pBA-IL-7, mIL-7 is under the control of the β-actin promoter (BA).

pWLneo (J558-neo). The IL-7 activity in the supernatant of the pBA-IL-7 transfectants could be completely blocked by an anti-IL-7 mAb. Both, parental J558L and J558-neo cells did not produce IL-7. The IL-7-producing clones differed from parental J558L cells neither morphologically nor with respect to their in vitro growth kinetics.

IL-7 Production by Tumor Cells Leads to Tumor Rejection in Syngeneic BALB/c but Not in Nude Mice. The tumor growth of the IL-7-producing cell lines was compared to that of parental or mock-transfected J558L cells. First, various numbers of cells were injected into mice. The tumorigenic potential of IL-7-producing clones markedly differed from parental and mock-transfected J558L cells (Table 2). In syngeneic BALB/c mice, J558-IL-7 cells did not give rise to tumors at all if cell numbers up to 10^6 were injected, although all mice injected with 10^6 parental J558L cells developed a tumor. At higher cell doses, small tumors appeared at the same time as in mice injected with parental J558 cells, but these stopped growing and began to regress between days 8 and 12, and completely disappeared at the beginning of or during the third week. A second clone, J558-IL-7A, which produces a comparable amount of IL-7, showed similar growth characteristics. In contrast, mice injected with J558-IL-7^{low} cells, which produce ~10 times less IL-7 than the two other clones, all developed tumors, some of which disappeared after a much longer time than observed with J558-IL-7-injected animals or even progressively continued to grow (Table 2). This indicates that the tumorigenicity inversely correlates with the amount of IL-7 produced by the tumor cells.

Table 1. *Expression of IL-7 in Transfected Cell Lines*

Expression vector	Cell line	IL-7 activity
		U/ml
pBA-IL-7	J558-IL-7	50
	J558-IL-7A	65
	J558-IL-7^{low}	4
pLTR-IL-7	TS/A-IL-7	50
pWLneo	J558-neo	–

Table 2. *Tumorigenicity of J558L Cells and Transfectants in Syngeneic BALB/c and Nude Mice*

		No. of mice with tumor/no. of injected mice*		
Cell type	Injection dose	BALB/c		Nude
	$\times 10^6$			
J558L	4	5/5		5/5
	2	13/15		5/5
	1	5/5		
	0.5	3/5		
	0.25	2/5		
J558-IL-7	4	0/5	(3)‡	5/5
	2	0/25	(6)‡	5/5
	1	0/5		
	0.5	0/5		
	0.25	0/5		
J558-IL-7A	4	0/5	(3)‡	5/5
	2	0/5		5/5
J558-IL-7^{low}	2	3/10	(7)‡	
J558-neo	4	5/5		
	2	5/5		

* Mice were observed up to 4 mo.
‡ Number of mice that transiently developed tumors that disappeared later on.

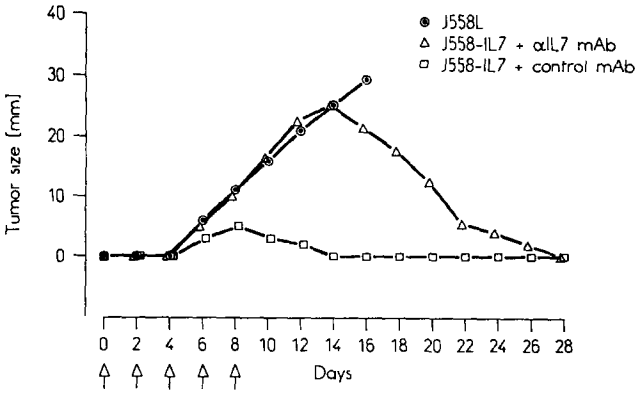

Figure 2. Growth of J558-IL-7 tumors in the presence of a mAb against IL-7. Groups of five mice were injected subcutaneously with 4 × 10^6 J558L or J558-IL-7 cells. Mice injected with J558-IL-7 cells received intravenously either the anti-IL-7 or the control mAb in intervals of 2 d from days 0 to 8 (*arrows*). Symbols represent the mean tumor size of all mice in each group. SD was <15% in all cases.

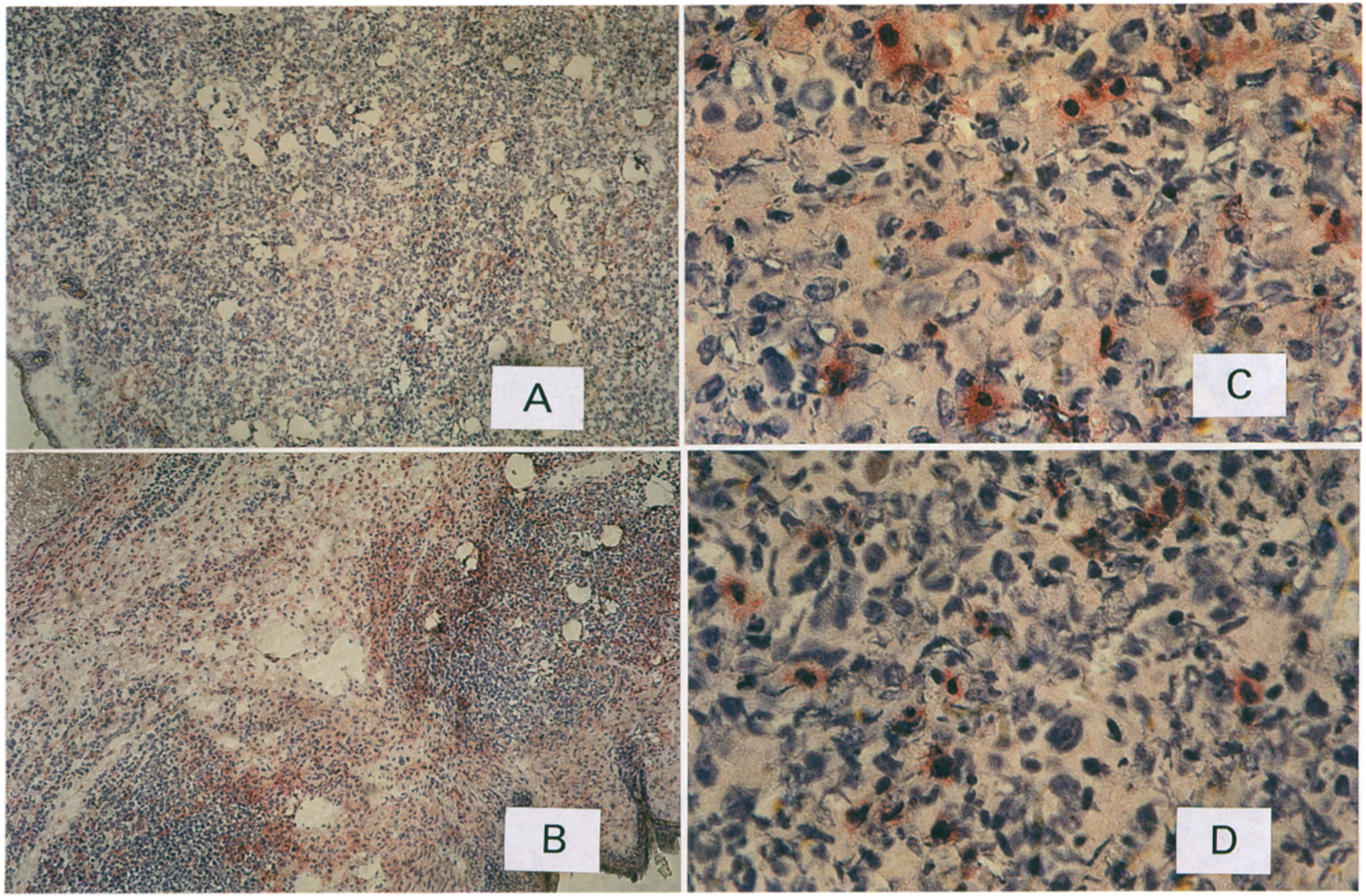

Figure 3. Immunohistochemical analysis of the tumor tissue 6 d after subcutaneous injection of 4 × 10^6 cells. Staining of (*A*) J558L and (*B*) J558-IL-7 tumors with the αCR3 mAb Mac-1 revealed a strong infiltrate of Mac-1+ cells in the J558-IL-7 tumor, whereas only a moderate infiltrate is present in parental J558L tumor. Staining of J558-IL-7 tumor with (*C*) αCD4 and (*D*) αCD8 mAbs demonstrates an infiltrate of CD4+ as well as CD8+ T cells. In J558L tumors, T cells were virtually absent (not shown) (*A* and *B*, ×100; *C* and *D*, ×630).

To determine whether indeed IL-7 was responsible for the tumor rejection, mice receiving J558-IL-7 cells were injected in parallel with a neutralizing anti-IL-7 mAb. As shown in Fig. 2, mice injected with J558-IL-7 cells and an isotype-matched control antibody transiently developed tumors, which remained <0.5 cm in diameter and completely disappeared by the end of the second week after injection of the tumor cells. In contrast, mice receiving the anti-IL-7 mAb in parallel to J558-IL-7 cells developed tumors indistinguishable from those formed in mice injected with parental J558L cells during the first 2 wk. Then, however, ~6–7 d after the last injection of the antibody, tumors stopped growing most likely due to the clearance of the mAb and subsequently regressed, whereas J558L tumors continued to grow and the mice had to be killed. After 4 wk, the tumors of J558-IL-7-injected animals, which had been grown to a size of >2 cm in diameter up to day 14, completely disappeared.

In contrast to the results observed in syngeneic animals, the tumor incidence in nude mice injected with either the IL-7-producing transfectants or parental cells was the same (Table 2). This suggested a host-dependent rejection mechanism most likely involving T cells.

Immunohistochemistry of the IL-7-dependent Cellular Tumor Infiltrate. To investigate the cellular mechanism of the IL-7-induced tumor rejection, immunohistologic analysis was performed on days 4, 6, 9, and 11 after subcutaneous injection of 4×10^6 J558L or J558-IL-7 cells (Fig. 3). The presence of T cells was assessed by staining sections of the tumor tissue with anti-CD4 and anti-CD8 mAbs. In J558L tumors, only occasionally did single cells stain positively with either antibody, regardless of the stage of the tumor. However, in J558-IL-7 tumors, the frequency of $CD4^+$ and $CD8^+$ cells on day 4 was already elevated and the number of either cell type progressively increased after day 6. The frequency of the $CD4^+$ as compared to the $CD8^+$ cells in the tumor tissue appeared approximately equal at all observed time points. Furthermore, a progressively increasing number of $CR3^+$ cells both infiltrating and surrounding the J558-IL-7 tumor could be detected, whereas only a moderate staining was found in J558L tumors (Fig. 3). The $CR3^+$ cells elevated in J558-IL-7 tumors were morphologically identified to be predominantly macrophages, whereas granulocytes that are also stained by the anti-CR3 mAb were not significantly increased as compared to J558L tumors.

Analysis of the Cellular Mechanism of the IL-7-mediated Tumor Rejection. Since the J558-IL-7 tumors failed to undergo rejection in nude mice, and furthermore, since immunohistologic analysis identified both $CD8^+$ and $CD4^+$ T cells during the tumor rejection, we further analyzed the contribution of either subset (Table 3; Fig 4). Depletion of both cell types by the anti-Thy-1.2 mAb resulted in the complete abrogation of the antitumor response (Fig. 4). The immunohistological analysis in Thy-1.2$^+$ cell–depleted animals revealed the absence of $CD4^+$, $CD8^+$ T cells and macrophages in the J558-IL-7 tumor tissue (Table 4). This demonstrates that the presence of T cells is a prerequisite for the macrophages to infiltrate the tumor.

Table 3. *Tumorigenicity of J558-IL-7 Cells in mAb-treated Mice*

mAb treatment	Cell line	No. of mice with tumor/ no. of mice in experiment		
		Exp. 1	Exp. 2	Exp. 3
αThy-1.2	J558-IL-7	5/5	ND	5/5
αCD4		4/5	5/5	5/5
αCD8		0/5	0/5	0/5
αCR3		ND	ND	5/5
Control mAb		0/5	0/5	0/5
–	J558L	5/5	4/5	5/5

Likewise, depletion of $CD4^+$ cells abrogated the tumor rejection (Fig. 4). However, a direct comparison of J558-IL-7 tumor growth in Thy-1.2 vs. $CD4^+$ cell–depleted animals showed a slightly retarded growth in the latter animals. The immunohistology showed that $CD4^+$ cells and macrophages were virtually absent in J558-IL-7 tumors of $CD4^+$ cell–depleted mice, whereas $CD8^+$ cells were not diminished as compared to untreated mice (Table 4). Yet, the $CD8^+$ infiltrate was not sufficient to suppress the tumor growth.

In contrast to $CD4^+$ cell depletion, depletion of $CD8^+$ cells did not interfere with IL-7-mediated tumor rejection (Fig. 4). Immunohistology of regressing tumors from $CD8^+$ cell–depleted mice confirmed the absence of $CD8^+$ and showed the presence of both $CD4^+$ cells and macrophages (Table 4). Therefore, $CD8^+$ cells were obviously not necessary for the tumor rejection.

To further analyze the role of $CR3^+$ cells in IL-7-producing tumors, J558-IL-7 cells were injected in parallel

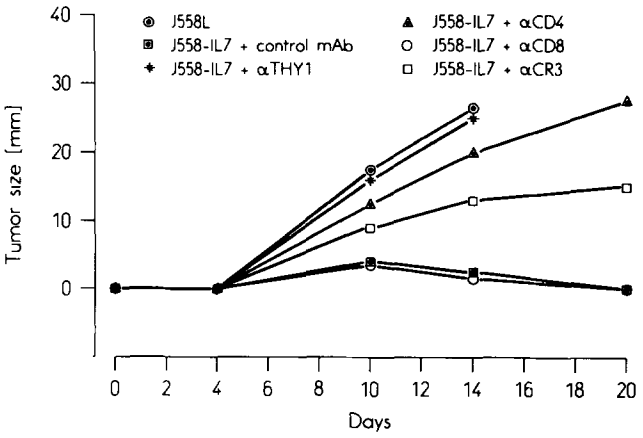

Figure 4. Kinetics of tumor growth of 4×10^6 J558-IL-7 cells in the presence of indicated mAbs in comparison to J558L cells. For details on the application of the mAbs, see Materials and Methods. One out of three experiments (Exp. 3) that are summarized in Table 3 is shown. Symbols represent the mean tumor size. SD was <15% in all cases.

Table 4. *Composite Results of Immunohistology from J558-IL-7 Tumors of mAb-treated Mice*

Staining	Tumors* of mice treated with mAb against:			
	Thy-1.2	CD4	CD8	CR3
αCD4	−‡	−	+	+
αCD8	−	+§	−	+
αCR3	−	−	+	+/−

* Tumors were excised in case of Thy-1.2$^+$, CD4$^+$, and CD8$^+$ cell depletion at day 14, in case of CR3$^+$ cell depletion at day 10.
‡ No positive cells were detected (αCD8, αCD4), or the number of positive cells was not increased (αCR3) in comparison to those detected in J558L tumors.
§ Number of positive cells was similar to those found in J558-IL-7 tumors of nontreated mice.

with the anti-CR3 mAb 5C6, which has been previously shown to inhibit the migration of inflammatory cells, such as macrophages and granulocytes in vivo (33, 36). The anti-CR3 treatment interfered with tumor rejection but the effect was incomplete in comparison to Thy-1.2$^+$ or CD4$^+$ cell–depleted mice. Immunohistologic analysis on day 10 after tumor cell injection revealed that although decreased in number (Table 4), CR3$^+$ cells had infiltrated the J558-IL-7 tumor. This indicates that the anti-CR3 mAb treatment had not completely prevented CR3$^+$ cell recruitment and may explain why the inhibition of tumor rejection was incomplete.

IL-7 Gene Transfer into a Second Tumor Cell Line of Different Cellular Origin Likewise Leads to Tumor Rejection. To confirm the observed antitumor effect, the IL-7 gene was expressed in the mammary adenocarcinoma cell line TS/A, which has been shown to be low- or nonimmunogeneic (25). Therefore, TS/A cells were transfected with plasmid pLTR-IL-7 along with the neomycin gene containing plasmid pWLneo, selected for G418 resistance, and cloned. Clone TS/A-IL-7, which released 50 U/ml IL-7 in the supernatant, was injected subcutaneously into syngeneic BALB/cAnN mice at doses of 2×10^5 cells, and tumor progression was analyzed in comparison to mice that received 2×10^5 parental TS/A cells. The mice injected with TS/A-IL-7 cells remained tumor free (five mice, observation time of 4 wk), whereas all TS/A-injected animals developed tumors within 2 wk. Additionally, TS/A-IL-7 cells grew as tumors in CD4$^+$ cell–depleted mice (5/5) like parental TS/A cells, indicating a similar mechanism of tumor rejection as observed with J558-IL-7 cells.

Discussion

The data presented here demonstrate that the local production of IL-7 obtained by gene transfer into tumor cells can induce an immune response that leads to the destruction of an otherwise progressively growing tumor. T cells play the central role in the antitumor response because it is completely abrogated in mice depleted of T cells. This is in agreement with the direct proliferative effects on both activated CD4$^+$ and CD8$^+$ T cells described for IL-7 in vitro (4, 5) and may explain the observed T cell infiltrate in the tumor. Depletion of either T cell subset revealed the absolute dependence of the IL-7-mediated antitumor effect on CD4$^+$ cells. Furthermore, CD4$^+$ cells can promote tumor rejection in the absence of CD8$^+$ cells. In view of the in vitro data showing that IL-7 is a potent inducer of LAK activity of CD8$^+$ cells (6, 7) and is able to induce MHC-restricted cytotoxic cells in vitro (7, 8), it is surprising that the CD8$^+$ T cells do not decisively contribute to the antitumor response in our experimental setting. Yet, although CD8$^+$ T cells are not necessary for tumor rejection, a contribution of CD8$^+$ cells can not be excluded. Their presence in J558-IL-7 tumors of CD4$^+$ cell–depleted mice could explain the slightly retarded growth of these tumors in comparison with tumors in mice depleted of both T cell subsets (Fig. 4).

The potential of CD4$^+$ T cells to induce the reduction of established tumors without any requirement for cytotoxic T cells has previously been described by other investigators (37, 38) and has been explained by a delayed-type hypersensitivity (DTH)-like mechanism in which other cells like NK or macrophages finally destroy the tumor. Consistent with a DTH-like mechanism, J558-IL-7 tumors are infiltrated by CR3$^+$ cells and, notably, CR3$^+$ cells are absent in tumors of CD4$^+$ cell–depleted mice. Additionally, an anti-CR3 mAb interfered with the IL-7-induced antitumor response. This mAb has been shown to block migration of CR3$^+$ cells (macrophages, granulocytes, and NK cells) to the site of inflammation (33, 36, 39). Since granulocytes were not enriched in J558-IL-7 tumors, and anti-asialo GM1 treatment, which abrogates NK cell activity, did not interfere with J558-IL-7 tumor rejection (preliminary results), the effects of anti-CR3 mAb treatment was most likely due to the interference with macrophage recruitment. The incomplete abrogation of tumor suppression by the anti-CR3 mAb could be explained by the fact that infiltration by macrophages was not completely inhibited in J558-IL-7 tumors of anti-CR3 mAb–treated mice. This would not be in conflict with the assumption of a DTH-like mechanism because the same antibody also blocks macrophage migration in classical DTH only in part (39). Alternatively, it cannot be excluded that further effector cells or even a direct tumoricidal activity of CD4$^+$ T cells may be responsible for the incomplete effect of anti-CR3 mAb treatment.

Tumor cell–targeted gene transfer has proved to be an extremely successful approach in search for antitumor activities of cytokines in vivo. In particular, this approach also facilitated the analysis of the effector mechanisms responsible for tumor destruction and revealed characteristic differences for the cytokines analyzed. Yet, a final comparison of the effector mechanisms is still difficult because the cellular pathways leading to the tumor suppression are not completely understood in most cases, and the experimental setting (tumor cell line, mouse strain, level of cytokine expression) may account for some of the differences.

Nevertheless, the requirement of CD4$^+$ cells and the fact that CD8$^+$ cells are not necessary for the IL-7-mediated

tumor suppression distinguishes IL-7 from all other cytokines analyzed in an analogous form (IL-2, IL-4, TNF, IFN-γ, G-CSF). Thus, IL-2 production by tumor cells conversely induces an immune response that requires $CD8^+$ but not $CD4^+$ T cells (10). Similarly, the rejection of IFN-γ-producing tumor cells is also dependent on cytotoxic $CD8^+$ T cells (15). TNF produced by tumor cells has been shown to induce a host-dependent antitumor response that involves macrophages (17), but obviously also depends on the action of both $CD4^+$ and $CD8^+$ T cells (19). Consistently, in nude mice, TNF leads to tumor suppression but not complete rejection (18). In contrast to these results, we could not detect any indication of IL-7-mediated tumor rejection in the absence of T cells. This also distinguishes IL-7 from IL-4 and G-CSF, since gene transfer experiments with these cytokines have shown that the respective antitumor mechanisms were not impaired in athymic nude mice (13, 20). Therefore, IL-7 induces a cellular pathway leading to tumor destruction that has not yet been described to be activated by any other cytokine gene transfer into tumor cells.

We gratefully acknowledge the excellent technical assistance of Gisela Schulz, Maria-Virgilia Odenwald, and Magelie Gärtner. Plasmids pBAneo, Aptagl, and cell line T/SA were kindly provided by R. Mulligan, J. Flanagan, and G. Forni, respectively. We also thank Hugh Rosen for kindly supplying us with the antibody 5C6, and M. Mielke for 2.43 ascites and advice. We thank E. Adam for typing the manuscript.

This work was supported by a grant from the Deutsche Krebshilfe, Mildred Scheel Stiftaug, e.V.

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Received for publication 13 August 1991.

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