Interleukin 7 Generates Antitumor Cytotoxic T Lymphocytes against Murine Sarcomas with Efficacy in Cellular Adoptive Immunotherapy

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Summary

Interleukin 7 (IL-7) is a 25-kD cytokine that was initially described as a pre-B cell growth factor. This cytokine has also been shown to have T cell proliferative and differentiation effects. In this report, we demonstrate that antitumor cytotoxic T lymphocytes (CTL) generated by secondary in vitro sensitization of draining lymph node cells in IL-7 are effective in treating 3-day syngeneic methylcholanthrene (MCA) sarcoma pulmonary metastases in mice. In vivo titrations comparing IL-7 to IL-2 antitumor CTL show that they have equivalent potency in adoptive immunotherapy. IL-7 antitumor CTL generated against MCA sarcomas of weak immunogeneity are also tumor specific in their in vivo efficacy. This study represents the first successful use of a cytokine other than IL-2 for the generation of cells with in vivo efficacy in cellular adoptive transfer.

Generation of antitumor cells with activity in vitro and therapeutic efficacy in vivo has been described with lymphokine-activated killer (LAK)[1] cells, tumor-infiltrating lymphocytes (TIL), and secondary in vitro sensitized CTL (1). In all of these methods of effector cell generation for adoptive immunotherapy, IL-2 has played a necessary role in lymphocyte culture and as a supportive agent for the transferred cells in vivo. While IL-2 is of prime importance in the generation of antitumor cytotoxic T cells, further search has uncovered other cytokines with T cell–activating and –proliferative capacity, including IL-4 (2, 3) and IL-6 (4, 5).

Recently, after the identification of a soluble molecule with pre-B cell growth factor activity, IL-7 was cloned (6–8) and shown to promote the proliferation of murine thymocytes (9–11), mature T cells (12–14), as well as the generation of lytic CTL with alloreactivity from thymocytes (15). We have recently shown that IL-7 was capable of generating antitumor CTL with similar cytolytic activity and enhanced specificity in ^{51}Cr release when compared to those generated with IL-2 (Jicha, D. L., S. Schwarz, J. J. Mulé, and S. A. Rosenberg, manuscript submitted for publication). In this report, we now demonstrate the in vivo efficacy and specificity of IL-7-generated antitumor CTL in mice bearing tumor metastases and compare this efficacy to that of antitumor CTL generated in IL-2.

[1] *Abbreviations used in this paper:* DLN, draining lymph node; LAK, lymphokine-activated killer; MCA, methylcholanthrene; TIL, tumor-infiltrating lymphocytes.

Materials and Methods

Mice. Female C57BL/6n (denoted B6) mice were obtained from the Animal Production Colonies of the National Cancer Institute, Frederick facility (Frederick, MD), and from the Charles River Breeding Laboratories, Inc. (Wilmington, MA).

Tumors. The methylcholanthrene (MCA)-induced 203 and 205 tumors are weakly immunogenic fibrosarcomas of B6 origin maintained in vivo by subcutaneous passage in syngeneic mice. All tumors were used fresh and were prepared as described previously (16).

Cytokines. Purified human rIL-7 was a gift of Sterling Drug, Inc. (Malvern, PA). Two lots of IL-7 were used, one with a specific activity of 4.7×10^7 U/mg of protein and another with a specific activity of 5.6×10^7 U/mg, as determined by bioassay using the IL-7-dependent IxN/2b pre-B cell line (6). Human rIL-2 was a gift of Cetus Corp. (Emeryville, CA). Lyophilized IL-2 was reconstituted in sterile water to 10^6 U/ml, as confirmed in a bioassay using IL-2-dependent CTLL (17).

Antitumor CTL Cultures. Popliteal draining lymph nodes (DLN) were removed in sterile conditions 7 d after footpad inoculation of B6 mice with 10^6 MCA 203 or 205 tumors in 0.05 ml. Single-cell suspensions were prepared by crushing the DLN with the blunt end of a syringe plunger and subsequent filtration through nylon mesh. Cultures were carried out in 8-ml, six-well plates (Costar, Cambridge, MA) with 4×10^5 DLN/ml. DLN were cocultured with 2×10^5/ml MCA 203 or 205 (as appropriate per footpad inoculation) previously irradiated with 2,000 rad. Cultures were generated in IL-7 (100 ng/ml), IL-2 (20 U/ml), IL-7 (100 ng/ml) plus IL-2 (20 U/ml), or no cytokine as noted. In all cases, cells were cultured for 11 d before use in adoptive transfer experiments.

Adoptive Immunotherapy Models. B6 mice were injected intravenously, via lateral tail vein, with 5×10^5 MCA 203 or 205 tumor cells in 1 ml of HBSS, to induce pulmonary metastases. On day 3 after delivery of tumor cells, therapeutic cells were injected intra-

venously, via lateral tail vein, at varying doses as noted. On day 14–17 after initial tumor delivery, pulmonary metastases were enumerated as described previously (17).

Statistical Analysis. Significant differences in the number of pulmonary metastases among groups were analyzed by the Wilcoxon rank sum test. Two-sided p values are presented in all cases.

Results and Discussion

To test the in vivo efficacy of IL-7-generated antitumor CTL obtained from DLN, we used a 3-d pulmonary metastatic model with the syngeneic MCA 203 sarcoma. Cell cultures were established in complete media without added cytokine in parallel with IL-7, IL-2, and IL-7 plus IL-2-supplemented cultures. Antitumor CTL were delivered intravenously once followed by IL-2 10,000 U given twice daily for 5 d beginning immediately after adoptive cell transfer. As shown in Table 1, IL-7 and IL-2 cultures were equally effective in eradicating metastatic disease at titrated cell doses. IL-7 plus IL-2 cultures were equivalent to or slightly poorer in eradicating metastatic disease than cells cultured in either cytokine alone. Importantly, cells cultured without a cytokine during secondary in vitro sensitization did not eradicate metastatic disease even at the highest dose (3×10^6) of cells delivered. In one of two experiments, a small but significant decrease below HBSS treatment controls was seen with 3×10^6 and 10^6 cells derived from cultures with no cytokine, but this was significantly less than the therapeutic effect obtained with IL-7 or IL-2 antitumor CTL at the equivalent cell dose. Fresh DLN, directly harvested from mice 7 d after footpad inoculation with the syngeneic autologous MCA 203 tumor, also had no therapeutic effect in three of three experiments. This latter finding established the necessity of secondary in vitro sensitization for the generation of therapeutic effector cells.

Supportive cytokine treatment with IL-2 in adoptive therapy with secondarily stimulated antitumor CTL has been shown to augment the efficacy of cells in vivo, generally twofold (16). In adoptive immunotherapy with TIL, a three- to fivefold augmentation in efficacy has been reported (19). The effect of supportive IL-2 on IL-7 antitumor CTL was evaluated by comparing the effect of delivering IL-2 (60,000 U/dose) or HBSS intraperitoneally twice daily for 5 d after titrated cellular adoptive transfer with CTL generated from DLN. As shown in Table 2, IL-2 augmented the efficacy of IL-7 and

Table 1. *Comparison of IL-7, IL-2, and IL-7 Plus IL-2 Versus No Cytokine in Generation of Antitumor CTL*

Group	Culture condition‡	No. of cells transferred§	IL-2‖	Mean no. of pulmonary metastases (SEM)*		
				Exp. 1	Exp. 2	Exp. 3
A	–	HBSS	–	232 (18)	248 (2)	250 (0)
B	–	HBSS	+	213 (36)	242 (4)	250 (0)
C	IL-7	3×10^6	+	0 (0)	0 (0)	0 (0)
D	IL-7	10^6	+	3 (2)	27 (20)	ND
E	IL-7	5×10^5	+	89 (14)	108 (24)	ND
F	IL-2	3×10^6	+	1 (1)	0 (0)	ND
G	IL-2	10^6	+	33 (6)	52 (28)	ND
H	IL-2	5×10^5	+	214 (34)	96 (36)	ND
I	IL-7 + IL-2	3×10^6	+	0 (0)	0 (0)	ND
J	IL-7 + IL-2	10^6	+	134 (18)	35 (13)	ND
K	IL-7 + IL-2	5×10^5	+	234 (16)	97 (36)	ND
L	No cytokine	3×10^6	+	238 (12)	187 (32)	ND
M	No cytokine	10^6	+	242 (8)	176 (34)	ND
N	No cytokine	5×10^5	+	250 (0)	250 (0)	ND
O	Fresh DLN¶	3×10^6	+	208 (32)	174 (38)	250 (0)

* Statistical significance of differences: Exp. 1, A vs. C, D, E, F, G, I, or J, $p_2 < 0.05$; A vs. B, H, K, L, M, N, or O, not significant; C vs. E, G, H, J, K, L, M, N, or O, $p_2 < 0.05$; C vs. D, F, or I, not significant; E vs. F, G, I, K, L, M, N, or O, $p_2 < 0.05$; E vs. H or J, not significant. Exp. 2, A vs. C, D, E, F, G, H, I, J, K, L, or O, $p_2 < 0.05$; A vs. B, M, or N, not significant; C vs. D, E, G, H, J, K, L, M, N, or O, $p_2 < 0.05$; C vs. F or I, not significant; E vs. F, I, J, or N, $p_2 < 0.05$; E vs. G, H, K, L, M, or O, not significant. Exp. 3, A vs. B or O, not significant; C vs. A, B, or O, $p_2 < 0.05$.

‡ B6 mice popliteal DLN were harvested 7 d after footpad inoculation with MCA 203. DLN cells were restimulated in vitro and cultured for 11 d before adoptive transfer.

§ B6 mice were inoculated intravenously with 5×10^5 203 MCA tumor. 3 d later, antitumor CTL were given intravenously in 1 ml of HBSS.

‖ IL-2, 10,000 U/dose, was delivered twice daily for 5 d intraperitoneally.

¶ B6 mice popliteal DLN cells were harvested 7 d after footpad inoculation with MCA 203 and adoptively transferred without further in vitro culture.

IL-2 antitumor CTL over HBSS in two of three experiments. IL-7 antitumor CTL delivered without cytokine support were as effective as those CTL generated with IL-2 in eradicating metastases. Overall, five comparisons of IL-7 vs. IL-2 antitumor CTL without supportive cytokine treatment showed them to be equivalent in antitumor CTL efficacy, and in one case, the IL-7 antitumor CTL were significantly better.

IL-7 was also tested as a supportive cytokine. The IL-7 dose used (20 μg/dose) matched the IL-2 dose of 60,000 U on the basis of the weight of the protein content of cytokine administered. IL-7 as a supportive cytokine for IL-2 antitumor CTL resulted in significantly less metastatic disease than HBSS control-treated groups in two of three experiments. In no case did IL-7 support of IL-7 antitumor CTL result in significantly better antitumor effects than was seen with HBSS administration (data not shown).

To determine the in vivo specificity of IL-7 antitumor CTL, cells generated against MCA 203 and 205 tumors were adoptively transferred in a 3-d pulmonary metastatic model with 10,000 U IL-2 administered intraperitoneally twice daily for five consecutive days. As shown in Table 3, adoptively transferred cells effectively treated only the respective autologous tumor when tested in the crossover pattern. These results demonstrated the specificity of MCA 203 and 205 IL-7-generated antitumor CTL in vivo.

In this report, we studied the in vivo efficacy of antitumor CTL generated with IL-7. Our findings now extend the mature T cell–activating effects of IL-7 (12–14; and Jicha, D. L., S. Schwarz, J. J. Mulé, and S. A. Rosenberg, manuscript submitted for publication) to include the induction of antitumor effector cells with therapeutic efficacy in vivo. We demonstrate that IL-7-generated antitumor CTL are effective in treating 3-d pulmonary metastatic disease from syngeneic, autologous tumor. Further, cells generated in IL-7 are as po-

Table 2. *IL-2 as a Supportive Cytokine with IL-7 and IL-2 Antitumor CTL*

Group	Culture condition‡	No. of cells§	Cytokine support‖	Mean no. of pulmonary metastases (SEM)*		
				Exp. 1	Exp. 2	Exp. 3
A	-	HBSS	HBSS	250¶ (0)	250 (0)	250 (0)
B	-	HBSS	IL-2	250 (0)	250 (0)	250 (0)
C	IL-7	3×10^6	HBSS	0 (0)	0 (0)	0 (0)
D	IL-7	10^6	HBSS	16 (6)	30 (22)	3 (3)
E	IL-7	5×10^5	HBSS	221 (20)	138 (38)	66 (38)
F	IL-2	3×10^6	HBSS	1 (1)	0 (0)	0 (0)
G	IL-2	10^6	HBSS	249 (1)	59 (26)	27 (26)
H	IL-2	5×10^5	HBSS	250 (0)	204 (29)	143 (43)
I	IL-7	3×10^6	IL-2	0 (0)	0 (0)	1 (1)
J	IL-7	10^6	IL-2	1 (1)	3 (3)	2 (1)
K	IL-7	5×10^5	IL-2	91 (35)	53 (20)	8 (5)
L	IL-2	3×10^6	IL-2	0 (0)	1 (1)	0 (0)
M	IL-2	10^6	IL-2	44 (15)	56 (28)	3 (2)
N	IL-2	5×10^5	IL-2	226 (13)	154 (39)	94 (34)

* Statistical significance of differences: Exp. 1, B vs. C, D, F, I, J, K, L, M, or N, $p_2 < 0.05$; B vs. A, E, G, or H, not significant; C vs. D, E, G, H, K, M, or N, $p_2 < 0.05$; C vs. F, I, J, or L, not significant; D vs. E, F, G, H, I, J, K, L, or N, $p_2 < 0.05$; D vs. M, not significant; E vs. F, I, J, K, L, or M, $p_2 < 0.05$; E vs. G, H, or N, not significant; I vs. K, M, or N, $p_2 < 0.05$; I vs. J or L, not significant; J vs. K, M, or N, $p_2 < 0.05$; J vs. L, not significant; K vs. L or N, $p_2 < 0.05$; K vs. M, not significant. Exp. 2, A vs. C, D, E, F, G, I, J, K, L, M, or N, $p_2 < 0.05$; A vs. B or H, not significant; C vs. E, G, H, K, M, or N, $p_2 < 0.05$; C vs. D, F, I, J, or L, not significant; D vs. E or H, $p_2 < 0.05$; D vs. F, G, I, J, K, L, M, or N, not significant; E vs. F, I, J, or L, $p_2 < 0.05$; E vs. G, H, K, M, or N, not significant; I vs. K, M, or N, $p_2 < 0.05$; I vs. J or L, not significant; J vs. K, M, or N, $p_2 < 0.05$; J vs. L, not significant; K vs. L, $p_2 < 0.05$; K vs. M or N, not significant. Exp. 3, A vs. C, D, E, F, G, H, I, J, K, L, M, or N, $p_2 < 0.05$; A vs. B, not significant; C vs. E, H, or N, $p_2 < 0.05$; C vs. D, G, I, J, K, L, or M, not significant; D vs. E, H, or N, $p_2 < 0.05$; D vs. F, G, I, J, K, L, or M, not significant; E vs. F, G, I, J, K, L, or M, $p_2 < 0.05$; E vs. H or N, not significant; I vs. N, $p_2 < 0.05$; I vs. J, K, L, or M, not significant; J vs. N, $p_2 < 0.05$; J vs. K, L, or M, not significant; K vs. L, $p_2 < 0.05$; K vs. M or N, not significant.

‡ B6 mice popliteal DLN were harvested 7 d after footpad inoculation with MCA 203. DLN cells were restimulated in vitro and cultured for 11 d before adoptive transfer.

§ B6 mice were inoculated intravenously with 5×10^5 203 MCA tumor. 3 d later, antitumor CTL were given intravenously in 1 ml of HBSS.

‖ IL-2, 60,000 U/dose, or HBSS was delivered twice daily for 5 d intraperitoneally.

¶ $n = 4$, in all other groups $n = 5$ or greater.

Table 3. *IL-7 Antitumor CTL Are Specific*

Group	In vitro MCA tumor‡	No. of cells transferred§	IL-2‖	MCA tumor treated¶	Mean no. of pulmonary metastases (SEM)*	
					Exp. 1	Exp. 2
A	–	HBSS	–	203	250 (0)	250 (0)
B	–	HBSS	+	203	250 (0)	250 (0)
C	203	3×10^6	+	203	0 (0)	0 (0)
D	205	3×10^6	+	203	250 (0)	250 (0)
E	–	HBSS	–	205	228 (22)	250 (0)
F	–	HBSS	+	205	250 (0)	250 (0)
G	203	3×10^6	+	205	248 (2)	250 (0)
H	205	3×10^6	+	205	0 (0)	0 (0)

* Statistical significance of differences: Exp. 1 and 2, C vs. A, B, D, E, F, or G, $p_2 < 0.001$; C vs. H, not significant; H vs. A, B, D, E, F, or G, $p_2 < 0.001$.

‡ B6 mice popliteal DLN were harvested 7 d after footpad inoculation with MCA 203 or 205. DLN cells were restimulated with their respective irradiated tumors in vitro, as noted, and cultured for 11 d in IL-7 before adoptive transfer.

§ B6 mice were treated intravenously with antitumor CTL or HBSS 3 d after tumor was delivered to mice.

‖ IL-2 (10,000 U/dose) or HBSS was delivered intraperitoneally twice daily for 5 d.

¶ Mice were inoculated intravenously with 5×10^5 MCA 203 or 205 tumor as specified.

tent as IL-2-generated antitumor CTL. We confirmed the necessity of IL-7 or IL-2 in our antitumor CTL cultures for the generation of antitumor cells at the cell titrations delivered. Specificity of IL-7 antitumor CTL in vivo in a crossover experiment with the MCA 203 and 205 tumors is also shown.

To our knowledge, these results represent the first successful therapeutic adoptive transfer of cells grown in a cytokine other than IL-2. This work further suggests that IL-7 may have an important role in adoptive immunotherapy.

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References

1. Yang, J.C., and S.A. Rosenberg. 1991. Adoptive cellular therapy: preclinical studies. *In* Biologic Therapy of Cancer. V.T. DeVita, S. Hellman, and S.A. Rosenberg, editors. J.B. Lippincott Company, Philadelphia, PA. pg. 197.
2. Widmer, M.B., and K.H. Grabstein. 1987. Regulation of cytolytic T-lymphocyte generation by B-cell stimulatory factor. *Nature (Lond.).* 326:795.
3. Mulé, J.J., C.A. Smith, and S.A. Rosenberg. 1987. Interleukin 4 (B cell stimulatory factor 1) can mediate the induction of lymphokine-activated killer cell activity directed against fresh tumor cells. *J. Exp. Med.* 166:792.
4. Garman, R.D., and D.H. Raulet. 1987. Characterization of a novel murine T-cell-activating factor. *J. Immunol.* 138:1121.
5. Uyttenhove, C., P.G. Coulie, and J.V. Snick. 1988. T cell growth and differentiation induced by interleukin-HP-1/IL-6, the murine hybridoma/plasmacytoma growth factor. *J. Exp. Med.* 167:1417.
6. Namen, A.E., A.E. Schmierer, C.J. March, R.W. Overell, L.S. Park, D.L. Urdal, and D.Y. Mochizuki. 1988. B cell precursor growth-promoting factor: purification and characterization of a factor active on lymphocyte precursors. *J. Exp. Med.* 167:988.
7. Namen, A.E., S. Lupton, K. Hjerrild, J. Wignal, D.Y. Mochizuki, A. Schmierer, B. Mosley, C.J. March, D. Urdal, S. Gillis, D. Cosman, and R.G. Goodman. 1988. Stimulation of B-cell progenitors by cloned murine interleukin-7. *Nature (Lond.).* 333:571.
8. Goodwin, R.G., S. Lupton, A. Schmierer, K.J. Hjerrild, R. Jerzy, W. Clevenger, S. Gillis, D. Cosman, and A.E. Namen. 1989. Human interleukin-7: molecular cloning and growth factor activity on human and murine B-lineage cells. *Proc. Natl.*

Acad. Sci. USA. 86:302.
9. Watson, J.D., P.J. Morrissey, A.E. Namen, P.J. Conlon, and M.B. Widner. 1989. Effect of IL-7 on the growth of fetal thymocytes in culture. *J. Immunol.* 143:1215.
10. Conlon, P.J., P.J. Morrissey, R.P. Nordan, K.H. Grabstein, K.S. Prickett, S.G. Reed, R. Goodwin, D. Cosman, and A.E. Namen. 1989. Murine thymocytes proliferate in direct response to interleukin-7. *Blood.* 74:1368.
11. Ozazaki, H., M. Ito, T. Sudo, M. Hattori, S. Kano, Y. Katsura, and N. Minato. 1989. IL-7 promotes thymocyte proliferation and maintains immunocompetent thymocytes bearing or T-cell receptors in vitro: Synergism with IL-2. *J. Immunol.* 143:2917.
12. Chazen, G.D., G.M. Pereira, G. LeGros, S. Gillis, and E. Shevach. 1989. Interleukin 7 is a T-cell growth factor. *Proc. Natl. Acad. Sci. USA.* 86:5923.
13. Grabstein, K.H., A.E. Namen, K. Shanebeck, R.F. Voice, S.G. Reed, and M.B. Widmer. 1990. Regulation of T-cell proliferation by IL-7. *J. Immunol.* 144:3015.
14. Morrissey, P.J., R.G. Goodwin, R.P. Nordan, D. Anderson, K.H. Grabstein, D. Cosman, J. Sims, S. Lupton, B. Acres, S.G. Reed, D. Mochizuki, J. Eisenman, P.J. Conlan, and A.E. Namen. 1989. Recombinant interleukin 7, pre-B cell growth factor, has costimulatory activity on purified mature T cells. *J. Exp. Med.* 169:707.
15. Bertagnolli, M., and S. Herrmann. 1990. IL-7 supports the generation of cytotoxic T lymphocytes from thymocytes: Multiple lymphokines required for proliferation and cytotoxicity. *J. Immunol.* 145:1706.
16. Shu, S., T. Chou, and S.A. Rosenberg. 1987. Generation from tumor-bearing mice of lymphocytes with in vivo therapeutic efficacy. *J. Immunol.* 139:295.
17. Barth, R.J., S.N. Bock, J.J. Mulé, and S.A. Rosenberg. 1990. Unique murine tumor-associated antigens identified by tumor infiltrating lymphocytes. *J. Immunol.* 139:295.
18. Rosenberg, S.A., P. Spiess, and R. Lafreniere. 1986. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science (Wash. DC).* 133:1318.