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Macrophage Tumor Cells Can Help to Cure Murine Lymphoma[1] (41150)

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Abstract. Intraperitoneal injection of immune syngeneic lymphocytes can prolong the survival time of DBA/2 mice bearing SL2 lymphoma. Addition of induced peritoneal macrophages or macrophage-like J774A tumor cells to the immune lymphocytes enchanced this antitumor effect. Enhancement of antitumor activity with J774A cells occurred in a local system as well as in a systemic system.

In several experimental and human systems, lymphocytes from immunized donors (immune lymphocytes) injected into tumor-bearing hosts may suppress the growth of the tumor (1–3). Peritoneal macrophages added to immune lymphocytes enhanced the local antitumor effect (4). Mixtures of immune lymphocytes and macrophages from immunized mice (immune macrophages) had a more potent antitumor effect than purified immune lymphocytes or immune macrophages (5). *In vivo* antitumor effects of transferred immune macrophages described up to now were local. DBA/2 mice with SL2 tumors can be cured by *in vivo* or *in vitro* activated macrophages (5, 6). We wanted to investigate whether transfer of immune lymphocytes can also give rise to prolonged survival times, using the DBA/2 SL2 system, and whether addition of macrophages to the transferred immune lymphocytes can enhance the antitumor effect of lymphocytes in this system. Furthermore, we wanted to know whether we could use macrophage-like tumor cells instead of macrophages to enhance the antitumor effect of immune lymphocytes.

Materials and Methods. Immune spleen cells: DBA/2 or BALB/c mice (H-2^d) were immunized with two intraperitoneal (ip) injections of 10^7 irradiated (5000 rad) SL2 lymphosarcoma cells (H-2^d) and one ip injection of 5×10^6 unirradiated SL2 cells, at intervals of 10 days. Six days after the last injection immune spleen cells were collected.

Macrophages. DBA/2 or BALB/c mice were injected ip with 2 ml Brewer's Medium (0236-02 Difco Inc.). Four days later peritoneal exudate macrophages were collected. J774A macrophage-like cells (BALB/c; H-2^d) grow as an ascitic tumor in the peritoneal cavity of the syngeneic host. The cells can be obtained in large quantities. The cells have a series of characteristics with normal macrophages in common, e.g., phagocytosis, immunoglobulin receptors, complement receptors, enzymatic patterns, antibody-dependent cell-mediated cytotoxicity, glass adherence (7, 8). DBA/2 mice were able to reject 5×10^7 but not 5×10^8 J774A cells injected ip.

MOPC 195 plasmacytoma cells (BALB/c; H-2^d) grow in the peritoneal cavity of the syngeneic mice.

To prevent replication of J774A and MOPC 195 the cells were treated with mitomycin-C (Christiaens, Brussels, Belgium; 10^6 cells/ml were incubated in medium with 25 μg/ml mitomycin-C at 37° for 45 min). Immune spleen cells (10^6/ml) were T cell depleted by incubation with a 1/250 dilution of antithymocyte serum (Microbiological Associates, Bethesda, Md.) at 4° for 45 min, then they were washed and incubated with a 1/10 dilution of guinea pig complement at 37° for 45 min.

To deplete immune spleen cells of macrophages and B cells, immune spleen cells were purified with glass wool and nylon wool columns as described by Julius *et al.* (9).

Experimental designs. I. DBA/2 mice were injected ip with SL2 cells (10^3), immune spleen cells (10^6), and macrophages (10^5); II. DBA/2 or BALB/c mice were injected subcutaneously (sc) with SL2 cells

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(10^5) on Day 0. On Day 3, 4, 5, 6, and 7 immune spleen cells (10^8) and macrophages (10^7) were injected ip daily.

Results. We investigated whether immune (vs SL2) DBA/2 spleen cells could prolong the survival time of SL2 bearing DBA/2 mice. Immune (vs SL2) DBA/2 spleen cells were mixed with SL2 lymphosarcoma cells. The mixture was injected into the peritoneal cavity of DBA/2 mice. This resulted in survival times which are significantly different from the survival of control mice (Table I). Throughout these studies we never observed control mice surviving an ip injection of 10^3 SL2 cells. Addition of Brewer's medium induced peritoneal DBA/2 macrophages (10^5) or BALB/c macrophages (10^5) to the mixture of immune DBA/2 spleen cells and SL2 cells significantly enhanced the antitumor effect (Table I). Addition of J774A cells (10^5) to the mixture of immune spleen cells and SL2 cells also enhanced the antitumor effect (Table I). Thus, J774A macrophage-like tumor cells gave similar results as Brewer's medium induced peritoneal macrophages.

We also investigated whether J774A cells could enhance the systemic antitumor effect of immune spleen cells. DBA/2 mice were inoculated with 10^5 SL2 cells sc. On Days 3–7 the mice were injected with immune spleen cells ip. This resulted in a significant prolongation of the survival time of the tumor bearing mice (Table II). Rejection of the tumor did not occur. J774A cells added to the immune spleen cells improved the results of this systemic adoptive immunotherapy. Thus, J774A macrophage-like cells are also able to cooperate with immune spleen cells in the systemic antitumor effect.

Neither Brewer's medium induced peritoneal macrophages (Table 1) nor J774A cells alone produced any antitumor effect in both the local and systemic model (Table I, II). This indicates that the antitumor effect was not an additive effect of the macrophages or the macrophage-like cells and the immune spleen cells, but it seems to be due to a synergism of both cell types.

J774A cells are antigenically different from the DBA/2 recipients and from DBA/2 immune spleen cells, since they are tumor

TABLE I. LOCAL EFFECT OF BREWER'S MEDIUM INDUCED PERITONEAL MACROPHAGES, J774A CELLS, AND IMMUNE (VERSUS SL2) DBA/2 SPLEEN CELLS ON THE INTRAPERITONEAL GROWTH OF SL2 LYMPHOSARCOMA IN DBA/2 MICE

Transferred cells[a]	Survival time in days[d,e,f]	Percentage survivors (>150 days)
Normal spleen cells	19 ± 1 (25)	0
Immune spleen cells[b]	20 ± 1 (21), >150 (4)[g]	16
Immune spleen cells[b] + Brewer's induced DBA/2 macrophages[c]	20 ± 1 (5), >150 (5)[h]	50
Immune spleen cells[b] + Brewer's induced BALB/c macrophages[c]	21 ± 2 (4), >150 (6)[h]	60
Immune spleen cells[b]+ J774A cells	35 ± 4 (11), >150 (14)[h]	56
Brewer's induced DBA/2 macrophages[c]	21 ± 1 (10)	0
Brewer's induced BALB/c macrophages[c]	21 ± 1 (10)	0
J774A cells	20 ± 1 (25)	0

[a] SL2 cells (10^3) were mixed with normal or immune DBA/2 spleen cells (10^6) and Brewer's medium induced peritoneal macrophages or J774A cells (10^5). The cells were injected ip in DBA/2 mice.

[b] DBA/2 mice were immunized with two ip injections of 10^7 irradiated (5000 rad) SL2 cells and one injection of 5×10^6 nonirradiated SL2 cells at intervals of 10 days. Immune spleen cells were collected 6 days after the last injection.

[c] Brewer's medium induced peritoneal macrophages (plus contaminating lymphocytes) were collected 4 days after ip injection of 2 ml Brewer's medium.

[d] The experiments were stopped after 150 days.

[e] Mean survival time ± SE.

[f] The number of mice in parentheses.

[g] $P < 0.05$ compared to normal spleen cells.

[h] $P < 0.05$ compared to immune spleen cells only.

TABLE II. SYSTEMIC EFFECT OF INTRAPERITONEAL INJECTION OF J774A CELLS, MOPC 195 CELLS, BALB/c SPLEEN CELLS, AND IMMUNE (VS SL2) SPLEEN CELLS ON THE SUBCUTANEOUS GROWTH OF SL2 LYMPHOSARCOMA

Transferred cells[a]	Recipient mice carrying SL2	Survival time in days[d,e,f]
Normal DBA/2 spleen cells	DBA/2	25 ± 1 (25)
Immune DBA/2 spleen cells[b]	DBA/2	31 ± 1 (25)[g]
Immune DBA/2 spleen cells[b] + J774A cells	DBA/2	41 ± 5 (14), >150 (11)[h]
Immune DBA/2 spleen cells[b] + MOPC 195 cells	DBA/2	26 ± 1 (10)
Immune DBA/2 spleen cells[b] + BALB/c spleen cells	DBA/2	28 ± 2 (10)
J774A cells	DBA/2	25 ± 1 (25)
Normal BALB/c spleen cells	BALB/c	28 ± 2 (10)
Immune BALB/c spleen cells[b]	BALB/c	25 ± 2 (7), >150 (3)[g]
Immune BALB/c spleen cells[b] + mitomycin-C treated J774A cells[c]	BALB/c	26 ± 3 (4), >150 (6)[h]
Mitomycin-C treated J774A cells[c]	BALB/c	24 ± 2 (10)

[a] SL2 cells (10^5) were inoculated sc (Day 0). Normal or immune DBA/2 or BALB/c spleen cells (10^8) and/or J774A, MOPC 195 or BALB/c spleen cells (10^7) were injected ip daily on Days 3–7.
[b] Immune spleen cells were collected as described in Table I.
[c] 10^6 cells/ml were incubated in medium with 25 μg/ml mitomycin-C for 45 min at 37°.
[d] The experiment was stopped after 150 days.
[e] Mean survival time ± SE.
[f] Number of mice in parentheses.
[g] $P < 0.05$ compared to normal spleen cells.
[h] $P < 0.05$ compared to immune spleen cells only.

cells of BALB/c origin. This difference in antigenicity might cause some form of aspecific stimulation of the injected immune DBA/2 lymphocytes or of the lymphocytes of the recipients or of both. However, cells of BALB/c origin other than macrophage-like cells (MOPC 195 cells or BALB/c derived spleen cells) did not enhance the antitumor effect of immune spleen cells (Table II). Neither control takes into consideration differences in lethality and dividing time of BALB/c spleen cells and MOPC 195 cells in the recipient DBA/2 mice compared with J774A cells. Therefore, mitomycin-C-treated J774A and MOPC 195 cells were tested for their ability to enhance the antitumor effect of injected immune DBA/2 spleen cells. Mitomycin-C-treated J774A cells gave positive results. In contrast, mitomycin-C-treated MOPC 195 cells did not enhance antitumor activity (Table III). Lysed J774A cells were not ef-

TABLE III. EFFECT OF MITOMYCIN-C-TREATED J774A CELLS, MITOMYCIN-C-TREATED MOPC 195 CELLS, AND IMMUNE (VS SL2) DBA/2 SPLEEN CELLS ON THE INTRAPERITONEAL GROWTH OF SL2 LYMPHOSARCOMA

Transferred cells[a]	Survival time in days[d,e,f]
Immune spleen cells[b]	22 ± 1 (19), >150 (1)
Immune spleen cells[b] + mitomycin-C treated J774A cells[c]	28 ± 3 (15), >150 (5)[g]
Immune spleen cells[b] + mitomycin-C treated MOPC 195 cells[c]	24 ± 1 (9), >150 (1)
Mitomycin-C treated J774A cells[c]	18 ± 1 (10)
Mitomycin-C treated MOPC 195 cells[c]	22 ± 1 (10)

[a] Transfer schedule as described in Table I.
[b] Immune spleen cells were collected as described in Table I.
[c] Cells were treated with mitomycin-C as described in Table II.
[d] The experiment was stopped after 150 days.
[e] Mean survival time ± SE.
[f] Number of mice in parentheses.
[g] $P < 0.05$ compared to immune spleen cells only.

fective either (tested in both the local and the systemic system).

To further elucidate the problem of the possible stimulation of the immune DBA/2 spleen cells by the antigenically different J774A cells, the ability of mitomycin-C-treated J774A cells to enhance the antitumor effect of immune (versus SL2) BALB/c spleen cells when injected in BALB/c mice carrying SL2 was tested (Table II). The results indicate that J774A cells were able to enhance the antitumor effect of injected immune spleen cells in the DBA/2-SL2 system, as well as in the BALB/c-SL2 system. Thus, the influence of addition of J774A cells to immune spleen cells on the antitumor effect seems to be dependent on J774A cells being viable functional macrophages.

Treatment of the immune spleen cells with antithymocyte serum and complement abrogated the antitumor effect of immune spleen cells in combination with J774A cells (Table IV). Passage of immune spleen cell populations through glass wool columns and nylon wool columns caused a decrease of the percentage of adhering cells; the resulting lymphocyte population was T cell enriched (73% T cells compared to 23% in the nonenriched T-cell population). This procedure did not lead to abrogation of the antitumor effect (Table IV). Thus, interaction between J774A cells and immune spleen cells probably consists of synergism between J774A and immune T cells.

Discussion. This report describes for the first time that macrophage-like tumor cells (J774A) can enhance the local and systemic effect of an adoptive lymphocyte therapy, as do Brewer's medium induced peritoneal macrophages. The nature of the essential function of macrophages and J774A cells in this cooperation with immune T cells is currently being investigated. The effect of immune T cells alone may be explained by direct cytotoxicity of the cells toward the SL2 cells. The immune T cells may also act as initiator T cells (10). The immune T cells may render the added macrophages or the added J774A cells cytotoxic (6, 7, 11–14). It is also possible that the immune T cells stimulate production of lymphocyte activating factor (LAF) by the added macrophages or by the added J774A cells (15–17). This LAF may be involved in stimulation of the immune T cells. Finally, both pathways may occur simultaneously. The cycle thus provided may be a very effective mechanism to inhibit tumor growth.

The data do not give information on the influence of immune (versus SL2) spleen cells on the host reaction to J774A cells. As DBA/2 mice are able to reject substantial numbers of J774A cells, experiments to establish this influence are not feasible, as one cannot expect lymphocytes to be effective in relatively low effector to target (J774A) ratios.

We have preliminary data on another macrophage-like line (WEHI-3) which has

TABLE IV. EFFECT OF NYLON WOOL PURIFICATION AND ANTITHYMOCYTE SERUM TREATMENT OF IMMUNE (VS SL2) DBA/2 SPLEEN CELLS ON THE SYSTEMIC ANTITUMOR EFFECT WITH J774A CELLS

Transferred cells[a]	Survival time in days[e,f,g]
Immune spleen cells[b] + J774A cells	33 ± 2 (8), >150 (2)[h,i]
Immune spleen cells[b] treated with ATS[c] + J774A cells	23 ± 1 (10)
Nylon wool purified immune spleen cells[d] + J774A cells	27 ± 2 (7), >150 (3)[h]
J774A cells	19 ± 1 (10)

[a] Transfer schedule as described in Table II.
[b] Immune spleen cells were collected as described in Table I.
[c] Immune spleen cells (10^6/ml) were incubated with a 1/250 dilution of ATS for 45 min at 4°, washed and incubated with a 1/10 dilution of guinea pig complement.
[d] Immune spleen cells were purified according to the method of Julius *et al.* (9). 10^8 purified spleen cells were injected daily.
[e] The experiment was stopped after 150 days.
[f] Mean survival time ± SE.
[g] Number of mice in parentheses.
[h] $P < 0.05$ compared to J774A only.
[i] $P > 0.05$ compared to nylon wool purified immune spleen cells + J774A.

the same capabilities as the J774A cells described in this paper, and which grows much easier in DBA/2 mice. We were not able to find any effect of immune (versus SL2) spleen cells on in vivo growth of WEHI-3 cells.

J774A cells form a rather homogeneous population of macrophage-like cells and are easily obtained in large quantities. This makes them a useful tool in studying the antitumor effects of macrophages in vivo as well as in vitro. Extension of the data base with observations on the J774A-immune T-cell interactions may lead to a refinement of adoptive immunotherapy models.

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