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Leukemia × Fibroblast Hybrid Cells Prolong the Lives of Leukemic Mice*

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Abstract—*ASL-1 leukemia × LM(TK-) fibroblast hybrid cells prolong the lives of leukemic (A/J×C3H/HeJ)F$_1$ mice. The hybrid cells, like the fibroblast cells used in forming the hybrid, have lost malignant growth properties in immunocompetent recipients and are rejected. Mice receiving hybrid cells along with ASL-1 cells exhibit immunity toward the leukemia cells; approximately 50% of the animals injected with 10^6 or more hybrid cells along with ASL-1 cells survive more than 60 days; animals in the control group injected with leukemia cells alone invariably die in shorter intervals. The immunity generated is persistent for at least 6 months. Some leukemic mice receiving doses of combination chemotherapy which are insufficient to cure them of the disease survive for prolonged and at times indefinite periods if they are injected with hybrid cells. The immunity generated in mice receiving hybrid cells is directed toward a leukemia-associated antigen of leukemia cells expressed by hybrid cells as well. In mixed lymphocyte culture a heightened stimulation of spleen cells from hybrid cell-injected mice toward ASL-1 cells is observed.*

INTRODUCTION

SOMATIC hybrids of neoplastic and non-neoplastic cells are rejected by immunocompetent histocompatible recipients; they induce specific partial immunity in mice who are otherwise susceptible to the neoplastic cell line used as one of the parents in forming the hybrid [1-11]. The presence of active immunity is detected by the capacity of hybrid cell-injected animals to resist tumor development by the neoplastic cell parents. Host immunity toward the malignancy is partial, i.e. it can be overcome if sufficient numbers of neoplastic cells are transferred into animals who have rejected hybrid cells [6, 12].

The resistance induced by injections of hybrid cells is immune-mediated since it can be transferred passively with lymphoid cells from hybrid cell-injected mice [13-16].

Because hybrid cell immunotherapy may be a useful adjunct to conventional approaches to the management of patients with neoplastic disease, we determined the survival of leukemic mice receiving chemotherapy and leukemia × fibroblast hybrid cells prepared using the leukemia cells as one parental source.

Here we report that leukemic (A/J×C3H/HeJ)F$_1$ mice (F$_1$ mice), a consequence of the injection of highly malignant ASL-1 cells [17-19], survive for prolonged and at times indefinite periods if both chemotherapeutic drugs and ASL-1×LM(TK-) hybrid cells are used in the treatment protocol. The hybrid cells used in the therapy have lost malignant growth properties in the histocompatible recipients and are rejected by them.

MATERIALS AND METHODS

Mice

A/J and C3H/HeJ mouse strains were obtained from the Jackson Laboratory (Bar Harbor, ME); F$_1$ offsprings of A/J and C3H/HeJ mice were bred in our animal quarters or were obtained from the Jackson Laboratory. All mice were maintained in

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Abbreviations: Hybrid cells, ASL-1×LM(TK-) cells; F$_1$ mice, (A/J×C3H/HeJ)F$_1$ mice; i.p., intraperitoneal; MLR, mixed lymphocyte reaction; cytosine arabinoside and methotrexate, combination chemotherapy; s.c., subcutaneously.

air-conditioned rooms and fed mouse chow (Purina) and water *ad libitum*. They were 6–12 weeks old when used in experimental studies, unless otherwise specified.

Cells

Murine leukemia ASL-1 is maintained by serial transfer in A/J mice. Typically, approximately 5×10^4 cells from the spleens of mice terminally ill with leukemia are transferred by intraperitoneal (i.p.) injection to recipient mice of the same inbred strain. More than 95% of the cells are neoplastic by morphologic criteria at the time of transfer. The cells stain positively with H-2^a antiserum and fluorescein-conjugated rabbit antimouse immunoglobulin serum.

Fusion of ASL-1 cells with LM(TK-) fibroblast cells (H-2^k), a thymidine-kinase-negative variant of LM cells of C3H mouse origin, was accomplished with the aid of HAT medium (containing hypoxanthine, aminopterin and thymidine), as described previously [20]. Proof of hybridization is dependent upon the presence of a hybrid karyotype including 'marker' chromosomes identified as originating in each parental source and on the presence of H-2 antigens of both parental sources associated with hybrid cells. In no instance has there been evidence of a heterogeneous population of cells, e.g. a mixture of hybrid and parental cells, in the cell population used. ASL-1×LM(TK-) hybrid cells used in these experiments grow adherent to plastic culture flasks (Corning, Corning, NY); they have been in continuous culture for more than 18 months and are maintained *in vitro* at 37°C in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum. The cells, like (A/J×C3H/HeJ)F_1 mice, express the determinants of both H-2^a and H-2^k antigen complexes and at the time of the experiment possessed 76 ± 8 chromosomes.

Survival of (A/J×C3H/HeJ)F_1 mice receiving ASL-1 leukemia cells, chemotherapy and ASL-1×LM(TK-) hybrid cells

(A/J×C3H/HeJ)F_1 mice exhibit no apparent immunity toward the progressive growth of passively transferred ASL-1 cells. The period of survival following an injection of ASL-1 cells is roughly dependent (linear-log) on the number of neoplastic cells injected.

To determine the effect of injections of ASL-1×LM(TK-) hybrid cells on the survival of F_1 mice injected previously with ASL-1 leukemia cells, ASL-1 cells freshly obtained from the spleens of A/J mice terminally ill with leukemia are injected i.p. into F_1 mice followed by treatment with chemotherapy and hybrid cells. Before injecting ASL-1 cells, differential counts are performed on the leukemia-spleen cell suspension and appropriate corrections are made for the proportion of non-neoplastic cells present. Typically, about 95% of the cells are malignant at the time of cell transfer. (No attempt is made to separate the neoplastic from the non-neoplastic cells.) ASL-1×LM(TK-) hybrid cells in log phase growth are removed from the culture flasks with 0.01 M Tris buffer, (pH 7.4) containing 2% EDTA (tetrasodium salt) and 0.85% NaCl. They are injected i.p. into the leukemic mice according to the schedule described in the legends that accompany the relevant figure and tables. Animals receiving chemotherapy were injected i.p. with a mixture of cytosine arabinoside and methotrexate (combination chemotherapy) [23–21] in addition to hybrid cells, as described. In each instance all the mice in each experiment received ASL-1 and hybrid cells as aliquots of the same cell suspension. 'Simultaneous' injections of the two cell types were administered within a short time interval of each other, usually within 60 min.

Mixed lymphocyte reaction

Mixed lymphocyte reaction (MLR) was performed to detect the presence of spleen cells that are reactive to ASL-1 or hybrid cells. Approximately 2.5×10^5 responder cells obtained from the spleens of F_1 mice injected previously with hybrid cell or ASL-1 cells (treated with 50 μg/ml mitomycin-C for 45 min at 37°C beforehand to prevent transfer of leukemia to the recipients) were incubated in the presence of various numbers of hybrid or ASL-1 cells used as the stimulator population. In each instance the cells used for stimulation were treated with 50 μg/ml mitomycin-C (Sigma Chemical Co., St. Louis, MO) for 45 min before they were added to the reaction mixture. (Further details accompany the relevant table legend.) The mixture of responder and stimulator cells was cultured in 96-well flat-bottom microculture plates (Co-Star, Cambridge, MA) containing MLR medium (RPMI 1640 supplemented with 20 mM HEPES, 5×10^{-5} M 2-mercaptoethanol, 5% fetal calf serum, 1% non-essential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, Gibco). The cell mixtures were incubated for 48 hr at 37°C in a humidified atmosphere of 7% CO_2 in air. Cell division occurring in the responder population was detected by the incorporation of 1 μCi of [^{3}H]thymidine (6.7 Ci/mM, Research Products International, Mount Prospect, IL) added to each well for the final 16 hr of incubation. The cultures were harvested onto glass fiber filter paper (M.A. Bioproducts, Walkersville, MD) by means of a

multiple automated sample harvester (Mash II, M.A. Bioproducts). After drying, the paper discs were placed into a scintillation cocktail and the radioactivity present was measured in a liquid scintillation counter (Packard Instrument Co., Morton Grove, IL).

RESULTS

The response in a mixed lymphocyte reaction of spleen cells of hybrid cell-injected mice toward ASL-1 cells

Mixed lymphocyte reactions were performed to determine if cells were present in the spleens of hybrid cell-injected mice that were responsive to ASL-1 cells. Spleen cells from mice injected previously with hybrid cells were cocultured with ASL-1 cells, hybrid cells or BW5147 cells, an antigenically distinct leukemia cell line. As indicated (Table 1), cells from hybrid cell-injected mice reacted positively to hybrid or ASL-1 cells but not to BW5147 cells. These results indicate that hybrid and ASL-1 cells share common antigenic determinants, confirming in mixed lymphocyte culture data obtained previously [16] by immunofluorescence microscopy.

ASL-1 cells express a leukemia-associated antigen [24, 25] which is immunologically cross-reactive (immunofluorescence) with an analogous antigen expressed by ASL-1×LM(TK-) hybrid cells as well. In two-dimensional gel electrophoresis, the leukemia-associated antigen of hybrid and ASL-1 cells does not indicate a charge heterogeneity and is likely to be structurally identical (unpublished data). To determine if prior exposure of target cells to antiserum toward this antigen affects the reaction, mitomycin-C-

Table 1. Spleen cells from mice injected with hybrid cells respond to leukemia cells

Group	Secondary MLR Responder cells*	Stimulator cells†	Responder:stimulator ratio	[^{3}H]Thymidine incorporation (mean cpm ± S.D.)	Stimulation index
1	Spleen cells from F_1 mice injected previously with hybrid cells§	hybrid cells	30:1	5808 ± 219	14.89
		BW 5147 cells	30:1	166 ± 50	0.43
		F_1 spleen‡	30:1	390 ± 164	
		hybrid cells	100:1	2925 ± 564	12.82
		F_1 spleen‡	100:1	288 ± 130	
2	Spleen cells from F_1 mice injected previously with hybrid cells§	ASL-1 (*in vitro*)‖	30:1	1194 ± 89	3.06
		F_1 spleen‡	30:1	390 ± 164	
		ASL-1 (*in vitro*)§	100:1	856 ± 66	3.75
		F_1 spleen§	100:1	228 ± 130	
3	Spleen cells from F_1 mice injected previously with hybrid cells	hybrid cells +5% anti-leukemia-associated antigens	10:1	268 ± 126	0.54
		hybrid cells +5% NMS	10:1	2281 ± 649	4.61
		F_1 spleen‡	10:1	495 ± 228	

Responder and stimulator cells were incubated in 200 μl of RPMI 1640 medium containing 20 mM HEPES, 5×10^5 M 2-mercaptoethanol, 5% FCS and antibiotics. After 48 hr at 37°C in a 7% CO_2: air mixture, 1 μCi of [^{3}H]thymidine was added to the reaction mixture and the incubation was continued for an additional 16 hr before harvesting.

$$\text{Stimulation index} = \frac{\text{cpm incorporated by responder cells in the presence of stimulator cells}}{\text{cpm incorporated by responder cells in the presence of self stimulation}}$$

*2.5×10^5 responder cells per well used.

†Stimulator cells (2×10^6 cells/ml) were incubated in RPMI 1640 medium containing 5% FCS and mitomycin-C (50 μg/ml) for 45 min at 37°C in a 7% CO_2: air humidified atmosphere. After incubation, the cells were washed three times with RPMI 1640 medium supplemented with 5% FCS before they were added to the reaction mixture in the numbers indicated.

‡Self stimulation: spleen cells treated with mitomycin-C (50 μg/ml) from F_1 mice injected i.p. 10 days previously with 1×10^7 hybrid cells, as described.

§(A/J×C3H/HeJ)F_1 mice were injected with 1×10^7 hybrid cells 10 days previously.

‖ASL-1 cells adapted to *in vitro* growth treated with mitomycin-C before addition to the reaction mixture, as described. 1×10^7 hybrid cells were incubated with 100 μl of undiluted leukemia-associated antiserum** or NMS for 2 hr before treatment with 40 μg/ml mitomycin-C for 45 min at 37°C and addition to the culture wells.

**Raised in A/J mice injected i.p. with approximately 5×10^6 mitomycin-C-treated ASL-1 cells weekly for 12 weeks. Antiserum titer, as determined by immunofluorescence staining of ASL-1 cells, was 1:64.

The data presented are the mean ± S.D. of triplicate cultures.

treated hybrid cells were incubated for 2 hr in medium containing a 1:20 dilution of antiserum before they were added to the reaction mixture. The results (Table 1, group 3) indicate that hybrid cells after antiserum treatment fail to stimulate, i.e. the leukemia-associated antigen is among the 'targets' of the reaction. Thus mice rejecting leukemia × fibroblast hybrid cells develop immunity which is directed at least in part toward a cross-reactive leukemia-associated antigen which has been 'inherited' by the hybrid cells.

The injection of at least 10^6 hybrid cells is required to successfully treat leukemic mice

F_1 mice receiving ASL-1 cells along with hybrid cells survive for prolonged periods if at least 10^6 hybrid cells are used for treatment, without chemotherapy.

In the experiment, F_1 mice were injected i.p. with 2×10^3 ASL-1 cells and at the same time with varying numbers of hybrid cells, ranging from 10^2 to 10^7. The results (Fig 1) indicate that mice receiving 1×10^6 hybrid cells or more survived

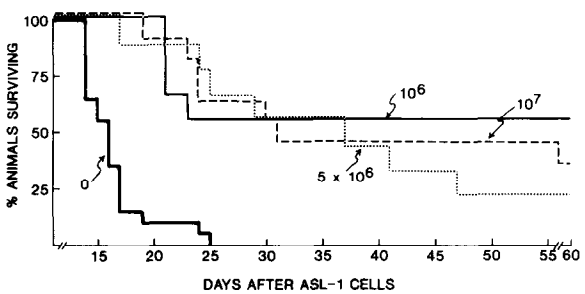

Fig. 1. (A/JxC3H/HeJ)F_1 mice received an injection of 2×10^3 ASL-1 cells from the spleens of F_1 mice terminally ill with leukemia. Within 1 hr they received a second injection of varying numbers of ASL-1×LM(TK-) hybrid cells, as indicated. None of the animals receiving less than 1×10^6 hybrid cells survived longer ($P < 0.01$) than animals in the control group receiving ASL-1 cells alone.

significantly longer than animals in the control group receiving ASL-1 cells alone. Approximately one-half of the animals treated in this fashion survived indefinitely and apparently rejected the neoplastic cells. Animals receiving lesser numbers of hybrid cells died at about the same time as animals in the control group injected with ASL-1 cells alone. The median survival of animals receiving simultaneous injections of less than 1×10^6 hybrid cells and 2×10^3 ASL-1 cells did not exceed 21 days.

Survival of F_1 mice receiving leukemia cells, followed by low-dose chemotherapy and then hybrid cells

In prior experiments [7] mice with well established tumours failed to survive for prolonged periods if injected solely with hybrid cells. Conceivably, treatment with chemotherapy before injecting hybrid cells to reduce the total number of neoplastic cells present might lead to enhanced results. In the experiments a dose of combination chemotherapy was chosen that was insufficient by itself to cure leukemic mice but was capable of prolonging life. Low-dose chemotherapy was added as an experimental 'variable'.

For the first therapeutic regimen F_1 mice were injected i.p. with 2×10^2 or 2×10^4 ASL-1 cells, and 24 hr later they received a single i.p. injection of combination chemotherapy (300 mg/kg cytosine arabinoside, 3 mg/kg methotrexate) followed in 4 days by a single i.p. injection of 5×10^6 hybrid cells. Animals in the control groups received the same number of ASL-1 cells and the same dose of combination chemotherapy but did not receive hybrid cells (group 2), or were injected only with ASL-1 cells (group 3).

The results of two separate experiments (Table 2) indicate that animals in the groups receiving leukemic cells followed by chemotherapy and

Table 2. Survival of leukemic mice receiving low-dose chemotherapy and hybrid cells

Experiment	Group	No. of ASL-1 cells injected	No. of mice	Chemotherapy	Hybrid cells	Mean survival time (days)	Range (days)
1	1	1×10^2	10	+	+	32	20-53
	2	2×10^2	19	+	(-)	19	14-20
	3	2×10^2	10	(-)	(-)	15	14-16
2	1	2×10^4	8	+	+	29	21-31
	2	2×10^4	5	+	(-)	19	15-24
	3	2×10^4	10	(-)	(-)	9	9-10

(A/JxC3H/HeJ)F_1 mice in group 1 were injected i.p. with 2×10^2 or 2×10^4 ASL-1 cells from the spleens of F_1 mice terminally ill with leukemia. Twenty-four hours later they received a single i.p. injection of combination chemotherapy (300 mg/kg cytosine arabinoside, 3 mg/kg methotrexate) and then 4 days later an i.p. injection of 5×10^6 ASL-1×LM(TK-) hybrid cells. Animals in group 2 were treated in the same way except that they did not receive hybrid cells. Animals in group 3 received 2×10^2 or 2×10^4 ASL-1 cells alone. The statistical difference (P) between the median survival time of animals in group 1 and animals in either of the control groups 2 or 3 is <0.01.

hybrid cells survived longer ($P < 0.01$) than animals in either the group receiving ASL-1 cells and only combination chemotherapy or ASL-1 cells alone. The median period of survival of animals in group 1 receiving ASL-1 cells followed by chemotherapy and hybrid cells was 32 and 29 days for mice receiving 2×10^2 and 2×10^4 ASL-1 cells respectively. The median survival of leukemic animals in control group 2 receiving chemotherapy alone was 19 days; in group 3 it was 15 days (Table 2). In none of the animals was there evidence of a progressively growing tumour of hybrid cells.

Are the immunotherapeutic effects of hybrid cells persistent for prolonged periods? To investigate this question, surviving mice with leukemia who were treated successfully with hybrid cells were challenged a second time 6 months later. The animals were challenged by receiving an i.p. injection of 10^3 ASL-1 cells; their median survival exceeded 90 days (range, 20–>90) whereas normal animals in a previously untreated control group receiving only ASL-1 cells died in approximately 17 days (range, 15–18; Table 3).

Comparison of the immunoprotective effects of hybrid cells with mitomycin-C-treated ASL-1 cells

To compare the immunoprotective properties of ASL-1 × LM(TK-) hybrid cells with those of mitomycin-C-treated ASL-1 cells, (A/J × C_3H/He)F_1 mice were injected with 5×10^6 cells of either type 21, 14 and 7 days before receiving an i.p. injection of 2×10^2 viable ASL-1 cells.

Control animals receiving 2×10^2 ASL-1 cells alone died in 13 ± 1 days, mice receiving mitomycin-C-treated ASL-1 cells before challenge survived 18 ± 6 days and animals receiving equivalent numbers of hybrid cells before ASL-1 cells survived more than 35 days, free of disease.

In no instance were mixtures of two cell types, e.g. inactivated leukemia cells and allogeneic cells or inactivated leukemia cells and histocompatible fibroblast cells, equally effective in stimulating immunity to viable leukemia cells.

DISCUSSION

F_1 mice rejecting semiallogeneic somatic hybrids of murine leukemia cells develop partial immunity toward the parental leukemia cell line used in forming the hybrid [9, 26]. Rejection of the hybrid cells is directed in part toward foreign histocompatibility contributed to the hybrid by the allogeneic parental cells along with leukemia-associated antigens.

ASL-1×LM(TK-) hybrid cells have lost malignant growth properties in histocompatible recipients; they fail to proliferate in histocompatible mice. In no instance involving multiple injections of up to 10^7 hybrid cells in various protocols into several hundred (A/J×C3H/HeJ)F_1 mice has there been evidence of a progressively growing tumor of hybrid cells. This has been the case even if the recipients have received prior injections of leukemic cells. In some instances a small, 2- to 4-mm, palpable tumor has appeared at the injection site. Invariably, it has regressed spontaneously. Deliberate intramuscular or intravenous injections of the hybrid cells have not resulted in progressive tumor growth.

The precise explanation for the rejection of the hybrid cells by F_1 mice is unknown; it does not appear to be the result of a loss of the cells' neoplastic capacity. ASL-1×LM(TK-) hybrid cells do form progressively growing tumors in

Table 3. Survival of (A/J×C3H/HeJ)F_1 mice injected with ASL-1 cells* 6 months *after receiving ASL-1×LM(TK-) hybrid cells and ASL-1 cells*

Group	Treatment	Median survival	Treatment	Median survival
1	Hybrid cells and ASL-1 cells six months previously, followed by (20–>90)§ ASL-1 cells†	>90‡ (13)‖	ASL-1 cells alone* (15–18)§	17‡ (4)‖

*All mice received 1×10^3 ASL-1 cells from the spleens of A/J mice terminally ill with leukemia.
†All mice received 5×10^6 ASL-1×LM(TK-) hybrid cells.
‡Median survival, in days.
§Range of survival, in days.
‖No. of animals used.
(A/J×C3H/HeJ)F_1 mice received three injections of approximately 5×10^6 ASL-1×LM(TK-) hybrid cells at weekly intervals. One week after the last injection they received approximately 2×10^3 ASL-1 cells from the spleens of F_1 mice terminally ill with leukemia. For at least the next 6 months the mice received no further treatment and were maintained in our animal quarters. At the end of this period the animals were injected a second time with 1×10^3 ASL-1 cells.

congenitally athymic nu/nu (BALB/c) mice or in F_1 mice which are subjected to high doses (550 Gy) of X-irradiation before cell transfer. Furthermore, the hybrid cells are immortalized, i.e. they grow indefinitely in culture without the deliberate addition of soluble growth factors and do not exhibit contact inhibition.

The results of experiments reported in Table 1 indicate the immunologic cross-reactivity of hybrid and ASL-1 cells. They indicate as well that the 'target' of reactivity is as least in part a leukemia-associated antigen of ASL-1 cells which is inherited by the leukemia × fibroblast hybrid cells.

The heightened antigenicity of hybrid, *vis-à-vis* ASL-1 cells, is illustrated by the extent of spleen cell reactivity in a secondary mixed lymphocyte reaction. Spleen cell proliferation of hybrid cell-injected mice in the presence of hybrid cells invariably exceeded that occurring in the presence of ASL-1 cells. ASL-1 cells may stimulate immune suppression in short-term culture or the 'target' of immune-mediated attack may be less accessible in ASL-1 cells than it is in hybrid cells. As determined by quantitative immunofluorescence methods involving the flow cytofluorograph, the 'density' of the tumor-associated antigen of ASL-1 cells, associated with hybrid cells, exceeds that of ASL-1 cells by approximately two-fold.

The immunoprotective effects of ASL-1× LM(TK-) hybrid cells toward passively transferred ASL-1 leukemia in F_1 mice are readily apparent. Mice receiving a single injection of approximately 10^6 or more hybrid cells along with ASL-1 cells survive for prolonged and at times indefinite periods. A single injection of hybrid cells followed in 1 week by ASL-1 cells reveals protective effects. Quantitative aspects are important. Greater numbers of ASL-1 cells, or a delay in the administration of hybrid cells, allowing the tumor to increase in size, affect the results obtained. Mice terminally ill with leukemia show no response to hybrid cell immunotherapy, although animals with minimal tumor could be 'treated' successfully.

The use of chemotherapy along with hybrid cells to 'treat' animals bearing ASL-1 leukemia, closer to clinical situations, led to prolonged survival. Some leukemic mice receiving chemotherapeutic agents to reduce the tumor 'load', followed by hybrid cells, survived for prolonged periods, pointing to the possible use of hybrid cell immunotherapy in the treatment of human disease. This is most appropriate if group-specific tumor-associated antigens are present or if hybrid cells are prepared from malignant cells obtained from the patient chosen for immunotherapy. The presence of active immune suppression or antigenic modulation, among other documented tumor 'escape' devices, surely will affect the potential success of hybrid cells as immunotherapeutic agents.

REFERENCES

1. Harris H, Klein G. Malignancy of somatic cell hybrids. *Nature* 1969, **224,** 1314-1316.
2. Harris H, Miller OJ, Klein G, Worst P, Taschibana T. Suppression of malignancy by cell fusion. *Nature* 1969, **223,** 363-368.
3. Jami J, Ritz E. Nonmalignancy of hybrids derived from two mouse malignant cells. II. Analysis of malignancy of LM(TK-)×CI-ID parental cells. *JNCI* 1975, **54,** 117-121.
4. Jami J, Ritz E. Tumor-associated transplantation antigens in immune rejection of mouse malignant cell hybrids. *Proc Natl Acad Sci USA* 1975, **72,** 2130-2134.
5. Jami J, Ritz E. Nonmalignancy of hybrids derived from two mouse malignant cells. I. Hybrids between L1210 leukemia cells and malignant L cells. *JNCI* 1973, **51,** 1647-1651.
6. Liang W, Cohen EP. Resistance to murine leukemia in mice rejecting syngeneic somatic hybrid cells. *J Immunol* 1976, **116,** 623-626.
7. Liang W, Cohen EP. Resistance to murine leukemia in mice receiving simultaneous injections of syngeneic hybrid and parental neoplastic cells. *J Immunol* 1977, **118,** 903-907.
8. Parkman R. Tumor hybrid cells—an immunotherapeutic agent. *JNCI* 1974, **52,** 1541-1545.
9. Taffaletti DL, Darow TL, Scott DW. Augmentation of syngeneic tumor-specific immunity by semiallogeneic cell hybrids. *J Immunol* 1983, **130,** 2982
10. Wiener F, Klein G, Harris H. The analysis of malignancy of cell fusion. IV. Hybrids between tumor cells and a malignant L cell derivative. *J Cell Sci* 1973, **12,** 253-261.
11. Wiener F, Klein G, Harris H. The analysis of malignancy by cell fusion, III. Hybrids between diploid fibroblasts and other tumor cells. *J Cell Sci* 1971, **8,** 681-692.
12. Bortin MM, Rimm AA, Saltzstein EC. Grafts vs leukemia: quantification of adoptive immunotherapy in murine leukemia. *Science* 1973, **179,** 811-813.

13. Boyer PJ, Fahey JL. Stimulation of lymphoid cells from normal and immune mice by syngeneic BALB/c plasma cell tumors. *J Immunol* 1976, **116,** 202-209.
14. Hayry P, Defendi V. Mixed lymphocyte cultures produce effector cells: a model *in vitro* for allograft rejection. *Science* 1970, **168,** 133-135.
15. Kim BS, Liang W, Cohen EP. Tumor specific immunity induced by somatic hybrids. I. Lack of relationship between immunogenicity and tumorgenicity of selected hybrids. *J Immunol* 1979, **123,** 733-738.
16. Liang W, Cohen EP. Activation of specific cellular immunity toward murine leukemia in mice rejecting syngeneic somatic hybrid cells. *J Immunol* 1977, **119,** 1954-1961.
17. Obta Y, Stockert E, O'Donnell PV, Okubo S, Synder HW, Old LJ. ^{G}(RADA-1): a new cell surface antigen of mouse leukemia defined by naturally occurring antibody and its relationship to murine leukemia virus. *J Exp Med* 1978, **147,** 1089-1105.
18. Old LJ, Stockert E. Immunogenetics of cell surface antigens of mouse leukemia. *Annu Rev Genet* 1971, **17,** 127-131.
19. Old LJ, Boyse EA, Stockert E. Antigenic properties of experimental leukemias. I. Serological studies *in vitro* with spontaneous and radiation-induced leukemias. *JNCI* 1963, **31,** 977-986.
20. Liang W, Cohen EP. Somatic hybrid of thymus leukemia (+) and (-) cells forms thymus leukemia antigens but fails to undergo modulation. *Proc Natl Acad Sci USA* 1975, **72,** 1873-1876.
21. Avery TL, Roberts D. Dose-related synergism of cytosine arabinoside and methotrexate against murine leukemia L1210. *Eur J Cancer* 1974, **10,** 425-429.
22. Henderson ES, Samaha RJ. Evidence that drugs in multiple combinations have materially advanced the treatment of human malignancies. *Cancer Res* 1969, **29,** 2272.
23. Tyrer D, Kline I, Venditti JM, Goldin A. Effectiveness of combination therapy with cytosine arabinoside (Ara-C) and MTX-resistance leukemia L1210 cells. *Proc AACR* 1967, **8,** 68-70.
24. Yu AC, Cohen EP. Studies on the effect of specific antisera on the metabolism of cellular antigens. II. The metabolism of TL antigens during antigenic modulation. *J Immunol* 1974, **112,** 1296-1301.
25. Yu AC, Cohen EP. Studies on the effect of specific antisera on the metabolism of cellular antigens. I. The isolation of thymus-leukemia antigens. *J Immunol* 1974, **112,** 1285-1289.
26. Klein G, Friberg S, Wiener G, Harris H. Hybrid cells derived from fusion of TA3-Ha ascites carcinoma with normal fibroblasts. I. Malignancy, karyotype and formation of isoantigenic varients. *JNCI* 1973, **50,** 125-130.