Europ. J. Cancer, Vol. 16, pp. 687–694

0014-2964/80/0501-0687 $02.00/0

Tumor Immunity Against MOPC 104E: Leukocyte Adherence Inhibition (L.A.I.) Assay*

R. A. LUBET† and D. E. CARLSON‡
Department of Radiology, U.T.H.S.C.D., 5323 Harry Hines Blvd, Dallas, Texas 75235, U.S.A.

Abstract—*Cells taken from the spleens of tumor-cured (immunized), tumor-bearing or untreated control mice were employed in a test tube Leukocyte Adherence Inhibition (L.A.I.) assay. Immunized animals showed a strong L.A.I. reactivity to MOPC 104E tumor cell antigens but not to those from an unrelated lymphoma. The assay was quite sensitive to even limited tumor growth, and a positive L.A.I. index was observed at least 6 days prior to the appearance of a palpable tumor. The L.A.I. index decreased substantially, however, in animals bearing large tumors. When the L.A.I. assay was performed in conjunction with the Winn tumor neutralization assay to determine the kinetics of development of tumor immunity in spleens of tumor-bearing mice, relatively little agreement was observed between the two assays. In contrast, when spleen cells from groups of animals with varying levels of in vivo immunity were compared, L.A.I. indices showed a substantial correlation with the magnitude of in vivo immunity.*

INTRODUCTION

THE MURINE plasmacytomas are particularly appealing models for studying tumor immunity. Significant *in vivo* tumor immunity can be induced by a variety of methods including immunization with tumor-specific paraprotein [1], repeated immunizations with X-irradiated or modified tumor cells [2, 3], and cure of palpable tumors by chemotherapy [4, 5]. We have recently shown with the MOPC 104E plasmacytoma that, by altering either the mode of curative therapy [6] or the size of the tumor at the time of therapy [7], we can generate tumor-cured animals with substantially differing amounts of tumor immunity.

Because immunity can be induced against a number of defined plasmacytoma antigens including specific paraprotein [1], viral antigens [8, 9], PC 1 alloantigen [10], and less well-defined tumor-associated transplantation antigens [11], the inducibility of tumor immunity against these tumors has been widely studied. A primary measure of immunity has been the recognition and quantitation of a humoral response against these tumors [1, 10, 11]. In addition, assays for cell-mediated tumor immunity have been reported, including a modified M.L.C. based on tumor recognition by immunized spleen cells [12], macrophage migration inhibition [3, 13] and microcytotoxicity [3]. The latter two assays suggest the presence of immunoreactive cells in both immunized and tumor-bearing animals.

The Leukocyte Adherence Inhibition (L.A.I.) assay, initially described by Halliday and Miller [14], is based on the decreased adherence of antigen-sensitized lymphoid cells to a glass or plastic surface in the presence of the sensitizing antigen. This assay, recently used to measure immunity in both man [15, 16] and mouse [17], appears to have some predictive value as a measure of cell-mediated immunity. Because the murine plasmacytomas appeared to be a good model for studying the development of tumor immunity as well as for inducing varying degrees of *in vivo* immunity, the predictive efficacy of this assay was evaluated for the MOPC 104E tumor model.

MATERIALS AND METHODS

MOPC 104E tumor

The plasmacytoma employed is the IgM-secreting MOPC 104E, grown and passaged s.c. in syngeneic BALB/c female mice (Laboratory Supply Co., Indianapolis, IN).

Accepted 24 October 1979.
*Supported by NIH Grants 13371, 17065, 05136 and 09082 from the National Cancer Institute.
†Present address: National Institutes of Health, Bldg. 10, Rm 8B-07, Bethesda, Maryland 20014, U.S.A.
‡To whom correspondence should be sent.

P 1798, a T-cell lymphoma syngeneic to BALB/c mice, was kindly given to us by Dr. M. Prager (U.T.H.S.C.D.).

Preparation of a 3M KCl *extract*

Tumor or spleen cells were minced and suspended at concentrations of 3.0×10^7 cells /ml. The cell suspension was centrifuged at 500 ***g*** for 5 min. The cell pellet was resuspended at a final concentration of 3×10^7 cells/ml in phosphate buffered saline (PBS) plus 3M KCl. The tumor cell or spleen cell suspension in PBS–KCl was stirred for 16 hr at 4°C. The resulting suspension was centrifuged in a Ti 60 rotor (Beckman Inst.) at 45,000 rev/min (R avg. 165,000 ***g***) for 60 min. The pellet was discarded, and the supernatant from 165,000 ***g*** spin was dialyzed for 12 hr against PBS at a 200:1 (v/v) ratio. The supernatant was removed and stored as individual aliquots at −20°C until use in the L.A.I. assay. Protein content of the resulting extracts was determined by the Lowry assay [18]. The concentration of 3M KCl extracts employed in the L.A.I. assay was 240 μg/tube. This concentration yielded the highest L.A.I. indices when concentrations from 30 to 480 μg/tube were evaluated [19].

Generation of immune animals

Animals were injected s.c. with 1.25×10^5 MOPC 104E tumor cells. Fourteen days later these animals were treated with 10 mg/kg cyclophosphamide. This therapy resulted in tumor regression in 100% of the animals bearing palpable tumors [6]. Thirty to 45 days after cyclophosphamide injection, cured animals were rechallenged with 60,000–100,000 tumor cells (30–50 times the TD_{50} of control animals). Those animals resisting secondary challenge were designated "tumor immune" and were employed as positive control animals in the L.A.I. tests.

Leukocyte adherence inhibition assay

While performance of the L.A.I. test as described initially [20] requires considerable technical expertise, the simplified method of Holan *et al.* [21] was used in these studies. Spleen cells from groups of at least three normal, tumor-cured or tumor bearing animals were obtained by mincing spleen cells in DMEM. Erythrocytes were lysed by incubation of cells in 0.83% NH_4Cl. The spleen cells were then pelleted, counted and resuspended at 10^7 cells/ml in DMEM + 15 mM Hepes buffer + 7% fetal bovine serum (FBS) (Microbiological Associates, Bethesda MD). One tenth ml of a given spleen cell suspension (10^6 cells) was placed in a 20 ml test tube. Simultaneously, 3M KCl extracts of spleen cells, MOPC 104E plasmacytoma cells or P 1798 lymphoma cells were diluted to a final concentration of 2.4 mg/ml. In individual tubes were combined 0.1 ml of spleen cells, 0.1 ml of the diluted 3M KCl extract, and 0.3 ml of DMEM with Hepes and FBS, to a final volume of 0.5 ml. Tubes were gently agitated and incubated horizontally at 30°C for 2 hr. Following incubation, 0.5 ml of PBS was added and each tube was inverted gently 3–6 times to suspend loosely adherent cells. Cells in suspension (non-adherent cells) were counted in a hemacytometer. Numbers of non-adherent cells from samples incubated with tumor extract were compared with those incubated with control extract. The L.A.I. index is the ratio of the number of non-adherent cells, tumor extract incubation/number of non-adherent cells, control extract incubation. Statistical comparisons of varying indices were determined employing the Wilcoxon ranking method [22]. The specificity of the 3M KCl extracts was unknown to the individual reading the hemacytometer.

Winn tumor neutralization assay

Spleen cell suspensions from groups of three immune, control or tumor-bearing animals were prepared. Cell viability was determined by trypan blue dye exclusion. Viable spleen cells at concentrations of 22, 66 or 200 times that of the tumor cells were incubated together with tumor cells for 30 min at 37°C. Aliquots containing 6×10^4 tumor cells and 1.3×10^6, 4×10^6, or 1.2×10^7 spleen cells were injected s.c. into normal animals. Recipient animals were then examined daily for growth of palpable tumors.

RESULTS

Specificity of the L.A.I. assay

Extracts from two different sources were employed in testing specificity of the immune reaction to MOPC 104E. One, a 3M KCl extract of normal spleen cells, was employed as a baseline because it contained B-cell antigens which might cross react with non-tumor-specific antigens of the B-cell-derived plasmacytoma. An extract of P 1798 lymphoma was employed to determine if either plasmacytoma-immune or control spleen cells reacted to tumor cell extracts in a nonspecific

manner. As shown in Table 1, a substantial increase was observed in the numbers of non-adherent plasmacytoma-immune spleen cells exposed to plasmacytoma extract, while no increase occurred upon exposure to extract from P 1798 lymphoma. These experiments established that the plasmacytoma-immune spleen cells reacted to plasmacytoma antigens but not to the unrelated P 1798 lymphoma. Employing non-immune spleen cells, a reproducible increase in the L.A.I. index was found upon exposure to plasmacytoma antigens. In contrast, a minimal increase in the L.A.I. indices of non-immune spleen cells was found upon exposure to extracts of P 1798 lymphoma cells.

Immunoreactivity of immune and normal spleen cells in the L.A.I. assay

In Figure 1 are shown L.A.I. indices of individual groups of MOPC 104E immune animals, tested for reactivity to 3M KCl extracts of MOPC 104E. Such animals regularly showed a substantial increase in the L.A.I. test (1.30–1.66). The L.A.I. indices from immune animals were higher than those of the control groups ($P<0.01$). Preliminary experiments showed that nonsensitized spleen cells also showed an increase in numbers of non-adherent cells in the presence of a plasmacytoma extract, as compared with the numbers of non-adherent cells in the presence of control extracts. The data in Fig. 1 show that exposure of non-immune spleen cells to a plasmacytoma extract regularly increased numbers of non-adherent cells, implying that there may be some natural reactivity to antigens on plasmacytomas in spleen cells from unimmunized animals. The MOPC 104E extracts employed caused no increase in cellular cytotoxicity over a 4-hr period as determined by trypan blue dye exclusion [19]. Non-adherent cell numbers ranged from 620,000 to 880,000 per 10^6 plated cells for spleen cells from immune animals incubated with MOPC 104E extract, and from 420,000 to 600,000 for similar cells incubated with control spleen extract. These experiments used at least four preparations of MOPC 104E and control spleen extracts, all with similar results.

The relationship of the L.A.I. to various in vivo *states of tumor immunity*

Since groups of animals with significantly different levels of tumor immunity could be

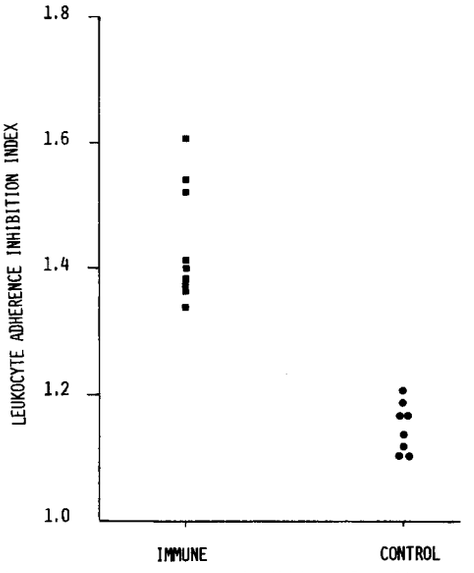

Fig. 1. Leukocyte Adherence Inhibition (L.A.I.) indices of MOPC 104E immune or control spleen cells. Spleen cells from MOPC 104E immune or control animals were employed in the L.A.I. assay. Each determination (square or circle) represents the average of triplicate determinations. Every determination employed pooled spleen cells from three similarly treated animals. Tumor immune animals were animals which had been cured of plasmacytoma with 10 mg/kg *cyclophosphamide and which had subsequently rejected secondary challenge with live tumor cells. Control animals had not been previously exposed to the MOPC 104E tumor. Tumor immune animals showed a significant increase ($P<0.01$) in the L.A.I. index relative to that of non-immune animals.*

Table 1. Reactivity of immune or control spleen cells toward MOPC 104E or P 1798 cell extracts

Source of spleen cells	Percentage of nonadherent cells in the presence of 3M KCl extracts from: (I) Spleen cells	(II) P 1798 lymphoma	(III) MOPC 104E	L.A.I.-P* (II/I)	L.A.I.-M† (III/I)
(A) Control‡	43.6±2.6	46.3±2.6	51.6±1.0	1.06	1.17
Immune§	39.0±2.2	42.7±1.4	53.6±1.2	1.08	1.37
(B) Control	51.5±2.0	54.0±2.3	56.7±1.05	1.05	1.10
Immune	46.3±3.0	44.6±1.1	61.0±2.8	0.96	1.34

*L.A.I. specificity toward P 1798 lymphoma cells relative to control spleen cell extracts.
†L.A.I. specificity toward MOPC 104E extracts relative to control spleen cell extracts.
‡Spleen cells derived from animals unexposed to MOPC 104E tumor.
§Spleen cells derived from animals who had been cured of a MOPC 104E tumor and had rejected a second tumor graft 30–55 days previously.

obtained [6, 7], it was possible to investigate the relationship between L.A.I. reactivity and *in vivo* immunity. Results from groups of animals with varying degrees of tumor immunity are shown in Table 2. Each value represents response from a spleen cell pool of at least three experimental mice.

Group I, a control group, showed an L.A.I. index of 1.10, consistent with the low but reproducible reactivity typically exhibited by non-immune spleen cells in this assay (Fig. 1). Animals of experimental group II had been cured of a palpable tumor with 10 mg/kg cyclophosphamide and had rejected a secondary challenge with 25,000 viable tumor cells. Since animals cured of a palpable tumor with low-dose cyclophosphamide show substantial immunity [6], a substantial L.A.I. index was expected and obtained. Animals in group III (Table 2) were exposed to 450 rad whole-body irradiation, injected with viable tumor cells, and treated with cyclophosphamide when tumors became palpable. Animals immunosuppressed both by whole-body irradiation and systemic cyclophosphamide might be expected to show relatively little tumor immunity; the L.A.I. index of 1.04 is consistent with this hypothesis. Animals in groups IV and V had been treated with 10 mg/kg cyclophosphamide. Group IV animals were injected with 1.25×10^5 viable tumor cells 4 days before cyclophosphamide therapy, while group V animals bore substantial tumors at the time of therapy. The L.A.I. index of mice cured of large tumors (group V) was substantial at 1.30, while mice from group IV, which maintained a much lower tumor burden at therapy, demonstrated a much lower L.A.I. index of 0.92. The explanation for the negative L.A.I. index in group IV is not obvious. Group VI animals had been cured of large tumors by cyclophosphamide (250 mg/kg) given 30 days before the L.A.I. assay. Such animals might be expected to have significant tumor immunity albeit less than low-dose-cured animals [6]. An L.A.I. index of 1.15 was observed, which was less than that found in the low-dose-cured animals of group V. Thus there appears to be some relationship between the L.A.I. index and *in vivo* tumor immunity.

Kinetics of development of tumor immunity

Two experiments were performed to relate the L.A.I. index to the duration of exposure to the tumor. The resulting data are presented in Fig. 2. In both experiments animals were injected with 1.25×10^5 tumor cells on day 0 and their spleens were removed on the indicated days following tumor inoculation. The circles on day 0 represent negative control responses, i.e., spleen cells were taken from animals which had no exposure to tumor. The squares on day 0 represent positive control responses, i.e., spleen cells from animals cured of palpable plasmacytoma by low-dose cyclophosphamide and which had rejected a secondary tumor challenge. The choice of cells as positive controls was based on earlier work in which such animals displayed a substantial degree of tumor immunity *in vivo* [6]. Immunologic reactivity as determined by an increase in the L.A.I. index rose sharply to day 6, followed by a decline (Fig. 2). At 4, 6 and 8 days following tumor injection, L.A.I. indices were significantly higher ($P<0.05$) than those of uninjected animals. Maximal L.A.I. indices for tumor-bearing animals were comparable to those of immune control animals. The sensitivity of this assay to even

Table 2. Leukocyte adherence inhibition in immune and non-immune mice

Group	Treatment	Percentage non-adherent cells (tumor) / Percentage non-adherent cells (control)	L.A.I.	2° Rejection*
I	Control	54±2 / 49±1.5	1.10	
II†	Low dose cured, 2° rejection	66±2 / 49±1.5	1.37	++++
III‡	WBI, cyclophosphamide cured	45.1±1 /43.5±1.5	1.04	N.T.
IV§	Small tumor low dose cured	43.5±1 /47.0±1.5	0.92	+
V§	Large tumor low dose cured	72.5±2.5/55.5±2.0	1.30	++++
VI§	Large tumor high dose cured	48.5±5 / 42±1.5	1.15	++

*Secondary rejection—this is a measure of an animal's ability to reject a live tumor cell challenge.

†Animals in group II had been cured of a palpable plasmacytoma with cyclophosphamide. Thirty days later these animals were rechallenged with a small number of tumor cells. Thirty days later these animals were employed in the L.A.I. test.

‡WBI = whole-body irradiation.

§Animals in groups IV–VI were cured of plasmacytomas 30 days prior to their use in the L.A.I. test.

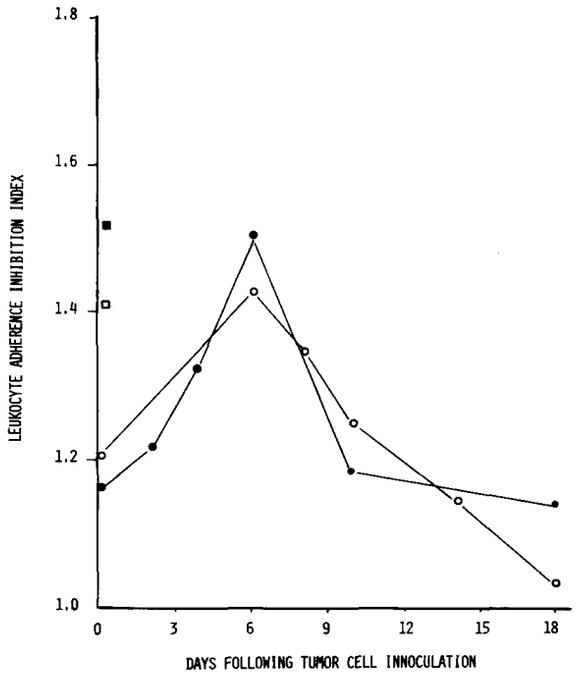

Fig. 2. The Leukocyte Adherence Inhibition (L.A.I.) index of spleen cells at various times following tumor cell inoculation. Spleen cells from mice who had been injected with 125,000 *viable tumor cells, at the times shown on the abscissa, were employed in the L.A.I. assay. The relative numbers of such cells that did not adhere in the presence of a* 3M KCl *extract of MOPC* 104*E relative to non-adherence in the presence of a control extract was determined to be the L.A.I. index. Open symbols, experiment* I*; closed symbols, experiment* II*; circles* (day 0)*, control spleen cells from mice not exposed to MOPC* 104*E tumor cells; square* (day 0)*, positive control spleen cells from animals who had been strongly immunized against MOPC* 104*E tumor cells.*

limited tumor exposure is emphasized by the positive reactivity of animals challenged 6 days previously. The substantial decrease in the L.A.I. index by day 10 is prior to the manifestation of a palpable tumor, which normally occurs between days 11 and 13. For animals bearing palpable tumors, the results in expt. I (closed circles) showed little difference when tested at either 10 or 18 days post-tumor inoculation. This finding, however, was not reproduced in expt. II or in two other experiments, [19] where L.A.I. indices of 1.02 and 1.10 were observed from groups of animals bearing large tumors. Thus, there appears to be a general decrease in the L.A.I. index after the tumor has reached a palpable stage.

To correlate the L.A.I. data with another more commonly employed assay for tumor immunity, the spleen cells used in expt. I (closed circles) for testing the L.A.I. index, were simultaneously used in the Winn assay. Both the average time of tumor appearance and the L.A.I. indices associated with each group are shown (Table 3). No differences were observed in the time of tumor appearance between incubation with control cells (group VI) and cells from mice bearing tumor for 2, 4 or 18 days (groups I, II and V). There was a slight but statistically insignificant increase in the time to tumor appearance in animals injected with 1.2×10^7 or 4.0×10^6 spleen cells from animals exposed to tumor for 6 or 11 days (groups III and IV). In no case, however, did spleen cells from tumor-bearing animals lead to substantial increases in the time of tumor appearance or a total abrogation of tumor growth. In the immune control group (group VII), ratios of spleen cells to tumor cells of as low as 22:1 yielded substantial delays in the time of tumor

Table 3. *Comparison of tumor immunity as assessed by the L.A.I. assay and the Winn neutralization test*

Groups	Days bearing tumor†	Average day of tumor appearance*			L.A.I. index
		200:1	66:1	22:1	
I	2	17.3±0.98	17.1±0.75	17.5±0.43	1.24
II	4	17.7±0.81	17.5±0.91	17.0±0.83	1.31
III	6	19.6±0.91	19.0±0.90	17.0±1.17	1.52
IV	11	19.5±0.50	19.3±0.57	18.7±0.56	1.20
V	18	17.1±0.27	18.0±0.61	17.9±0.59	1.17
VI	Control‡	17.5±0.81	17.6±1.0	16.4±0.91	1.17
VII	Immune control§	‖	26.1±1.2	22.7±1.1	1.54

*The values listed below are the average day of tumor appearance following injection of groups of 5 animals with 6×10^4 viable tumor cells, and 22:1, 66:1 or 200:1 excess spleen cells.

†Spleen cells were obtained from animals injected with 1.0×10^5 tumor cells on day 0.

‡Spleen cells from animals not exposed to MOPC 104E cells.

§Spleen cells from animals cured of a MOPC 104E tumor, and which had rejected a rechallenge with the same tumor.

‖For spleen cells from group VII animals at 200:1 ratios, tumors failed to grow out in 4 out of 5 animals; at 66:1 ratios, 1 of 5 animals failed to show tumor growth.

appearance, and 200:1 ratios totally abrogated tumor growth in four of five animals. The two assays thus yielded substantially different results. The L.A.I. results implied significant tumor recognition in spleens from animals bearing tumors for 6 days, yet no tumor inhibitory activity was seen with these cells in the Winn assay. Animals bearing tumors for 6 days and tumor-cured animals showed strikingly different degrees of immunity in the Winn assay yet showed similar L.A.I. indices.

DISCUSSION

We have used the murine plasmacytoma MOPC 104E to evaluate the L.A.I. assay under conditions of known immunoreactivity. Earlier we showed that autochthonous immune responses could abrogate substantial tumor burden following small doses of either X-rays or cyclophosphamide. Further, upon rechallenge, cured animals were shown to reject MOPC 104E cell inocula some 200 times that observed for previously untreated mice [6].

The question of specificity shown by immune spleen cells in the assay was addressed by comparing the activities of extracts from the original plasmacytoma with extract from the unrelated lymphoma P 1798. With P 1798 lymphoma extracts, the L.A.I. indices of 1.05 and 1.03 for plasmacytoma-immune and normal spleen cells suggest lack of cross reactivity. The indices are in contrast to results with MOPC 104E extracts, in which L.A.I. indices of 1.14 and 1.37 were seen for normal and plasmacytoma-immune spleen cells. Thus, it appears that the assay is specific for plasmacytoma-immune spleen cells.

Two lines of evidence suggest that normal spleen cells exhibit limited but reproducible reactivity to the plasmacytoma. Results from Fig. 1 show an L.A.I. index of 1.14 for normal cells. Further, when normal spleen cells were mixed with tumor cells (Table 2, control) and injected into control recipients, palpable tumor formation required 17 days, while tumor alone produced palpable tumors in 14.5 days [19]. Others have reported both cytotoxic serum [10] and *in vitro* cell-mediated immunity [12] against this and other plasmacytomas in control animals. The apparent specificity of normal cell immunoreactivity may involve naturally occurring cross-reactive differentiation antigens which are expressed on most plasma cells [10] or antigens from endogenous A or C type particles [9].

Experiments were performed to determine the development of tumor immunity in the spleens of tumor-bearing animals employing the L.A.I. assay. A peak L.A.I. index occurred 6 days following tumor cell inoculation followed by a decrease in reactivity. Since these tumors normally become palpable 12–13 days post-tumor implantation, a decline in the L.A.I. index occurs prior to palpable tumor formation. This early increase in immune reactivity followed by a later decline is characteristic of many *in vitro* models of tumor immunity [23]. A similar pattern of reactivity has recently been shown by Braun and Dray [24] when measuring immunity against MOPC 315 using an MMI assay. Previous work testing immunity, employing the L.A.I. microtest against either a s.c. melanoma [17] or MSV-induced tumors [25], also showed a rapid induction of reactivity followed by a decline with tumor growth. Since the L.A.I. assay is relatively new and any direct extrapolation of the results is problematic, it was compared to the more traditional Winn tumor neutralization assay. The somewhat unexpected results showed that, while the L.A.I. index of some tumor-bearing animals (i.e., group III, Table 3) approached that of the immune animals, spleen cells from the same cell pool were not nearly as effective in the Winn assay. This general lack of effectiveness of spleen cells from tumor-bearing animals in the Winn assay could be due to a variety of reasons including: (1) the lack of significant immunity in tumor-bearing animals; (2) the presence of cells in the spleens of tumor-bearing animals which suppress or inhibit the specific immunologic response against the tumor; or (3) the carryover of metastatic myeloma cells in the spleen during the Winn assay. It must be emphasized that the two assays are measuring different end-points, and therefore might not be expected to show concordance. While the L.A.I. assay presumably measures tumor antigen recognition by immune spleen cells, the Winn assay measures the ability of immune spleen cells to inhibit tumor growth *in vivo*.

Because variation in the amount of residual tumor immunity could readily be achieved in this tumor system, investigations relating the L.A.I. index to *in vivo* tumor immunity were undertaken (Table 2). Animals cured with a low dose of cyclophosphamide and showing strong *in vivo* immunity [6] had a high L.A.I. index. Similarly, animals cured of tumor with a high dose of cyclophosphamide and showing less *in vivo* immunity [6] also had a lower L.A.I. index. That the L.A.I. index of high-dose-cured animals is similar to that of con-

trol animals is surprising since such animals can reject more cells than control animals. One possible explanation for this difference is that although high-dose-cured animals showed reactivity *in vivo*, their spleens may include different relative numbers of T- and B-lymphocytes, monocytes and neutrophils than low-dose-cured or control animals. Thus, if the reactivity in the L.A.I. assay is dependent on the presence of certain cells, this reactivity may be altered in high-dose-cured animals.

Another method of inducing animals with significantly differing amounts of *in vivo* tumor immunity involves curing animals with tumors of differing sizes [7]. Animals cured of small tumors showed limited *in vivo* immunity and a low L.A.I. index. In contrast, animals cured of a large tumor by low-dose cyclophosphamide therapy exhibited relatively high *in vivo* tumor immunity and a high L.A.I. index. Similarly, animals bearing a large tumor and cured with a relatively high dose of cyclophosphamide showed intermediate levels of immunity both *in vivo* and in the L.A.I. assay. In general, the magnitude of the L.A.I. index does bear some resemblance to the magnitude of *in vivo* immunity. In summary, the L.A.I. assay appears to have some promise, although it requires further characterization to determine its exact biological significance.

REFERENCES

1. H. N. Eisen, N. Sakoto and S. J. Hall, Myeloma proteins as specific antigens. *Transplant Proc.* **7,** 209 (1975).
2. W. H. Williams and R. G. Krueger, Tumor associated transplantation antigens of myelomas induced in BALB/c mice. *J. nat. Cancer Inst.* **49,** 1613 (1972).
3. M. F. Poupon and G. Lespinats, Cell mediated immunity directed against a syngeneic plasma cell tumor in the mouse. Determination by macrophage migration inhibition test. *J. nat. Cancer Inst.* **48,** 1297 (1972).
4. J. L. McCoy, J. H. Dean, L. W. Law, J. Williams, N. T. McCoy and B. J. Holliman, Immunogenicity, antigenicity and mechanisms of tumor rejection in mineral oil induced plasmacytomas in syngeneic BALB/c mice. *Int. J. Cancer* **14,** 264 (1974).
5. M. Potter and J. L. Walters, Effect of intraperitoneal pristane on established tumor immunity to the Adj.PC-5 plasmacytoma. *J. nat. Cancer Inst.* **51,** 875 (1973).
6. R. A. Lubet and D. E. Carlson, Tumor immunity against MOPC 104E: effects of different therapeutic regimens. *Cancer Immunol. Immunother.* **2,** 267 (1977).
7. R. A. Lubet and D. E. Carlson, Tumor immunity against MOPC 104E: effects of tumor size and time post therapy on *in vivo* tumor immunity. *J. nat. Cancer Inst.* **60,** 1107 (1978).
8. R. G. Krueger, W. H. Williams and G. C. Miller, Cellular and viral antigens of BALB/c myelomas. *J. nat. Cancer Inst.* **52,** 1203 (1974).
9. E. L. Kuff, K. K. Leuders, H. L. Ozer and N. A. Wivel, Some structural and antigenic properties of intracisternal A particles occurring in mouse tumours. *Proc. nat. Acad. Sci. (Wash.)* **69,** 218 (1972).
10. R. B. Herberman and T. Aoki, Immune and natural antibodies to syngeneic plasma cell tumors. *J. exp. Med.* **136,** 94 (1972).
11. J. P. Kolb, M. F. Poupon and G. Lespinats, Tumor associated antigen (TAA) and anti-TAA antibodies in the serum of BALB/c mice with plasmacytomas. *J. nat. Cancer Inst.* **52,** 723 (1974).
12. P. J. Boyer and J. L. Fahey, Secondary *in vitro* lymphocyte proliferative responses to syngeneic plasma cell tumors. *Cancer Res.* **36,** 1492 (1976).
13. M. L. Padarathsingh, J. H. Dean, J. L. McCoy, D. D. Lewis, J. W. Northing, T. Natori and L. W. Law, Cell mediated immunity against particulate and solubilized tumor associated antigens of murine plasmacytomas detected by MMI assays. *Int. J. Cancer* **20,** 624 (1977).
14. W. J. Halliday and S. Miller, Leukocyte adherence inhibition: a simple test for cell mediated tumor immunity and serum blocking factors. *Int. J. Cancer*, **9,** 477 (1972).
15. W. J. Halliday, A. E. Maluish, J. H. Little and N. C. Davis, Leukocyte adherence inhibition and specific immunoreactivity in malignant melanoma. *Int. J. Cancer*, **16,** 645 (1975).

16. N. Grosser and D. M. P. Thompson, Cell mediated antitumor immunity in breast cancer patients evaluated by antigen induced leukocyte adherence inhibition in test tubes. *Cancer Res.* **35,** 2571 (1975).
17. P. G. Holt, J. Chcalmer and D. Keast, Development of two manifestations of T-lymphocyte reactivity during tumor growth: altered kinetics associated with elevated growth rates. *J. nat. Cancer Inst.* **55,** 1135 (1975).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265 (1951).
19. R. A. Lubet, *Therapy and Development of Tumor Immunity Against the Murine Plasmacytoma* MOPC 104E. Dissertation, The University of Texas Health Science Center at Dallas, Texas (1977).
20. W. J. Halliday, The leukocyte adherence inhibition assay. In In Vitro *Methods in Cell Mediated and Tumor Immunity.* (Edited by B. R. Bloom and J. R. David) p. 547, Academic Press, New York (1976).
21. V. Holan, M. Hasek, J. Bubenik and J. Chutna, Antigen mediated macrophage adherence inhibition. *Cell. Immunol.* **13,** 107 (1974).
22. J. R. Rohlf and R. R. Sokal, *Biometry.* Freeman, San Francisco (1969).
23. S. B. Howell, J. H. Dean, E. C. Esber and L. W. Law, Cell interactions in adaptive immune rejection of a syngeneic tumor. *Int. J. Cancer* **14,** 662 (1974).
24. D. P. Braun and S. Dray, Immune RNA mediated transfer of tumor antigen responsiveness to unresponsive peritoneal exudate cells from tumor-bearing mice. *Cancer Res.* **37,** 4138 (1977).
25. R. F. Mortensen, Detection of cellular immunity to murine sarcoma induced tumors by leukocyte adherence inhibition. *J. nat. Cancer Inst.* **62,** 157 (1979).