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Murine Leukemia Cell Hybrids: The Quantity of TL Antigens Expressed by Parental and Hybrid Cells Fails to Correlate With Their Sensitivity to TL Antibody and Complement

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The quantity of thymus-leukemia (TL) antigens expressed by murine leukemia cells is significantly greater than that expressed by somatic hybrids of such cells. Based upon the results of ^{125}I-lactoperoxidase labeling and antibody absorption procedures, and corrected for size differences between the two cell types, the quantity of TL antigens expressed by RADA-1 cells, a radiation-induced murine leukemia cell line of strain A/J mice, is approximately 5.0 times greater than that of somatic hybrids of RADA-1 and LM(TK)$^-$ cells. LM(TK)$^-$ cells are a thymidine kinase-deficient TL(−) mouse fibroblast cell line. The quantity of TL antigens expressed is related only in part to their susceptibility to lysis by TL antibodies and guinea pig complement (GPC). RADA-1 cells resist lysis. The quantity of TL antigens expressed by RADA-1 cells is analogous to that formed by nonneoplastic thymocytes obtained from F_1 hybrids of two strains of TL(+) and TL(−) mice; cells from both strains are sensitive to TL antiserum and GPC. ASL-1 cells, a spontaneously occurring leukemia cell line of A/J mice, express TL antigens in significantly higher quantities than any of the cell types examined. Exposed to TL antisera, the quantity of TL antigens of ASL-1 cells, but not that of hybrid cells, gradually diminishes. ASL-1 cells convert over a 6-h period of exposure to antibody and guinea pig complement (GPC) resistance; hybrid cells remain sensitive. However, ASL-1 cells converted to TL antibody and GPC resistance continue for a time to express TL antigens in quantities similar to that of sensitive F_1 thymocytes and resistant RADA-1 cells. RADA-1 × LM(TK)$^-$ hybrid cells, which are sensitive to TL antibodies and GPC, express the lowest quantities of TL antigens of any of the cell types examined. It is likely that differences in the quantities of TL antigens expressed by different cell lines reflect genetic mechanisms controlling TL antigen expression. The failure of TL antisera to affect the quantities of TL antigens expressed by hybrid cells is taken as an indication that genetic controls governing antigen expression may be distinguished from those involved in regulating responsiveness to specific antiserum.

Key words: thymus-leukemia antigens, murine leukemia cells, quantity of antigens expressed, susceptibility to TL antiserum, antigenic modulation, somatic hybrid cells

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Little is known of the regulatory controls which govern the formation, expression, and metabolism of membrane-associated proteins of murine leukemia cells. In prior studies, investigating three antigenically distinct membrane proteins of ASL-1 cells, a spontaneously occurring leukemia of strain A mice, we found [1] that the metabolic turnover of each protein was distinctly different from those of the other two. The antigens – H-2, thymus-leukemia (TL) and a tumor-associated protein characteristic of these neoplastic cells – are representative of three categories of membrane-associated proteins. H-2 is a genetic polymorphic complex of determinants widely distributed throughout tissues of the organism. TL is a differentiation antigen associated in the tumor-free animal with immature thymus cells. Mature thymus cells, capable of engaging in antibody "helper" and cytotoxic immune reactions, no longer form or express TL antigens [2]. The tumor antigen is a protein characteristic of neoplastic ASL-1 cells. It may be virally specified [3].

TL antigens reversibly disappear from the membranes of ASL-1 cells exposed to TL antibodies. Cells incubated further in medium without TL antibodies reexpress TL antigens (antigenic modulation); the process is analogous to down-regulation of hormone receptors. Not all antigens of ASL-1 cells undergo modulation. H-2 antigens and the tumor antigen are continually expressed even after prolonged incubation in medium containing specific antibodies.

In previous publications [4, 5], we reported that RADA-1 cells, a radiation-induced murine leukemia cell line of A/J mouse origin, formed TL 1, 2, 3 antigens but resisted the cytolytic effects of GPC and high titers of antisera specific for various determinants of the TL complex. Under the circumstances of the experiments performed, other lines of TL(+) cells invariably lysed.

RADA-1 cells are not nonspecifically resistant to antibodies and GPC; they lyse in the presence of antisera specific for other membrane-associated determinants, as $H\text{-}2^a$ or Thy 1.2. The cells do form TL antigens, as detected by immunofluorescence, by the cellular reduction by absorption of the titer of TL antiserum, and by the direct isolation of TL antigens from NP-40 cell extracts [4, 5]. Complement is consumed during the nonlytic reaction of RADA-1 cells, TL antiserum, and GPC [4].

A comparison of the relative quantities of TL antigens "exposed" to the external environment of various TL(+) cell types is presented. Their sensitivity or resistance to TL antiserum and GPC is correlated with the quantity of TL antigens present. The results indicate a) that ASL-1 cells, converted by prior exposure to TL antibody and complement (C) resistance, retain TL antigens on their surface membranes at a density equivalent to other lines of TL(+)-sensitive cells; b) that somatic hybrids of [TL(+)] RADA-1 × [TL(–)] LM $(TK)^-$ cells form TL antigens at significantly lower density than RADA-1 cells; and c) that RADA-1 × LM$(TK)^-$ cells expressing TL antigens at the lowest density of all the cell lines tested, are sensitive to TL antibodies and C.

MATERIALS AND METHODS

Cells

ASL-1 and RADA-1 murine leukemia cells are maintained by serial transfer in histocompatible recipients, as described previously [5, 6].

Somatic hybrids of RADA and LM$(TK)^-$ or ASL-1 and LM$(TK)^-$ cells, are selected in hypoxanthine, aminopterin, thymidine-containing medium [6, 7]. LM$(TK)^-$ cells are a thymidine kinase deficient mutant of mouse LM cells, a sustained fibroblast cell line.

Proof of cellular hybridization is dependent upon the sharing of H-2 antigens of parental sources and a hybrid karyotype. Clonal isolates are selected after two subcultures.

Antisera

TL 1,2,3 antiserum is raised in congenic TL(–) A/J mice injected weekly for 10 weeks with approximately 5×10^7 thymocytes from A/J mice (TL 1,2,3).

TL 1,3 antiserum is raised in (Balb/c × C_3H)F_1 mice injected according to a similar schedule with mitomycin-C-treated ASL-1 cells [5]. H-2^a antiserum is raised in C_3H/HeJ mice (H-2^k); H-2^k antiserum is raised in A/J mice (H-2^a). (H-2^a and H-2^k antigen complexes share some determinants.)

Assays for the sensitivity of murine cells to specific antisera. Sensitivity of the cells to specific antiserum is detected in microcytotoxicity tests involving incubations of the cells to be tested, antiserum in varying dilutions, and GPC as described previously [5–7]. As controls, the cultures are treated in the same way except that normal mouse serum or antiserum with specificity toward other membrane-associated determinants is substituted. Cell lysis is detected with the aid of trypan blue dye.

Solubilization and immunoprecipitation of radioactively labeled TL antigens of murine leukemia cells. Membrane-associated proteins, exposed to the external milieu, are labeled in the presence of lactoperoxidase with ^{125}I. The method of Marchalonis [8], and Phillips and Morrison [9] is followed as described previously [10]. In brief, approximately 6×10^6 cells to be iodinated are washed and resuspended in 1.0 ml phosphate-buffered saline (PBS), 250 μg lactoperoxidase (Calbiochem, La Jolla, California) and 0.5 mCi/ml carrier-free ^{125}I-sodium (New England Nuclear, Boston). H_2O_2 (40 μl) is added and the mixture is agitated at room temperature. The cells are washed with Hank's balanced salt solution before they are extracted with nonidet P-40 (NP-40; Shell Chemical).

Immunoprecipitation of isotopically labeled proteins from NP-40 cell extracts. The antigenicity of certain hydrophobic membrane-associated proteins is preserved in NP-40-containing solutions; they may be selectively immunoprecipitated with specific antisera. TL antigens of radioactively labeled cells may be recovered by this method [10, 11]. TL antigens are complexed with mouse TL antisera and precipitated with rabbit antimouse immunoglobulin. To remove all TL antigen-antibody complexes the immunoprecipitations are performed at immune equivalence, as determined by prior testing. Immune precipitates, consisting of radioactively labeled TL antigens, mouse TL antisera, and rabbit antimouse immunoglobulins, are washed and dissolved in tissue solubilizer (NCS, Amersham/Searle) and then counted in an Auto-gamma spectrometer (Amersham/Searle). "Background" radioactivity, as determined by substituting normal mouse serum (NMS) for TL antisera, is subtracted from each experimental sample to yield "specific" cpm. For most experiments, the background ranged between 5% and 10% of that obtained with specific antiserum.

Quantitative determinations of TL antigen expression. The relative capacity of various cell types to reduce by absorption TL antisera of known titers is used as an estimate of the quantity of antigens expressed. This method detects "exposed" antigens and does not reflect the presence of determinants inaccessible to the antiserum used for absorption. The technique of Cikes and Klein [12] is used, as described previously [7]. Preliminary antiserum titration is performed using C-mediated cytotoxicity of TL(+) thymus cells from A/J mice. The concentration of antiserum chosen for antigen quantitation is four times greater than the highest dilution leading to lysis of 50% of A/J thymocytes. After incubation with varying numbers of the cells to be tested, the antiserum is retitered

using freshly isolated thymocytes; changes in titer are noted. As controls, TL(−) cells are substituted for TL(+) cells.

RESULTS

Exposing RADA-1 Cells to TL Antibodies Leads to a Reduction in the Quantities of TL Antigens Expressed

Treatment of RADA-1 cells resistant to the cytolytic effects of TL antiserum and GPC with TL antiserum leads to a diminution in the quantity of TL antigens recovered subsequently. The cells are incubated for varying periods with TL 1,2,3 antiserum and then labeled with ^{125}I, followed by extraction with NP-40. TL antigens are recovered from the cell lysates by specific immunoprecipitation. Within 2 h of prior antiserum exposure, the quantity of radioactivity present in immunoprecipitates formed subsequently is reduced by approximately one-half (Fig. 1). Cells preincubated in medium in which NMS is substituted for TL antiserum continue to express TL antigens as detected by this method. Prolonged incubation of the cells in medium containing TL antiserum leads to a progressive reduction in the quantity of TL antigens recovered.

The Density of TL Antigens Formed by RADA-1 and RADA-1 × LM(TK)$^-$ Somatic Hybrid Cells

In prior experiments it was determined that stable somatic hybrids of ASL-1 cells, a leukemia cell line of A/J mice (TL 1,2,3) and LM(TK)$^-$ cells [TL(−)] formed TL antigens [13]. Hybrid cells, unlike ASL-1 cells failed to undergo antigenic modulation even under more stringent conditions, ie, higher antiserum concentrations and longer periods of incubation, than required to stimulate modulation of ASL-1 parental cells.

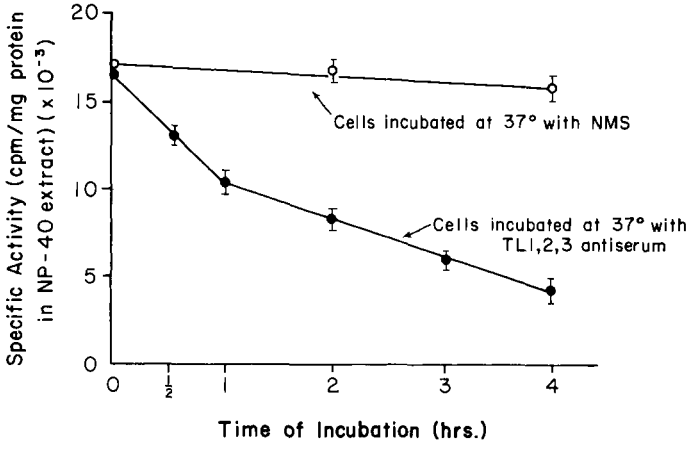

Fig. 1. Detection of membrane-"exposed" TL antigens of RADA-1 cells resistant to TL antibodies and GPC. The cells are incubated at 37°C in medium containing TL 1,2,3 antiserum, after which they are washed and then labeled with ^{125}I (lactoperoxidase). TL antigens released from the cells during extraction with NP-40 are recovered by immunoprecipitation with mouse TL 1,2,3 antiserum and rabbit antimouse Ig.

To determine the effect of cell fusion on the density of TL antigens formed by hybrid cells and their susceptibility to TL antiserum and GPC, somatic hybrids of RADA-1 and LM(TK)$^-$ cells were prepared [5]. Like ASL-1 X LM(TK)$^-$ hybrids, RADA-1 X LM(TK)$^-$ cells formed TL antigens; however, the density of TL antigens present was significantly less than that of RADA-1 cells. Based upon the relative absorptive capacity of the cells for TL 1,2,3 antiserum of known titer, the quantity of TL antigens present on the membranes of hybrid cells was about one-half that of RADA-1 cells (Fig. 2). Approximately 6×10^6 RADA-1 cells were required to reduce a known titer of TL antiserum by one-half; about 12×10^6 hybrid cells were required to reduce the antiserum titer to the same extent. The numbers of TL(+) thymocytes of A/J [TL(+)] X C3H/HeJ [TL(−)] F_1 mice to reduce the antiserum by 50% was equivalent to that of ASL-1 cells, higher than that of hybrid cells.

Differences in the absorptive capacity of hybrid and parental cells for TL antiserum are not corrected for differences in the sizes of the two cell types. The mean diameter of RADA-1 cells [as determined with an electronic sizing device (Coulter Electronics, Hialeah, Florida)] is approximately 9.5 mμ; the mean diameter of RADA-1 X LM(TK)$^-$ hybrid cells is 15.2 mμ. Corrected for differences in the sizes of hybrid and parental cells, the relative density of TL antigens of hybrid cells is approximately 20% of RADA-1 cells. Using similar criteria, thymocytes of (A/J X C3H/HeJ)F_1 animals form TL antigens at approximately the same cellular density as RADA-1 cells. It should be noted that these calculations do not take into account such important known variables as changes in antigen density with various phases of the cell cycle or the nonrandom distribution of specific membrane associated determinants over the surfaces of the cells. The local density of the antigens may vary considerably.

Complement Sensitivity of Somatic Hybrids of RADA-1 X LM(TK)$^-$ Somatic Hybrid Cells

RADA-1 X LM(TK)$^-$ hybrid cells, unlike RADA-1 cells used as one parent in forming the hybrid, lyse in the presence of GPC and antisera specific for varying determinants

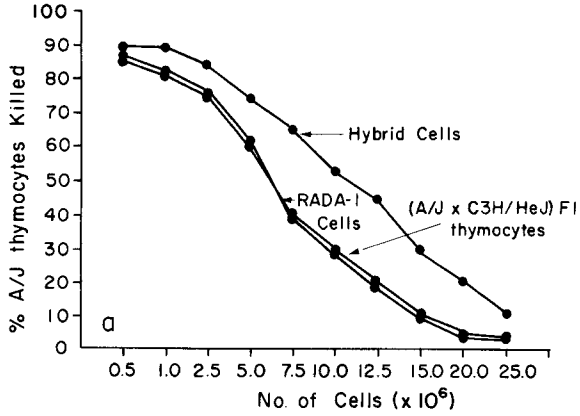

Fig. 2. Reduction in the cytolytic titer of TL 1,2,3 antiserum for TL(+) thymocytes from A/J mice by prior absorption with RADA-1 or RADA-1 X LM(TK)$^-$ hybrid cells. Appropriately diluted TL antiserum is incubated at 4°C for 30 min with varying numbers of cells. The residual antibody activity in the supernatant is determined by complement-mediated cytotoxicity toward thymocytes from A/J mice.

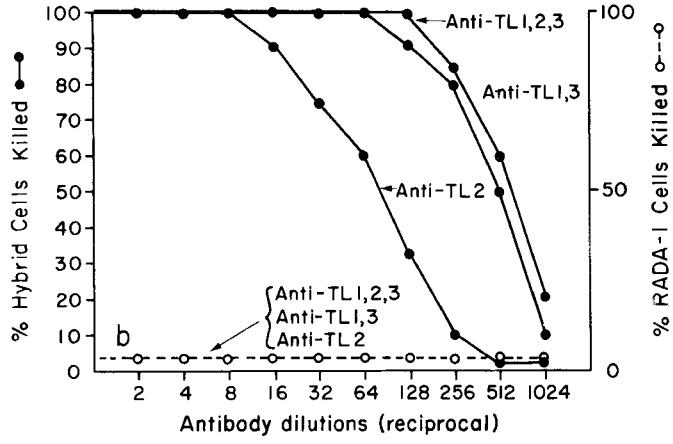

Fig. 3. RADA-1 × LM(TK)$^-$ hybrid cells, unlike RADA-1 cells used as one parent in forming the hybrid, lyse in the presence of TL antisera and GPC. RADA-1 or hybrid cells were incubated at 20°C for 15 min with varying dilutions of TL 2, TL 1,3, or TL 1,2,3 antiserum before the addition for 20 min at 37°C of active GPC. The proportion of cells killed by the procedure was determined under low power by magnification with the aid of trypan blue dye.

of the TL antigen complex. TL 1,3,TL 2 or TL 1,2,3 antisera along with active GPC leads to lysis of hybrid cells (Fig. 3). Under similar conditions, aliquots of the antiserum lyse thymocytes of A/J mice as well as ASL-1 cells.

It is conceivable that the sensitivity of RADA-1 × LM(TK)$^-$ hybrid cells to TL antiserum and GPC results from the appearance of an antigen whose synthesis is directed by the LM(TK)$^-$ genome. Even though the TL antiserum we used showed no cytotoxicity toward LM(TK)$^-$ cells, the antisera of mouse origin could contain isoantibodies specific for this putative determinant. To test this possibility, the TL antisera were absorbed with LM(TK)$^-$ cells before use by overnight incubation at 4°. Incubations of hybrid cells with absorbed antiserum along with GPC were performed subsequently. The results were equivalent to those obtained previously; hybrid cells failed to resist TL antiserum and GPC.

Eleven clonal isolates of RADA-1 × LM(TK)$^-$ cells were tested for their susceptibility to TL antiserum and GPC. All formed TL antigens and shared a hybrid karyotype, although the number of chromosomes present varied between 82 and 86. Nine of 11 clones were sensitive, two were resistant (Table I).

The Density of TL Antigens of ASL-1 Cells Exposed Previously to TL Antisera

ASL-1 cells lyse upon the addition of TL antisera and GPC. Exposed to TL antiserum, but without C, the cells undergo antigenic modulation and convert to antiserum and C resistance [13]. Conversion to TL antibody and C resistance correlates with a diminution of the density of TL antigens expressed by the cells.

Quantitative absorption procedures indicate (Fig. 4) that ASL-1 cells incubated for 6 h with TL 1,3 antiserum and converted to antibody and C resistance retain TL antigens on their surface membranes, as indicated by their capacity to reduce by absorption known titers of TL 1,2,3 antiserum. The quantity of TL antigens retained by the cells is approximately 25% of that present on aliquots of the cell suspension incubated for similar periods with NMS. After further exposure to TL 1,3 antiserum, the cells, like TL(–) cells, no longer affect the antiserum titer.

TABLE I. Summary of Studies of Different Clones of RADA-1 × LM(TK)$^-$ Hybrid Cells

RADA-1 × LM(TK)$^-$ clones	Chromosome No[a]	TL antigen expression	Antigenic modulation	Sensitivity to lysis by TL antiserum and GPC
1	86	+	–	+
2	84	+	–	+
3	85	+	–	–
4	85	+	–	+
5	86	+	–	–
8	85	+	–	+
10	84	+	–	+
11	83	+	–	+
13	82	+	–	+
14	87	+	–	+
15	82	+	–	+

[a]Based on analysis of at least 20 metaphase spreads.

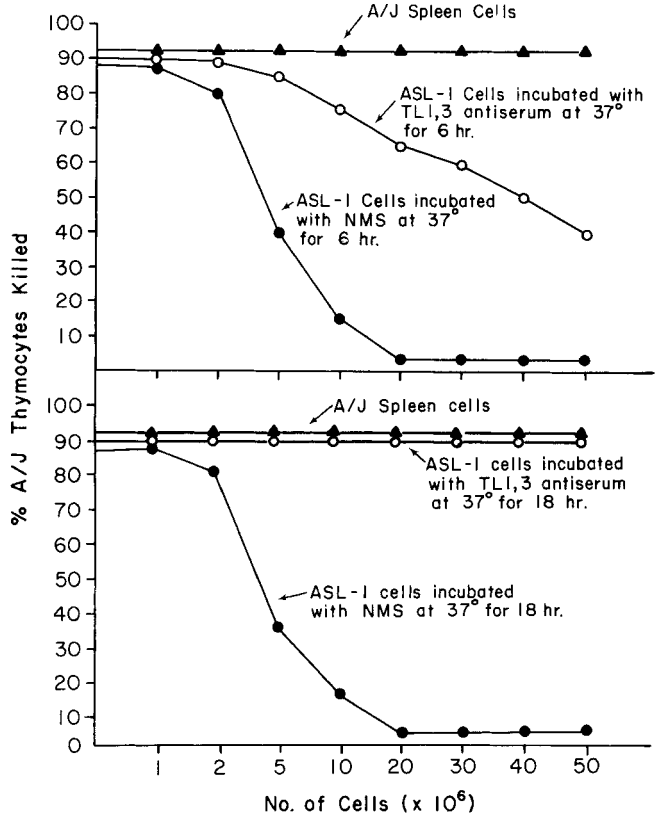

Fig. 4. Changes in the cytolytic titer of TL 1,2,3 antiserum after incubation with ASL-1 cells or TL(–) spleen cells from A/J mice.

Immunofluorescence tests provide further evidence that cells exposed to TL antiserum for periods that, while brief, are sufficient to convert to antibody and C resistance retain TL antigens on their surface membranes. ASL-1 cells resistant to TL 1, 3 antibodies and C stain positively, at lessened intensity than sensitive cells. Under similar conditions, TL(−) spleen cells from A/J mice fail to stain. Further exposure of ASL-1 cells to TL antiserum leads to a complete absence of immunofluorescence.

Immunoprecipitation of ^{125}I-Labeled TL Antigens From NP-40 Extracts of Modulated ASL-1 Cells

TL antigens labeled with ^{125}I are recoverable by specific immunoprecipitation from NP-40 extracts of TL antibody and C-resistant modulated cells. The labeling material used, ^{125}I-lactoperoxidase, labels "exposed" membrane-associated proteins [8]. Approximately 35% of the radioactivity present in extracts of cells not previously exposed to TL antisera is found in extracts of cells converted during a 5-h incubation period to TL antibody and C resistance (Fig. 5). Continued incubation of the cells in medium containing TL antibodies leads to a progressive diminution in the radioactivity recoverable; by 20 h, radioactivity present in the immunoprecipitates falls to "background" levels.

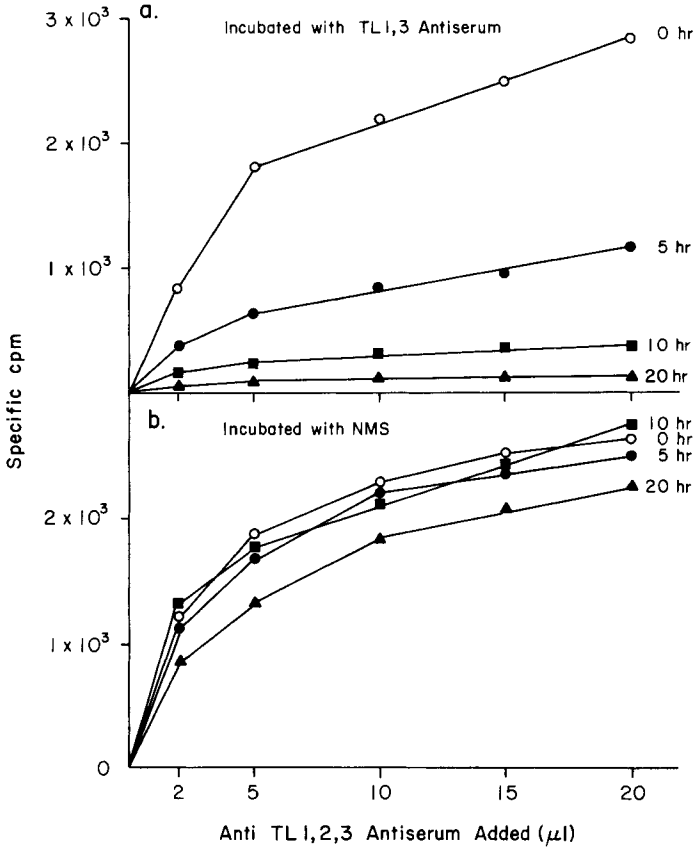

Fig. 5. Immunoprecipitation of ^{125}I-labeled TL antigens from NP-40 extracts of ASL-1 cells incubated previously at 37°C for varying periods with TL 1,3 antiserum. The radioactivity in immunoprecipitates formed with normal mouse serum is subtracted from that formed with TL 1,2,3 antiserum to yield "specific cpm."

These experimental methods designed to detect "exposed" antigenic determinants do not rule out the possibility of continued presence of "hidden" determinants or those which might be present intracellularly. TL antigens may be recovered from NP-40 extracts of ^{3}H-fucose-labeled cells modulated previously with no detectable "exposed" antigens [11].

The Sensitivity of Various TL(+) Cell Types Correlates Only in Part With the Quantity of TL Antigens Expressed

Two lines of evidence, reported here, support our contention that the sensitivity of the various lines of TL(+) cells investigated relates only in part to the quantity of TI antigens present.

ASL-1 cells, incubated for 5 h in medium containing TL antiserum, convert to antibody and C resistance. The absorptive capacity of the resistant cells parallels that of TL(+) thymocytes from two strains of F_1 mice, both of which are sensitive to TL antiserum and GPC (Fig. 6). In this instance, some lines of cells expressing similar quantities of TL antigens as resistant cells are sensitive. ASL-1 × LM(TK)$^-$ somatic hybrid cells, forming TL antigens at a quantity similar to that of modulated ASL-1 cells, like nonneoplastic thymocytes of F_1 mice, lyse in the presence of antiserum and GPC.

The absorptive capacity of RADA-1 cells for TL antisera, spontaneously resistant to TL antibodies and GPC, parallels that of the sensitive cells (Fig. 6). It exceeds by at least a factor of two the absorptive capacity of RADA-1 × LM(TK)$^-$ somatic hybrid cells for aliquots of the same antiserum.

Cells from each of nine clones of RADA-1 × LM(TK)$^-$ hybrid cells, expressing the least quantity of TL antigens of any cell lines examined, were sensitive to TL antiserum and GPC.

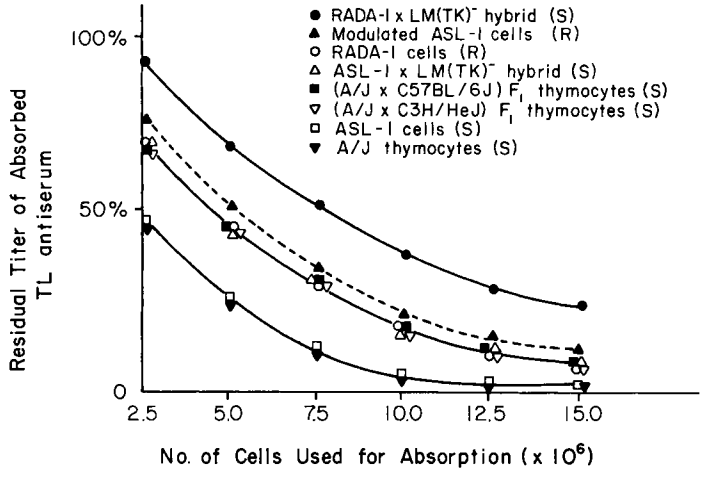

Fig. 6. The absorptive capacity of various types of TL(+) cells for TL antiserum of known titer. Appropriately diluted TL 1,2,3 antiserum was incubated at 4°C for 30 min with various numbers of cells. After centrifugation, the residual TL antibody titer was determined by C-mediated microcytoxicity tests using A/J thymocytes (TL 1,2,3) as "target" cells. The percentage of cells killed was determined by trypan blue dye exclusion. S) cells are sensitive to cytolytic effects of TL antibodies and GPC; R) cells are resistant to cytolytic effects of TL antibodies and GPC.

DISCUSSION

A variant of RADA-1 cells, a leukemia cell line of strain A mice, was detected which resists the cytolytic effects of TL antibodies and GPC. TL antigens are associated with the plasma membranes of the resistant cells, but in lower quantities than are found with ASL-1 cells, an independently arising leukemia cell line of the same mouse strain. ASL-1 cells are sensitive to TL antibodies and C. Other lines, notably somatic hybrids of RADA-1 and LM(TK)$^-$ cells, form TL antigens in lower concentration than RADA-1 cells. RADA-1 × LM(TK)$^-$ cells are sensitive, indicating that in this instance resistance to TL antisera and GPC fails to correlate with the quantities of TL antigens expressed by the cells.

The quantity of TL antigens expressed by the cells is estimated by antibody absorption procedures. Varying numbers of cells are incubated with TL antiserum of known titer, after which the serum is retitered. Changes in titer are detected, using TL(+) thymocytes from A/J mice as an "indicator" system. Control cells, such as TL(−) spleen cells from A/J mice, or other TL(−) cell types, are used as controls. They fail to affect the antiserum titer.

The TL antiserum used by us for quantitating membrane-associated TL antigens is raised in TL(−) congenic strain A mice immunized with thymocytes from TL(+) mice of the same strain. The only known antigenic disparity between donor cells and those of the recipient is present in the TL complex. Analyses by polyacrylamide gel electrophoresis of immunoprecipitates of NP-40 extracts of ^{125}I-labeled ASL-1 or RADA-1 cells prepared with such antisera indicate that the antiserum is monospecific [5–7].

Absorption procedures have been used successfully to quantitate "exposed" antigens. The reductions in antiserum titer are related inversely to the number of cells used. The result of quantitative absorption procedures for detecting "exposed" membrane-associated antigens correlate with those obtained by ^{125}I-labeling methods. The quantities of ^{125}I present in immunoprecipitates of cells extracted with NP-40 gradually decline if the cells are incubated prior to labeling with TL antibodies. The method used – indirect immunoprecipitation involving mouse TL antibodies and rabbit antimouse immunoglobulin – detects both membrane-associated proteins as TL as well as mouse immunoglobulin complexed on the cell surface.

RADA-1 and ASL-1 cells are of similar size (9 ± 0.3 μ in diameter). The significant differences in the absorptive capacity found between the two cell types may be taken as an indication that quantitative differences in TL antigen expression are present. It should be noted, however, that the total cellular content of antigens, including those present intracellularly, is not considered by this approach. Furthermore, although the absorption procedure is performed at low temperature (4°) for brief periods (30 min), it is conceivable nevertheless that antibody-induced effects on antigen expression have occurred. This is especially problematic in the study of antigens, as TL, which in the presence of TL antiserum disappear from the membranes of ASL-1 and RADA-1 cells. The spatial distribution on the cell of membrane-associated proteins, which may prove to be an important variable in studies of cellular sensitivity to immunoglobulins, is not considered by antibody absorption procedures.

Localized areas of high antigen density, such as those detected for immunoglobulin determinants in the trailing uropod of motile lymphoid cells as well as low density lipoprotein (LDL) receptors in "coated pits" of several cell types, are indications that the distribution of membrane-association determinants is not always random [14, 15]. It is quite likely that immune-mediated attacks upon the integrity of the cell occur "locally" in relatively small areas of the membrane.

Resistance of RADA-1 cells to TL antibody and GPC is specific – the cells lyse in the presence of antibodies for other membrane-associated determinants such as H-2 or Thy 1.2. The complexity of the interaction of antibody and C and its cytolytic effects is indicated by the observation that C is consumed in the nonlytic reaction of RADA-1 cells, TL antibody, and GPC. Whether C-fixation occurs at a site "distant" from the actual membrane itself or on the surface of the membrane at a rate failing to exceed the cells' capacity for repair has not been determined. Not every complement component may be activated. The susceptibility of mammary adenocarcinoma cells [16] and guinea pig hepatoma cell lines [17] is dependent on factors other than antigen density. The phase of the cell cycle is a significant factor.

Resistance of RADA-1 cells to TL antiserum and C may be the result of genetically determined factors. Somatic hybrids of RADA-1 and LM(TK)$^-$ cells, a TL(–) sustained cell line of C3H/He mouse origin, are sensitive to TL antiserum and GPC. In this instance, the quantity of antigens expressed by the sensitive hybrid cells is approximately 80% less than that of RADA-1 cells. Differences between the two cell types in antigen distribution on the membrane, possible association with cytoskeletal structures and other considerations which might affect complement sensitivity or resistance have not been determined.

In part, the susceptibility of cells to antibody- and C-mediated lysis correlates with the density as detected by quantitative antibody absorption techniques of target antigens associated with the cells [18–20]. Semiallogeneic somatic cell hybrids of mouse cells, forming lower densities of H-2 antigens than their sensitive parental sources, are resistant to H-2 antiserum and C [18]. Treatment of murine [19] or human [20] lymphoid cells with neuraminidase increases the densities of surface antigens and converts the cells to antibody and C sensitivity. As indicated here, however, this is not invariably the case for cells forming TL antigens exposed to TL antiserum and C. Thymus cells of F_1 hybrids of TL(+) and TL(–) mice sensitive to TL antibodies and C form TL antigens at the same density as resistant RADA-1 cells. Somatic hybrids of TL(+)-resistant cells and TL(–) mouse LM cells form TL antigens at a significantly lower density than their resistant parents. The hybrid cells lyse in the presence of TL antiserum and GPC.

ASL-1 cells, an independently arising TL(+) leukemia cell line of A/J mice, when exposed to antisera (without C) specific for various components of the TL antigen complex, convert over a 6-h period to TL antibody and C resistance. Resistant cells, nevertheless, retain, for a time, the capacity to reduce by absorption a known titer of TL antiserum, and thus retain TL antigens in exposed sites. (Continued incubation in TL antiserum leads to further reductions in antigen expression, until after 18 h the cells' absorptive capacity is lost.) The quantity of TL antigens associated with ASL-1 cells, converted to resistance to TL antiserum and C, parallels that of other lines of TL(+) cells which are sensitive.

After fusion, regulatory factors of one parent stimulate (or repress) analogous cellular functions of the other parent [21]. Fusion of an antibody-forming cell whose own antibody-forming capacity is repressed often leads to suppression of antibody synthesis [22]. In other instances, fusion of two antibody-forming cells, even from heterologous species, leads to persistance for indefinite periods of immunoglobulin formation. In some instances, the immunoglobulins are "hybrids" themselves, possessing characteristics of the immunoglobulins of both parents [23]. Similar phenomena have been described involving enzyme induction [24], pigment biosynthesis [25], and oncogenic potential [26]. Hybrids of modulation (+) ASL-1 cells and LM(TK)$^-$ cells lose the capacity to undergo antigenic modulation. Stackpole et al [27] reported that the modulation of TL antigens of RADA-1 cells requires the complement component C3. Conceivably, formation of the C3 binding site is suppressed in hybrid cells.

It is likely that conversion to complement sensitivity after hybridization of RADA-1 and LM(TK)$^-$ cells represents another example of an alteration in cellular phenotype resulting from cell fusion. This is supported by the observation that not every clonal isolate of hybrid cells is resistant. Two of the 11 clones tested are resistant to TL antiserum and GPC; the remaining are sensitive. The chromosomal complements of sensitive and resistant isolates are not significantly different in total number.

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