MUTANT LINES OF GUINEA PIG L2C LEUKEMIA

I. Deletion of Ia Alloantigens is Associated with a Loss in Immunogenicity of Tumor-Associated Transplantation Antigens

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The products of the major histocompatibility complex (MHC)[1] play a major role in a variety of immunological reactions including allograft rejection, graft vs. host reactions (reviewed in reference 1), and lymphocyte-macrophage cooperation for antibody production (2, 3) and for cellular immunity (4–6). In addition, recent studies have emphasized the requirement for compatibility for at least part of the MHC for effective T-cell-mediated lysis of virus-infected (7, 8) or trinitrophenyl-modified (9) target cells. Furthermore, Germain et al. (10) have performed experiments which demonstrate that alloantisera direct against MHC antigens can block T-cell-mediated lysis of syngeneic tumor target cells suggesting a close relationship between tumor-associated transplantation antigens (TATAs) and the antigens of the MHC.

In the present study, we had the opportunity to directly analyze the relationship between immune response-associated (Ia) antigens and a TATA by comparing the antigenicity of a series of mutant lines of a strain 2 guinea pig leukemia of B-lymphocyte origin. Five lines were studied for both the presence of an immunogenic TATA and Ia alloantigens. In the one leukemic line that lacked detectable surface Ia antigen, an immunogenic TATA was not found; however, cells of this line were rejected by strain 2 guinea pigs which had been immunized with cells of any of the other lines. Thus, the ability of different mutant leukemia lines to elicit a tumor-specific reactivity in syngeneic hosts was associated with the expression on the cell membrane of the Ia alloantigens of the guinea pig major histocompatibility complex (GPLA). Moreover, a correlation between membrane antigenic composition of the leukemic cells and their growth pattern in random-bred guinea pigs was observed, allowing a more precise definition of the role of the serologically defined alloantigens of the GPLA complex in allograft rejection.

Materials and Methods

Animals. Inbred strain 2 (St. 2), inbred strain 13 (St. 13), random-bred Hartley, and NIH-multipurpose guinea pigs, 300–400 g in weight, were obtained from the Animal Production

[1] *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; EAC, erythrocytes-antibody-complement complexes; GPLA, guinea pig major histocompatibility complex; HBSS, Hanks' balanced salt solution; Ia, immune response associated; MHC, major histocompatibility complex; St. 2, strain 2; TATA, tumor-associated transplantation antigen.

Section, National Institutes of Health, Bethesda, Md. The guinea pigs were age matched, pooled, and randomized at the beginning of each experiment.

Leukemias. Each of the leukemia lines used in this study presumably derive from the initial L2C leukemia, which arose sometime during the 1942–1953 Congdon-Lorenz study (11, 12). The original leukemia line had been carried since 1954 by serial cell-graft transfer in inbred St. 2 or in (St. 2 × Hartley)F_1 guinea pigs, independently by Dr. E. M. Nadel and Dr. C. W. Jungeblut who provided starter tumor material to several investigators (13, 14). The different L2C lines used in this study were recently obtained from different laboratories, either from actively transplanted animals or from samples preserved for several years in frozen state. In this paper the designation L2C will refer to the L2C leukemia in general, while the more specific designations used below will refer to the individual subtypes of the leukemia.

The L2C line referred as LE-L2C was obtained in 1969 from Dr. L. S. Kaplow, Yale University, New Haven, Conn., and maintained in our laboratory since then. The line referred as BZ-L2C was obtained in 1974 from Dr. B. Zbar, NCI, Bethesda, Md.; GH-L2C was obtained in 1974 from Dr. G. D. Hsiung, Veterans Administration Hospital, West Haven, Conn.; EN-L2C was obtained in 1975 from Dr. E. N. Nadel, Medical University of South Carolina, Charleston, S. C.; and LG-L2C was obtained in 1975 from Dr. J. W. Pearson, NCI, Bethesda, Md. After receipt, these leukemias were maintained by passage in St. 2 animals by the intradermal injection of 1×10^5 cells. When the animals appeared moribund and had leukocyte counts greater than $200{,}000/mm^3$ they were exsanguinated by cardiac puncture into a heparinized syringe. Erythrocytes were sedimented by addition of one-third volume of 3% gelatin in Hanks' balanced salt solution (HBSS). The buffy coats containing a large number of leukemic cells were removed, the cells washed three times in HBSS, and preserved by slow freezing and storage at −70°C. Except when otherwise specified, all the experimental challenges reported here were done by injecting 10×10^6 trypan blue-excluding cells in 0.2 ml of HBSS intradermally in the flank.

Detection of Membrane C3 Receptors. Membrane receptors for the third component of the complement (C3) were identified on normal lymph node and L2C cells, as previously described (15). Briefly, antigen-antibody-mouse complement complexes (EAC) were prepared by adding an equal volume of fresh mouse serum, diluted 1:10, to a suspension of sheep blood cells precoated with rabbit 19S anti-Forssman antibody, a generous gift of Dr. M. Frank, Laboratory of Clinical Investigation, NIAID, NIH, Bethesda, Md. An aliquot (0.4 ml) of the lymphoid cell suspension (2×10^6 cells/ml) to be tested was added to 0.4 ml of a suspension of EAC. The mixture was incubated at 37°C for 30 min with gentle agitation. The number of trypan blue-excluding viable lymphocytes with three or more erythrocytes attached to the cell surface (rosettes) were enumerated in a hemocytometer.

Preparation of Alloantisera. The immune sera used in this study were prepared by cross-immunization of animals of known phenotype with lymph node and spleen cells (approximately 100×10^6 cells per recipient) emulsified in complete Freund's adjuvant (CFA) containing 0.5 mg/ml of *Mycobacterium butyricum* (Difco Laboratories, Detroit, Mich.). 2 and 4 wk after primary immunization the animals were boosted by either subcutaneous or intraperitoneal injection of 100×10^6 lymph node and spleen cells in HBSS. 6 wk after primary immunization the animals were exsanguinated, the sera heat inactivated at 56°C for 45 min, and stored at −70°C. St. 13 anti-2 serum contains antibodies to two distinct GPLA specificities, Ia.2 and Ia.4; St. 2 anti-13 also contains antibodies to two distinct GPLA specificities, Ia.1 and Ia.3. Specific antisera to Ia.4 were raised by immunizing random-bred $Ia.2^+$, $Ia.4^-$ guinea pigs which possess the B.1 GPLA antigen with cells from inbred St. 2 (Ia.2,4, B.1) donors; antisera to Ia.2 were obtained by immunizing St. 13 (Ia.1,3, B.1) animals with cells from $Ia.2^+$ $Ia.4^-$, $B.1^+$ random-bred animals. Antisera to the B.1 alloantigen was prepared as previously described (16). Further details concerning the nature of these antibodies will be presented in the result section.

Immunofluorescence. A direct immunofluorescence technique was used to detect the presence of membrane immunoglobulins (Ig), as well as B and Ia alloantigens on the various L2C cell lines. Viable L2C and normal lymph node cells were incubated directly with fluorescinated antisera at 4°C for 30 min. The cells were washed three times, suspended in HBSS, and examined under a petroleum jelly-lined cover slip with a Leitz-Ortholux microscope equipped with a vertical illuminator (E. Leitz, Rockleigh, N. J.). The presence of IgM idiotype determinants was determined by indirect immunofluorescence. Viable L2C cells and control normal St. 2 lymph node cells were

incubated at 4°C for 15 min with a sheep antiserum to the idiotypic determinant(s) of membrane IgM of LE-L2C cells. This serum had been rendered specific by in vivo absorption in a normal St. 2 animal (17) and was kindly supplied by Dr. G. T. Stevenson, Tenovous Research Laboratory, General Hospital, Southampton, England. The cells were washed three times in HBSS and incubated with a fluorescein-conjugated rabbit antisheep gamma globulin antiserum at 4°C for 30 min. The cells were then washed three times and examined as described before.

Cytotoxicity Testing. The presence of individual alloantigens on L2C cell membranes was also tested by cytotoxicity. The ^{51}Cr-release assay was performed as previously described in detail (18). In brief, about 50 × 10^6 target cells were incubated in 1 ml of HBSS containing 10% fetal calf serum and 100 μCi of ^{51}Cr (sp act 200–500 μCi/μg, Amersham/Searle Corp., Des Plains, Ill.) for 60 min at 37°C. The cells were then washed twice and resuspended at a concentration of 10 × 10^6 cells/ml. 0.1 ml of cell suspension was then added to 10 × 75 mm tubes, followed by 0.1 ml of 1:10 dilution of antiserum and 0.1 ml of 1:2 dilution of lyophilized guinea pig serum (Grand Island Biological Co., Grand Island, N. Y.) as source of complement. The amount of radioactivity released was quantitated as follows:

$$^{51}\text{Cr release (\%)} = 100 \times \frac{\text{Radioactivity released by antiserum dilution} - \text{radioactivity released in absence of antiserum}}{\text{Radioactivity released by frozen-thawed cells} - \text{radioactivity released in absence of antiserum}}.$$

Absorption of Alloantisera. 0.1 ml of the different guinea pig alloantisera were mixed with 0.9 ml of an HBSS suspension containing 1 × 10^8 normal or leukemic lymphocytes and incubated for 4 h at 4°C on a rocking platform. The absorbed antisera were centrifuged at 30,000 rpm for 30 min.

Immunization and Subsequent Challenge. St. 2 guinea pigs were immunized with leukemic cells as previously described (19). Briefly, leukemic cells in HBSS were emulsified with an equal volume of CFA. An aliquot (0.4 ml) of the emulsion containing 20 × 10^6 leukemic cells was distributed among the foot pads. Control groups were injected with an emulsion of CFA-HBSS alone. The immunized and control animals were challenged 14 days after the immunization with 10 × 10^6 viable L2C cells injected intradermally, except when otherwise specified.

Assay of DNA Synthesis. Cultures were performed in round bottom microtiter plates (Cooke Laboratory Products Div., Dynatech Laboratories Inc., Alexandria, Va.) in RPMI supplemented with L-glutamine (0.3 mg/ml), 200 IU/ml of penicillin, 100 μg/ml of streptomycin, and 10% guinea pig serum. Responding lymph node cells were enriched in T lymphocytes by purification over adherence columns as previously described (16), and were cultured in triplicate at a concentration of 2.4 × 10^5 cells per well in a total vol of 0.2 ml. Stimulating cells were treated with 40 μg/ml of mitomycin C (Sigma Chemical Co., St. Louis, Mo.) for 40 min at 37°C, washed four times, and cultured at a concentration of 0.8 × 10^5 cells per well. Microtiter plates were incubated at 37°C, in a humidified atmosphere of 5% CO_2-95% air. 18 h before harvesting 1 μCi of tritiated thymidine (sp act 6.7 Ci/mmol; New England Nuclear, Boston, Mass.) was added to each well. Cultures were collected with the aid of a semiautomatic microharvesting device and the amount of tritiated thymidine incorporated was determined by liquid scintillation spectrometry and expressed as total counts per minute.

Results

Derivation of L2C Lines. The L2C leukemia is an acute leukemia of guinea pigs which arose in an old unirradiated control St. 2 female sometime between 1942 and 1953 during a research study by Congdon and co-workers (11, 12), whose primary goal was to analyze the biological effect of long-term whole body γ and X irradiation. This leukemia has been serially transplanted in inbred St. 2 and in (St. 2 × Hartley)F_1 guinea pigs for over 20 yr. Initially, L2C leukemia was maintained at the National Cancer Institute, then by C. W. Jungeblut (13), and on collaborative basis by E. M. Nadel and S. Burstein (14) who provided starter material of this leukemia line to several laboratories. The five

L2C lines analyzed in the present study were recently obtained from different laboratories in which L2C leukemia had been actively transplanted for various lengths of time. Since the L2C leukemia has been maintained and studied at various times for more than 20 yr by several investigators, it is difficult to identify the exact sequence of the transmission of the leukemia from one investigator to another. However, it should be noted and will be discussed in detail in another paper, that all the five different lines bear two X chromosomes and an identical extra metacentric chromosome indicating a common ancestral origin.[2]

Course of L2C Leukemias in Untreated St. 2 Guinea Pigs. The intradermal inoculation in St. 2 animals of 10×10^6 leukemic cells, in 0.2 ml of HBSS of all the five leukemic lines tested consistently induced a generalized acute leukemia with a rather characteristic clinic course. A hemorrhagic nodular leukemic tumor appears at the intradermal injection site about 7 days after inoculation. Shortly after, when the intradermal tumor reaches about 10–15 mm in diameter, a progressive systemic disease is manifested by increasing leukocytosis, with a doubling time of about 24 h, abdominal distension with palpable hepatosplenomegaly, marked ataxia, and death with neurological manifestations about 12–24 h after the leukemic cells have reached 200,000 cells/mm^3. Gross autopsy examinations revealed a spleen 6–10 times the normal size, considerable hepatomegaly, and enlarged lymph nodes. The pathological manifestations and cytomorphology were similar in all five of the L2C lines studied, and were similar to the findings previously reported by Opler (20).

Expression of C3 Receptors. In previous studies, we have demonstrated that LE-L2C cells bear on the membrane multiple markers (C3 receptors and surface Ig) characteristics of bone marrow-derived (B) lymphocytes and therefore the L2C leukemia was interpreted as a leukemia of B-cell origin (15). Therefore we have comparatively analyzed the presence of some membrane markers on the various L2C lines (Table I). The presence of complement (C3) receptor was determined by incubating the leukemic cells with EAC. As previously reported (15), EAC rosettes with three or more erythrocytes can be detected on 75–80% of LE-L2C cells. A substantial percentage (40–50%) of GH-L2C and EN-L2C cells also form EAC rosettes. Significantly fewer LG-L2C and BZ-L2C cells formed percentage of EAC rosettes.

Presence of Surface Immunoglobulins and a Common Idiotypic Determinant. Surface Ig were constantly detected by a direct immunofluorescence technique on more than 90% of cells from each of the five L2C cell lines using a polyspecific rabbit antiguinea pig Ig antiserum. These data are shown in Table I. Moreover, with an antiguinea pig IgM antiserum, the surface Ig was identified as IgM on both LE-L2C (17) and BZ-L2C leukemia cells (G. T. Stevenson, personal communication). Recent studies of solubilized membrane-associated immunoglobulin indicate that the surface IgM of LE-L2C, GH-L2C, and BZ-L2C cells is monomeric (F. Finkelman and B. Schwartz, unpublished observations).

[2] Whang-Peng, J., E. C. Lee, G. Forni, and I. Green. 1975. Mutant lines of guinea pig L2C leukemia. II. Comparative cytogenetic studies and banding analysis of normal and leukemic karyotypes. *J. Natl. Cancer Inst.* In press.

TABLE I
Surface Markers of the Five L2C Leukemic Lines

	Presence of C3 receptors	Presence of surface Ig	Surface IgM	Presence of a common idiotypic determinant
	% of EAC rosette-forming cells	% of fluorescent cells with an antiguinea pig Ig antiserum*	Cells positive with an anti-IgM antiserum‡	% of fluorescent cells with an anti-LE-L2C Ig idiotype serum§
LE-L2C	75–80	>90	Yes	>90
LG-L2C	40–50	>90	ND‖	>90
EN-L2C	40–50	>90	ND	>90
GH-L2C	10	>90	ND	>90
BZ-L2C	>3	>90	Yes	>90

* Determined by direct immunofluorescence technique using an antiserum with broad specificity.
‡ G. T. Stevenson, personal communication and reference 17.
§ Determined by indirect immunofluorescence using a highly specific anti-idiotypic antibody (see text).
‖ ND, not done.

The various L2C lines were then tested for a common IgM idiotypic determinant by indirect immunofluorescence using an anti-idiotype antiserum prepared against IgM extracted from LE-L2C cells (17). When the antiserum was tested against cells of the five L2C lines, each displayed an identical pattern of uniform circumferential staining with superimposed bright spots on more than 30% of the cells (Table I). In contrast, no staining of normal St. 2 or St. 13 lymph node cells, known to contain about 35% B lymphocytes, was observed. These results indicate the presence of a common idiotypic determinant on all the L2C lines studied and the absence of appreciable numbers of idiotype-bearing cells from normal lymphoid populations. However, in spite of the binding detected by immunofluorescence, the anti-idiotype antiserum was not cytotoxic for any of the L2C lines in presence of complement, when tested by a ^{51}Cr-release technique.

Expression of Alloantigens of the GPLA. Although the serological analysis of GPLA is not completely determined, recent serological, genetic (21), and physiochemical (22) studies have identified two linked regions or loci. The *B* region codes for at least four allelic specificities termed *B.1*, *B.2*, *B.3*, and *B.4* which appear to be the guinea pig equivalent of the murine *D*- or *K*-region gene products. Inbred St. 2 and St. 13 animals both bear the *B.1* allele and the sera raised by cross-immunization of these two inbred strains identify what appears to be the guinea pig equivalent of the murine Ia antigens. St. 2 anti-13 serum contains antibodies to two specificities termed Ia.1 and Ia.3, while St. 13 anti-2 serum contain antibodies to two distinct specificities Ia.2 and Ia.4. It has been demonstrated that the alloantigens coded by these two regions differ in their tissue distribution. The Ia alloantigens are restricted to lymphoid cells while the B alloantigens have a wide tissue distribution.

TABLE II
Presence of I- and B-Region Antigens on Normal Lymphocytes and L2C Cells

Target Cells	Percentage of ^{51}Cr released by different antisera*				
	St. 13 anti-2‡	Anti-Ia.2	Anti-Ia.4	St. 2 anti-13§	Anti-B.1
Normal St. 2 lymph node cells	59	30	28	0	85
Normal St. 13 lymph node cells	0	0	0	30	80
LE-L2C	75	ND‖	ND	0	88
GH-L2C	70	20	77	0	30
EN-L2C	73	85	80	0	88
LG-L2C	75	22	70	0	85
BZ-L2C	1	0	0	0	85
BZ-L2C after treatment with neuraminidase	3	ND	ND	1	90

* Final dilution 1:30.
‡ St. 13 anti-2 sera contain antibodies to both Ia.2 and Ia.4 alloantigens.
§ St. 2 anti-13 sera contain antibodies to both Ia.1 and Ia.3 alloantigens.
‖ ND, not done.

We next studied the different leukemic lines for the presence of *B*- and *I*-region gene products both by indirect immunofluorescence and by a complement-dependent ^{51}Cr-release cytotoxicity assay (Table II). St. 13 anti-2 sera, which contain antibodies directed to Ia.2 and Ia.4 specificities stain LE-L2C, GH-L2C, EN-L2C, and LG-L2C cells. Moreover, these Ia antigens could be capped independently of membrane immunoglobulins (I. Green, unpublished observations). In contrast no Ia.2–Ia.4-specific staining was detectable on BZ-L2C cells. Similarly, the ^{51}Cr-release technique performed with either St. 13 anti-2 antisera or with monospecific anti-Ia.2 and anti-Ia.4 antisera detected these two alloantigens on all L2C lines except the BZ-L2C line. Moreover, absorption of St. 13 anti-2 antisera with a large excess of BZ-L2C cells failed to remove the cytotoxic antibodies directed against St. 2 Ia specificities. However, it should also be noted that differences were repeatedly observed in the percentage of ^{51}Cr released from the various Ia-positive L2C lines with the monospecific anti-Ia.2 sera suggesting that these lines differed in the amount of Ia.2 antigen they contained. Finally to exclude the possibility that Ia.2, Ia.4 specificities were expressed on the BZ-L2C cell membrane, but were masked by sialic acid residues, often produced in higher amount in transformed cells, the BZ-L2C cells were treated with 100 μg of neuraminidase for 30 min at 37°C. Even after this treatment no Ia specificities were detectable on BZ-L2C cell membranes. As anticipated, no Ia.1,3 specificities were detectable on any of the L2C leukemic lines.

Alloantisera monospecific for B.1 alloantigens stained all the L2C lines equally well. In addition, anti-B.1 antisera, in the presence of complement, lysed cells from each of the five L2C leukemic lines. The amount of lysis obtained with the L2C cells was comparable to the degree of lysis observed with normal St. 2 and St. 13 spleen cells both of which express the *B.1* specificity (Table II). Moreover studies of ^{125}I-surface labeled and of ^{3}H-leucine internally

TABLE III
Growth and Rejection of GH-L2C and BZ-L2C in Guinea Pigs of Different Histocompatibility Type

Leukemic line injected	Recipient animal*	Identified histocompatibility antigens		Takes‡
		B	I	
GH-L2C (B.1, Ia.2, Ia.4)	St. 2	B.1	Ia.2, Ia.4	40/40
	St. 13	B.1	Ia.1, Ia.3	0/20
	Random bred	B.1	Ia.2,4§	0/8
	Random bred	B.1	Ia.1,3§	0/6
	Random bred	B.1	Ia.1,3; Ia.2,4§	0/3
	Random bred	B.1; B.3	Ia.1,3*; Ia.2,4§	0/3
	Random bred	B.3	Ia.1,3*	0/4
BZ-L2C (B.1, Ia absent)	St. 2	B.1	Ia.2; Ia.4	20/20
	St. 13	B.1	Ia.1, Ia.2	20/20
	Random bred	B.1	Ia.2,4§	5/5
	Random bred	B.1	Ia.1,3§	8/8
	Random bred	B.1	Ia.1,3§; Ia.2,4§	3/3
	Random bred	B.1; B.3	Ia.1,3§; Ia.2,4§	2/2
	Random bred	B.3	Ia.2,4§	0/4
	Random bred	B.3	Ia.1,3§	0/3

* Challenged with 10×10^6 leukemic cells.
‡ Leukemic animals 45 days after challenge.
§ In these animals Ia specificities have been identified by antisera directed to both Ia.2 and Ia.4, or to both Ia.1 and Ia.3 alloantigens and therefore an animal that lacked one of two Ia specificities could not be detected.

labeled GPLA antigens indicated no differences in the molecular characteristics of B.1 and Ia.2,4 alloantigens on LE-L2C, GH-L2C, and normal St. 2 lymphoid cells, at least when studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. By contrast a normal B.1 alloantigen but no Ia alloantigens were detected on BZ-L2C cells by these techniques (F. Finkelman and B. Schwartz, unpublished observations).

Transplantation of GH-L2C and BZ-L2C Leukemias into Guinea Pigs of Different Histocompatibility Type. The antigens of the GPLA have been defined mainly by serological and genetic studies. The transplantation data available were obtained by skin grafting (23), by transfer of nonlymphoid tumors which do not bear Ia alloantigens on the membrane (24), and by limited studies with LE-L2C leukemia cells (25). It was therefore of great interest to study the growth and rejection patterns of these leukemic lines in recipient animals that differed at either the *I* or *B* loci and to attempt to correlate the presence of absence of Ia antigens on the leukemic cells with differences in growth and rejection patterns. For these studies we have used GH-L2C cells as Ia-positive, and BZ-L2C as Ia-negative leukemic cell lines. The results of these studies are shown in Table III.

The inoculation of 10×10^6 GH-L2C cells into random-bred animals which differ at either, or both, the *I* or *B* loci, resulted in the formation of a small

erythematous papule 24–48 h after the challenge followed, in every case, by a complete rejection of the leukemia cells with the formation of a small cutaneous scar. Surprisingly, when injected in random-bred guinea pigs from the NIH colony, which initially were thought to possess the B.1, Ia.2, and Ia.4 alloantigens, the GH-L2C leukemia cells were also rejected. This rejection was at first thought to be due to the presence of an as yet unidentified alloantigen. However, recent studies of the alloantigens of the animals of the NIH colony done with monospecific anti-Ia.2 and Ia.4 antisera (not available before) indicated that the great majority of random-bred animals of the NIH colony which were thought to be both Ia.2 and Ia.4 positive are actually Ia.2 positive and Ia.4 negative. In retrospect, these data suggest that the rejection of GH-L2C leukemia in animals thought to have been B.1, Ia.2, and Ia.4 positive was actually dependent on the absence of Ia.4 alloantigen in the recipient animal.

A quite different growth-rejection pattern was observed upon challenge of allogeneic animals with the BZ-L2C leukemia, which does not express Ia antigens on the membrane. These leukemic cells could be transplanted into all random-bred animals that were B.1 positive without regard to which Ia antigens the recipient possessed. However, the BZ-L2C leukemia was uniformly rejected in B.1-negative animals (Table III). This growth-rejection pattern confirms the dominant role played by the B alloantigens in rejection of tumors that do not bear Ia antigens on their surface (24). Thus, the results with the GH-L2C and BZ-L2C leukemias indicate that antigens on the cell surface coded for by genes at both the *I* and *B* loci can act as transplantation antigens in the guinea pig.

Presence of TATA. The presence of TATA on L2C cells was determined in the present study by the results of immunization protection tests. Immunization was achieved by injecting the leukemic cells emulsified in CFA into the foot pads of St. 2 guinea pigs. Irradiated cells and unirradiated cells were equally effective. As can be seen in Table IV, St. 2 guinea pigs immunized 14 days earlier with LE-L2C, GH-L2C, EN-L2C, and LG-L2C cells emulsified in CFA, were almost completely protected against a subsequent challenge with viable cells of the line which has been used for immunization. Moreover, 24–48 h after the intradermal challenge a skin reaction with induration and erythema was evident. By contrast, guinea pigs which had been immunized and challenged with the Ia-negative BZ-L2C leukemia cells displayed no protection or increased survival as compared to unimmunized controls, confirming what previously has been reported for this particular leukemic line by Wepsic et al. (26). In a crisscross immunization-protection experiment we then examined whether cross-protection between the different leukemic lines could be obtained. St. 2 animals which had been immunized with GH-L2C, EN-L2C, or LG-L2C were completely protected against a challenge with any of these leukemic lines, indicating that these three lines share a common TATA (Table V). St. 2 guinea pigs preimmunized with BZ-L2C displayed no significant protection or prolonged survival upon challenge with any of the other leukemia lines compared to the control animal groups injected with HBSS-CFA. Surprisingly, however, animals preimmunized with GH-L2C, EN-L2C, or LG-L2C were completely protected against challenge with the apparently nonantigenic BZ-L2C leukemia cells. Thus, these crisscross protection tests indicate the presence of a common TATA in each of

TABLE IV
Immunogenicity of Different L2C Lines in Syngeneic St. 2 Guinea Pigs

Immunization	Challenge*	Surviving animals 45 days after challenge
20 × 10^6 LE-L2C cells in CFA	LE-L2C cells	14/15
HBSS in CFA	LE-L2C cells	1/16
20 × 10^6 GH-L2C cells in CFA	GH-L2C cells	14/14
HBSS in CFA	GH-L2C cells	0/12
20 × 10^6 EN-L2C cells in CFA	EN-L2C cells	5/5
HBSS in CFA	EN-L2C cells	0/5
20 × 10^6 LG-L2C cells in CFA	LG-L2C cells	5/5
HBSS in CFA	LG-L2C cells	0/4
20 × 10^6 BZ-L2C cells in CFA	BZ-L2C cells	0/9
HBSS in CFA	BZ-L2C cells	0/10

* Challenge was performed 14 days after the immunization with 10 × 10^6 cells injected intradermally.

the L2C lines. However the BZ-L2C appears to possess a "TATA" which is incapable of eliciting an immune response.

Stimulation of St. 2 Lymphocytes Proliferation By L2C Cells. We next analyzed the capacity of cells of the various L2C lines to stimulate proliferation of lymphocytes obtained from control or from preimmunized St. 2 guinea pigs.

Lymphocytes from control donors did not develop a detectable response against leukemic cells. By contrast, an anamnestic lymphocyte proliferative response was initiated in lymphocytes from St. 2 animals which had been preimmunized 14 days before with Ia-positive L2C leukemia cells by each of the various L2C lines tested, including the Ia-negative BZ-L2C line (Table VI). No significant differences were observed in the DNA-synthetic response of preimmunized St. 2 lymphocytes stimulated by the various L2C cell lines. Moreover, these data obtained with these four L2C cell lines tested were consistent with the data we have previously obtained with LE-L2C-stimulating cells (19). In contrast no anamnestic proliferative response against any L2C cell line was observed with lymphoid cells obtained from St. 2 animals which had been preimmunized with the Ia-negative BZ-L2C cells.

Discussion

The data obtained in the present study demonstrate that there are several different lines of L2C leukemia and that an important correlation can be made between the surface antigenic structure of these cells and their immunogenic potential. The presence of an identical chromosomal marker and the presence of the same idiotypic determinant on the surface Ig of the five different lines analyzed, overwhelmingly indicate that these lines are mutants of a single parental line. A detailed analysis of the karyotypes of each of these five leukemic lines, which will be reported elsewhere (see footnote 2) indicate, however, that each line presents a slightly different chromosomal pattern and therefore each line is a distinct mutant arising during the passage of the L2C leukemia in various laboratories.

TABLE V
Cross-Protection against the Various L2C Lines Induced by Preimmunization of Syngeneic Strain 2 Guinea Pigs with Different L2C Cells

L2C cell line used for immunization*	L2C cell line used for challenge‡	Surviving animals 45 days after challenge
None§	GH-L2C	0/7
	EN-L2C	0/6
	LG-L2C	0/9
	BZ-L2C	0/8
GH-L2C	EN-L2C	6/6
	LG-L2C	5/5
	BZ-L2C	7/7
EN-L2C	GH-L2C	6/6
	LG-L2C	4/4
	BZ-L2C	4/4
LG-L2C	GH-L2C	5/5
	EN-L2C	5/5
	BZ-L2C	5/5
BZ-L2C	GH-L2C	1/7
	EN-L2C	0/4
	LG-L2C	0/5

* Immunization with 20×10^6 cells emulsified in CFA.
‡ Challenge with 10×10^6 living cells. Challenge performed 14 days after the immunization.
§ Injected with HBSS-CFA only.

These lymphoid tumors of B-cell origin, most of which express both the GPLA B and Ia antigens, permitted us to undertake a more complete analysis of the role of the different GPLA specificities in tissue transplantation than had been previously possible. The GH-L2C leukemic line which bore both the B and Ia alloantigens would only grow in syngeneic St. 2 animals and failed to grow in B.1 compatible but Ia distinct St. 13 guinea pigs or in random-bred animals of various histocompatibility types. In contrast, the BZ-L2C line, which was Ia negative, grew readily in both St. 2 and St. 13 animals. Moreover the ability of this line to grow in random-bred guinea pigs was only related to the presence or absence of B.1 antigen in the recipient. The growth-rejection pattern of this Ia-negative leukemic line is therefore identical to the pattern previously observed with nonlymphoid, Ia-negative tumors (24). In the case of the Ia-positive variant (GH-L2C) the Ia antigen (and probably the B antigen) appears to function as a transplantation antigen, while in the case of the Ia-negative variant (BZ-L2C) the B.1 antigen functions as a transplantation antigen. Recent studies have also demonstrated that cell surface antigens coded by the *I* region of the murine MHC can act as transplantation antigens (27, 28).

Of particular interest is the data on the immunogenicity of the different

TABLE VI

DNA-Synthetic Response to L2C Leukemic Cells of Lymph Node Cells from St. 2 Guinea Pigs Preimmunized with GH-L2C or BZ-L2C

Effector lymphocytes from strain 2 animals preimmunized with:	Stimulator cells	St. 2 Lymph node cells [^{3}H]thymidine incorporation*		
		72 h	120 h	144 h
GH-L2C	–	489 ± 85	534 ± 37	565 ± 100
	GH-L2C	2,327 ± 150	13,614 ± 559	7,858 ± 1,336
	EN-L2C	4,767 ± 600	15,857 ± 432	9,274 ± 1,176
	LG-L2C	4,777 ± 760	11,802 ± 559	5,107 ± 1,230
	BZ-L2C	3,132 ± 169	12,075 ± 470	9,220 ± 637
BZ-L2C	–	403 ± 15	945 ± 138	709 ± 89
	GH-L2C	371 ± 32	975 ± 91	1,000 ± 105
	EN-L2C	290 ± 91	924 ± 89	768 ± 63
	LG-L2C	389 ± 35	763 ± 53	952 ± 110
	BZ-L2C	367 ± 88	853 ± 65	721 ± 98

* Results are expressed as mean counts per minutes ± SE of triplicate determinations.

leukemic lines. The present study demonstrates that the four Ia-positive leukemia lines bear a TATA which is able to induce specific resistance to a subsequent challenge with the leukemia cells of the same line in St. 2 hosts. In contrast, the Ia-negative mutant line (BZ-L2C) did not possess TATAs as measured by the same type of immunization and challenge experiments (Table V) demonstrated that it is not simply that BZ-L2C leukemia cells lack a TATA but rather the TATA of this particular mutant is not immunogenic. That is, St. 2 animals preimmunized with cells of any of the Ia-positive L2C leukemic lines can subsequently reject a challenge with the BZ-L2C leukemia cells. These results indicate that BZ-L2C cells share a common TATA with the other L2C leukemias, and that it can be recognized by immunized recipients, but that this TATA is not immunogenic when it is present on the BZ-L2C cell. Moreover, an in vitro anamnestic proliferative response of lymph node cells from St. 2 guinea pigs against the Ia-positive and Ia-negative leukemic lines can be obtained only with lymphocytes from animals preimmunized with the Ia-positive but not the Ia-negative L2C lines (Table VI). These in vivo and in vitro experiments point to a relationship between the immunogenicity of L2C cells and the expression of Ia alloantigens. In both, the in vivo and in vitro experiments, the immunization with the Ia-negative BZ-L2C cells failed to elicit a population of TATA-responsive T lymphocytes suggesting that the presence of Ia alloantigens is a necessary requirement for the elicitation of a primary immune response to the L2C TATA.

Although the role of the Ia antigens in the immune response in general has been incompletely defined, the Ia antigens or the products of closely linked genes appear to play a central role in the interaction of immunocompetent cells. Thus, a requirement for shared Ia antigens has been demonstrated for the interaction of macrophages and lymphocytes required for the generation of helper T-cell activity in mice (29), for the passive transfer of delayed hypersensitivity in mice (6), and for antigen-induced T-cell proliferation in guinea pigs (4,

5). Although it is well accepted that macrophages play a major role in the processing of soluble protein antigens, the role of the macrophage in the induction of the immune response to membrane alloantigens and tumor-antigens is less well established. Nevertheless, the lack of immunogenicity of the Ia-negative leukemic cells (despite the fact it bears a common TATA) can be explained in the context of what is known about the requirements for the response to more defined antigens. One possible mechanism for a successful immunological event is a direct interaction between the Ia antigens of lymphoid tumors and the host T cells. Thus, in the present study it is possible that Ia alloantigens are necessary structures to allow a physical contact between lymphocytes and leukemic cells bearing TATAs, which is required for an effective immune response to the TATA to be generated. A second possibility is that the TATA must be processed in vivo by host macrophages and that Ia antigens of the tumor cells act as an integral part of the TATA molecular organization and are required for immunogenicity. According to this hypothesis it would seem that Ia antigens of host macrophages (in which the tumor antigens are "processed") cannot substitute for the Ia antigens which are an integral part of the tumor cell membrane. Thus, the Ia antigens may be functionally equivalent to the carrier portion of the tumor-specific determinants, analogous to the protein portion of the immunogen in the case of hapten-protein conjugates. A similar carrier role of normal membrane antigens for TATAs has been recently reported on a murine adenocarcinoma (30). However the hapten-carrier analogy is imperfect in that the hapten-positive carrier-negative BZ-L2C cells were able to stimulate in a mixed leukocyte tumor interaction and presumably were lysed by specific cytotoxic T cells in vivo. In these situations perhaps the product of the guinea pig B region acted as carrier molecule (see below).

An analogy can be drawn between our findings obtained in vivo and the studies of the generation of cytotoxic effector cells in mouse mixed leukocyte culture in vitro. Here two conditions appear to be necessary; Ia differences must be present to generate a mixed leukocyte reaction and at the same time H2-K and/or H-2D differences are required on the cells undergoing the mixed lymphocyte reaction for the generation of killer cells. Finally the target cells must contain the relevant H2-K and/or H2-D alloantigen. In this in vitro system an effective cytotoxic activity does not develop in the absence of Ia differences (31, 32).

The data presented here are also consistent with recent observations which suggest that the most highly immunogenic TATAs may represent altered or modified H-2 antigens (10, 33–36). Thus, Germain et al. (10) demonstrated that alloantisera raised by immunization with normal lymphoid cells can inhibit lysis of tumor targets by syngeneic immune T lymphocytes. An intriguing possibility, suggested by our in vivo and in vitro results, is that the TATA associated with the products of the *I* region may be important for the induction of the immune response, while the same TATA associated with the products of the *B* region which is the guinea pig equivalent of the mouse *D*- and *K*-region products is important as the target for specific cytotoxic T lymphocytes. It would be of interest to evaluate the effect of anti-B.1 serum in an in vitro assay of T-cell cytotoxicity against L2C. However, we have been hampered in these studies

because of the present lack of a suitable assay of T-cell cytotoxicity in this particular system. Although the present data suggest that the deletion of Ia alloantigens is associated with a loss in immunogenicity of TATA, it is possible that such an association is merely coincidental. Studies are now in progress with solubilized membrane antigens to determine the requirement of Ia alloantigens in the immunological recognition of L2C TATA in syngeneic guinea pigs.

Studies of the relationship between Ia antigens and antigenicity of TATA in this series of guinea pig B-cell malignancies may provide a model for studies of this relationship in human leukemic cells and may be especially relevant since it has been recently demonstrated that a high proportion of acute human lymphocytic leukemias resemble B cells in carrying Ia-like antigens (37).

Summary

Five different lines of a strain 2 guinea pig leukemia (L2C) which had been carried in different laboratories share certain chromosomal markers and have a common surface immunoglobulin idiotypic determinant indicating that they have a common origin. All these leukemic lines have on their surface of the B alloantigen (equivalent of the murine H-2K and H-2D antigens) and four of these five lines have on their surface the Ia alloantigens normally present on the strain 2 lymphocytes. The result of a study of the growth and rejection patterns of these leukemias in inbred and random-bred guinea pigs of selected histocompatibility type indicates that both the B and Ia antigens can act as transplantation antigens in guinea pigs. Immunization protection tests in syngeneic animals demonstrated that the four Ia-positive leukemias possessed a tumor-associated transplantation antigen(TATA), while the one Ia-negative leukemia by this criteria did not appear to have TATA. However, crisscross immunization protection tests demonstrated that preimmunization of syngeneic animals with an Ia-positive L2C line lead to a subsequent protection against challenge with the Ia-negative leukemia. Immunization with the Ia-negative line never protected against a subsequent challenge with any of the leukemic cells of L2C lines. These results strongly suggest that the Ia-negative leukemia possessed a TATA that can be recognized but is not itself immunogenic, and also indicate that Ia antigens on L2C cells are functionally associated with TATA and can act as immunological carriers for tumor transplantation determinants.

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