# INDUCTION OF PARTIAL IMMUNOLOGIC TOLERANCE IN RATS AND PROGRESSIVE LOSS OF CELLULAR ANTIGENICITY IN GROSS VIRUS LYMPHOMA*

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Temporary changes in cellular antigenicity reflecting an adaptational alteration were originally observed in leukemic cells and termed antigenic modulation (1, 2). Subsequently, transitory (3–6) or permanent (7–8) loss of antigenic expression has been described in other systems and correlated with an increased capacity for allogeneic transplantation (9–11).

Such changes of cellular antigenicity represent a major modality by which growing tumors (12) and transplanted tissues (4) escape immunologic annihilation. It is therefore of great interest to study the mechanisms of antigenic variation and the correlation between transplantability and expression of various antigens. We have previously reported observations on Gross virus-induced rat lymphomas that lost virus replication and Gross murine leukemia virus (G-MuLV)[1] antigenic expression during serial transplantation and recovered both when explanted in vitro (5).

In the present work, attempts were made to establish a system in which changes of antigenicity can be deliberately induced in order to facilitate the study of their mechanics. This was achieved by injection of deaggregated antigen into newborn rats, resulting in a long-lasting state of partial immunologic tolerance to Gross virus-induced lymphoma cells. Subsequent to their transplantation in partially tolerant rats the lymphoma cells showed loss of membrane G-MuLV antigens with maintenance of cytoplasmic antigenicity. This antigenic disjunction made the cells serially transplantable in nonconditioned adult hosts, which further resulted in the loss of internal specific antigens.

## Materials and Methods

*Induction of Lymphoma.*—1- to 4-day old (W/Fu) rats were given one intraperitoneal (i.p.) injection of 0.1-ml stock rat-adapted Gross lymphoma virus (GLV) diluted 1:10 in medium 199. As previously reported (13) an incidence of nearly 100% induction of thymic lymphoma was obtained within an average latency period of 73 days.

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[1] *Abbreviations used in this paper:* GLV, Gross lymphoma virus; G-MuLV antigens, Gross murine leukemia virus antigens; PBS, phosphate-buffered saline; RaLV, rat leukemia virus.

*Induction of Partial Immunologic Tolerance.*—GLV-induced thymic lymphomas were finely minced and a 20% suspension in phosphate-buffered saline (PBS) was prepared. The latter was homogenized at 4°C in a Waring blender (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn.) and subsequently sonified for 60 s at 0°C in a 100 W ultrasonic MSE disintegrator (MSE Inc., Westlake, Ohio) to solubilize the antigenic components. This was followed by one 10,000 $g$ centrifugation for 10 min at 4°C. The supernate was spun at 100,000 $g$ for 1 h at 4°C and the resulting supernate passed through a 10 nm pore size millipore filter. 1- to 3-day old W/Fu rats were injected i.p. with 0.1 ml of this preparation.

*Transplantation.*—75- to 120-day old W/Fu rats were grafted with GLV-induced thymic lymphomas or subsequent generations of transplanted lymphomas. The grafts consisted of 3 mm³ of tumor tissue introduced subcutaneously by a cutdown in the skin of the left side of the thorax. Assessment of tumor acceptance was made after 30 days.

*Indirect Immunofluorescence.*—

*Membrane antigens:* to determine the presence of G-MuLV antigens on the cellular membranes the method of Möller (14) was used. The tumors to be assayed were minced and the cells suspended in 199 medium. 0.1 ml of this suspension containing $2 \times 10^6$ cells was added to 0.1 ml anti-G-MuLV serum and incubated for 30 min at 37°C. Subsequently, the cells were washed twice in 199 medium and incubated with 0.1 ml of 1:10 fluorescein-conjugated goat antirat globulin (Hyland Laboratories, Costa Mesa, Calif.) for 1 h at 37°C. After three washings with 199 medium the cells were mounted in 50% glycerol in PBS, coversliped, and examined under a Leitz fluorescence microscope equipped with a BG 12 exciter filter, K 530 barrier filters, and a high pressure HBO 200 W mercury bulb (E. Leitz, Rockleigh, N. J.). The G-MuLV antisera used in all immunologic assays were raised in (W/Fu $\times$ BN)F₁ hybrid rats grafted with GLV-induced W/Fu lymphomas, according to the method of Geering et al. (15).

*Cytoplasmic antigens:* To determine the presence of G-MuLV intracytoplasmic antigens, an indirect immunofluorescence technique was used. As previously described (5, 10) the tissues were minced and the cells suspended in 199 medium at $1 \times 10^6$ cells/ml and placed on microscopic slides which were incubated overnight in a $CO_2$ gassed incubator at 37°C. The cells were then washed in Hanks' solution, dried 1–2 h at 37°C, fixed for 10 min in acetone at room temperature, and used immediately or stored at $-20$°C. Drops of anti-G-MuLV serum at various dilutions were placed over the cells and the cells incubated at 37°C for 1 h, then washed twice with PBS at pH 7.2 and twice in distilled water. The slides were air dried, covered with fluorescein-labeled goat antirat globulin and incubated for 1 h at 37°C. Subsequently, they were washed twice in PBS and once in distilled water. The slides were counterstained with 0.06% Evans' blue stain for 1 min at 37°C, washed twice in distilled water, and dried. They were mounted in 50% glycerol in PBS, coversliped, and examined under a Leitz fluorescence microscope as described (E. Leitz).

*Gel Microimmunodiffusion.*—A modification of the Ouchterlony double-diffusion technique (16) previously described (10) was used for producing immune precipitates in gel. Soluble antigen fractions of tumor tissue were prepared by ultrasonic disruption at 0°C followed by centrifugal clarification at 70,000 $g$ for 2 h. The precipitating anti-G-MuLV homologous antisera were produced as previously described. The double-diffusion tests were performed in IDF cells (Cordis Laboratories Miami, Fla.).

*Antibody Cytotoxicity.*—An established rat thymic culture that permanently replicates the GLV (17) was used as the source for target cells (G cells). The cells were suspended in 1 ml of Puck's medium with 15% fetal bovine serum, assessed for viability with the trypan blue dye test, and adjusted to $1 \times 10^6$ cells/ml. 1 ml of the cell suspension was incubated with 200–300 $\mu$Ci of $^{51}$Cr (sodium chromate specific activity 10 $\mu$Ci/$\mu$g Cr₁ at a concentration of 10 $\mu$Ci/ml; The Radiochemical Centre, Amersham, England) at 37°C for 30–40 min as described by Herberman and Oren (18). The labeled cells were washed twice in 40 ml of Eagle's medium

and adjusted to $1 \times 10^6$ cells/ml, then 0.1 ml was added to 0.1 ml of serial twofold dilutions of anti G-MuLV rat antiserum inactivated at 56°C for 30 min. After incubation for 30 min at 37°C, 0.1 ml of noncytotoxic fresh rabbit serum was added as the source of complement and the incubation continued for another 30 min. 2 ml of cold Eagle's medium were added to each tube, the cells were sedimented by centrifugation at 500 $g$ for 10 min, and then 0.1 ml of the supernatant fluid of each tube was counted for released radioactivity in a Nuclear-Chicago automatic gamma scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.). The release of $^{51}$Cr in counts per minute was considered as the index of cell sensitivity to a known cytotoxic and anti-G-MuLV antiserum. The spontaneous release of $^{51}$Cr in the presence of complement alone was considered as the control baseline. The maximum $^{51}$Cr release was obtained by freezing and thawing the cells four times.

*Complement Fixation.*—The tests were conducted in the laboratory of Dr. R. J. Huebner (National Cancer Institute) according to a technique previously described (19).

*Induction of Focus-Forming RNA Virus.*—Investigation for the presence of rat leukemia virus (RaLV) was made in the laboratory of Dr. R. J. Huebner. The technique of focus formation induction in cloned nonproductive rat cell lines after treatment with 5′-bromodeoxy-uridine, as previously described (20), was used.

*Electron Microscopy.*—1-mm cubes of tumor tissue were fixed in 3% gluteraldehyde solution in 0.075 M sodium cacodylate buffer, pH 7.2, for 24 h at 4°C as previously described (13). Postfixation was carried out in 1% osmium tetroxide in veronal acetate buffer, pH 7.2, for 2 h. After dehydration in a series of graded ethanol solutions, tissue fragments were embedded in Epon. Sections were cut on an LKB ultrotome (LKB Instruments, Inc., Rockville, Md.) and thin sections were double stained with uranylacetate. The grids were examined in a Siemens Elmiskop 101 (Siemens Corp., Medical Industrial Div., Iselin, N. J.) and photographed at an initial magnification of 6,000–20,000 diameters.

## RESULTS

Rats injected at birth with soluble antigen and matched normal controls were grafted subcutaneously at age 75–120 days with GLV-induced lymphomas. In three separate experiments, a total of 10 recipients conditioned at birth with different pools of soluble antigens were grafted with GLV-induced lymphomas. Nine accepted the grafts which grew progressively and killed the recipients in contrast to all of 16 normal controls which rejected the same transplants. The growing tumors were then grafted into normal-nonconditioned adult W/Fu rats and the grafts took in 17 of 23, while none of 12 grafts of primary lymphomas used as controls took in similar normal recipients (Table I). Subsequently, the tumors originally grafted in neonatally conditioned rats became serially transplantable in nonconditioned recipients. In eight successive tumor generations a total of 146 tumors were grafted in normal adult recipients and 125 were accepted. The percentage of successful takes generally increased with the number of the tumor generation. However, qualities intrinsic to the original tumor seemed to be of even greater importance for future transplantability, since some lines of tumors resulted in less than 100% takes while others showed total takes in all grafts throughout their tumor generations. Thus, in the first experiment in which two lines of transplantable tumors originating in two-conditioned rats were followed, the rate of takes was 19/27 (70.3%) in the first line and 84/84

TABLE I

Correlation of G-MuLV Antigens with Transplantability of Lymphoma Cells

| Recipient | Transplant | | | | | Results | | |
| | Origin | Antigenicity | | | | L/T* | % | G‡ |
| | | MF§ | CF‖ | ID¶ | No.** | | | |
|---|---|---|---|---|---|---|---|---|
| Normal control | GLV-lymphoma | + | + | + | 16 | 0/16 | 0.0 | — |
| Conditioned at birth | GLV-lymphoma | + | + | + | 10 | 9/10 | 90.0 | $L_1$ |
| Normal—tr. gener. 1 | Lymphoma—$L_1$ | − | + | + | 7 | 17/23 | 73.9 | $L_2$ |
| Normal—tr. gener. 2 | Lymphoma—$L_2$ | − / − | + / − | + / − | 5 / 9 | 33/39 | 84.6 | $L_3$ |
| Normal—tr. gener. 3 | Lymphoma—$L_3$ | − / − | + / − | + / − | 2 / 9 | 32/38 | 84.2 | $L_4$ |
| Normal—tr. gener. 4 | Lymphoma—$L_4$ | − / − | + / − | + / − | 4 / 21 | 22/24 | 91.7 | $L_5$ |
| Normal—tr. gener. 5 | Lymphoma—$L_5$ | − / − | + / − | + / − | 6 / 23 | 13/13 | 100 | $L_6$ |
| Normal—tr. gener. 6 | Lymphoma—$L_6$ | − / − | + / − | + / − | 0 / 13 | 9/9 | 100 | $L_7$ |
| Normal—tr. gener. 7 | Lymphoma—$L_7$ | − / − | + / − | + / − | 0 / 9 | 6/6 | 100 | $L_8$ |

* L, lymphoma takes; T, total lymphoma transplanted.
‡ G, successful grafts used for subsequent transplantation.
§ MF, membrane immunofluorescence.
‖ CF, cytoplasmic immunofluorescence.
¶ ID, gel microimmunodiffusion.
** number of transplants assayed. Example: recipients in transplant generation 2 were grafted with lymphoma $L_2$ which was MF−, CF+, ID + in five animals tested and MF−, CF−, ID− in nine animals tested. 33 of 39 grafts took (84.6%). These tumors designated $L_3$ were subsequently grafted in recipients tr. gener. 3. Two of them were MF−, CF+, ID+ and nine were MF−, CF−, ID− etc.

(100%) in the second. In the second experiment the rates of takes were 6/16 (37.5%) in one tumor line and 16/19 (84.2%) in another.

To correlate tumor transplantability with antigenic experession, all graft recipients were examined for the presence and amount of anti-G-MuLV antibodies and all the tumors were studied for cellular antigenicity using five different assays. The lymphoma cells originally grafted in neonatally conditioned rats showed numerous GLV particles under the electron microscope (Fig. 1) and were strongly positive in indirect immunofluorescence for both membrane (Fig. 2) and cytoplasmic (Fig. 3) G-MuLV antigens. Antigens of these cells also reacted positively with anti-G-MuLV antiserum in gel microimmunodiffusion tests, antibody cytotoxicity assays, and complement fixation tests.

When the tumors which grew in the neonatally conditioned recipients were
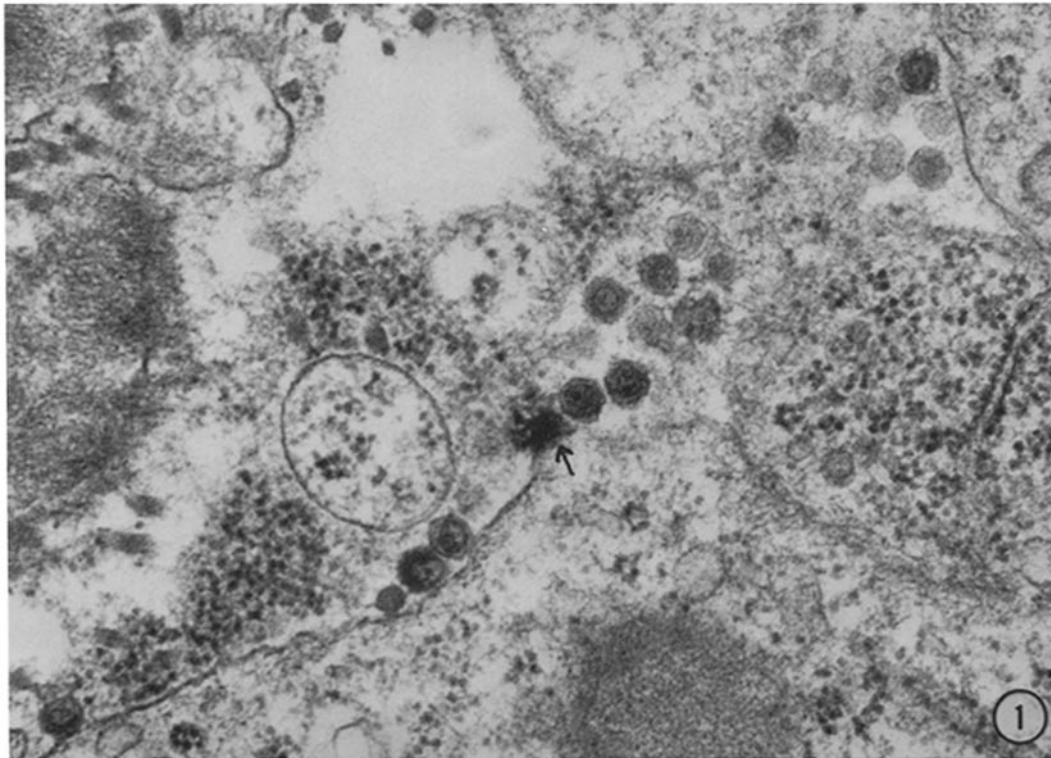
FIG. 1. GLV-induced thymic lymphoma cell showing numerous C-type virus particles at the plasma membrane and in the intercellular space. Budding virus particle at arrow. × 60,000

now tested in indirect immunofluorescence it was found that membrane and cytoplasmic G-MuLV antigenicity had dissociated, and that the cells were still displaying positive cytoplasmic fluorescence while the membrane fluorescence was no longer present. As the tumors were further transplanted it appeared that the membrane antigenicity had been permanently lost. The cytoplasmic fluorescence, indicating G-MuLV antigenicity, persisted in the serially transplantable tumors for two to three more generations and similarly disappeared thereafter. At one point in every experiment, after a number of transplant generations (3–6) all the transplanted tumors became negative for both membrane and cytoplasmic antigenicity in indirect immunofluorescence. The loss of antigenic expression in the transplanted tumor cells was confirmed by gel microimmunodiffusion, complement fixation, and antibody cytotoxicity tests. All these assays were consistently negative and no indication of persistent G-MuLV antigenicity was detected. Attempts to explant these cells in tissue cultures were generally unsuccessful and a few cultures which were grown for intervals of up to 90 days did not show recovery of cellular antigens.
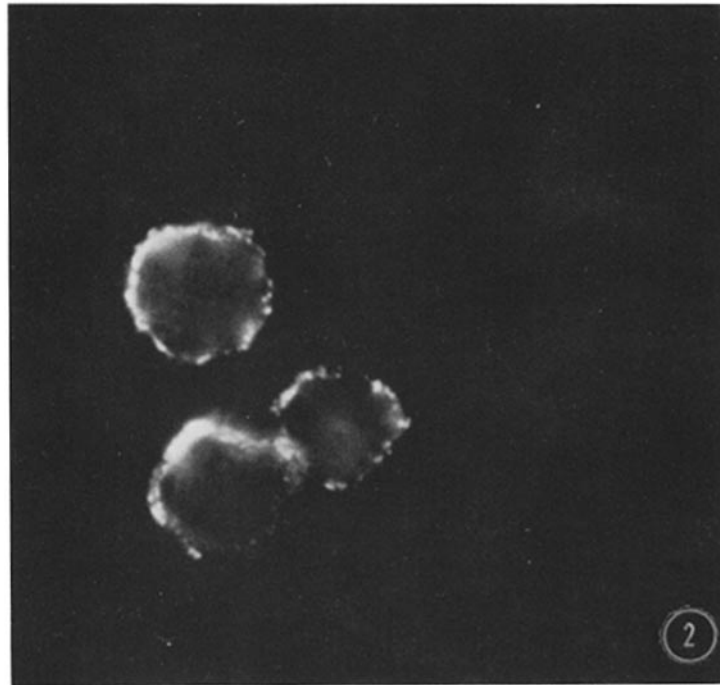
FIG. 2. GLV-induced thymic lymphoma cells showing positive membrane reaction in indirect immunofluorescence after staining with anti-G-MuLV antiserum. × 2,100

The loss of G-MuLV antigenic expression occurred progressively: that of membrane antigens in the first transplant generation and that of cytoplasmic antigens in an increasingly greater number of transplanted tumors in later generations. The acceptance of tumor grafts consistently correlated with antigenic expression. Tumors with positive membrane antigenicity such as primary GLV-induced lymphomas were rejected in close to 100% of the recipients, tumors with absence of both membrane and cytoplasmic antigenic expression were accepted in 100% of the recipients, and tumors with antigenic disjunction, membrane negative and cytoplasmic positive, were accepted in 37.5–84.2% of the recipients (Table II).

Sera of normal-nonconditioned rats who had accepted transplantable tumors were assayed for the presence of anti-G-MuLV antibodies in parallel with rats that had rejected primary GLV-induced lymphomas. It was found that normal-nonconditioned rats bearing the transplantable tumors that showed negative membrane fluorescence and positive cytoplasmic fluorescence, had sera with high anti-G-MuLV antibody titers (Table III). Such titers displayed a range of 1:8–1:1,024 with a mean reciprocal titer of 80 in indirect immunofluorescence, which was considerably higher than rats that rejected tumors with both positive

membrane and cytoplasmic antigenicity. Even more surprising, rats grafted with tumors showing, for the first time in their serial transplantation, absence of both membrane and cytoplasmic antigenicity still showed high anti-G-MuLV antibody titers with a mean reciprocal titer of 340. However, after two to three consecutive generations of negative membrane and cytoplasmic antigenicity, serum anti-G-MuLV antibody titers declined to ranges of 1:4–1:64 with a mean reciprocal titer of 20. The high titers of antibodies recorded in immunofluorescence were consistently confirmed by parallel microimmunodiffusion, antibody
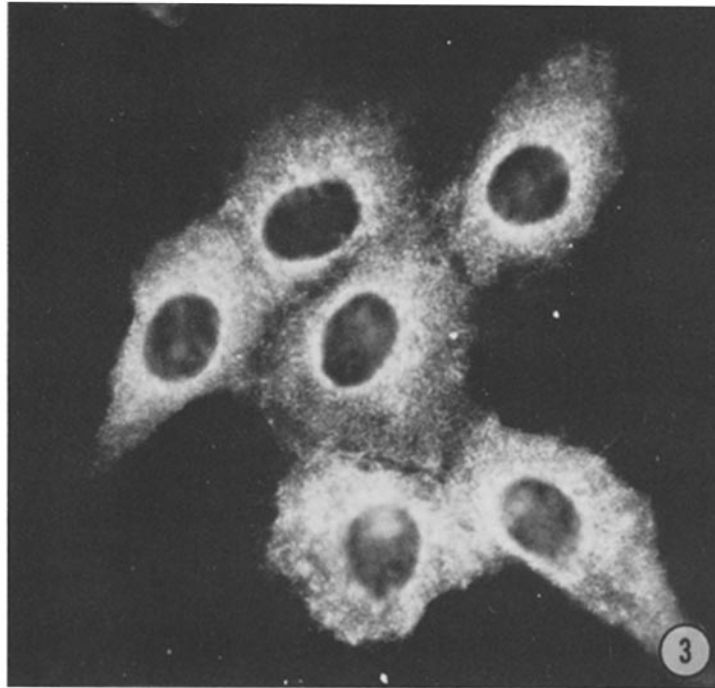


FIG. 3. GLV-induced thymic lymphoma cells showing positive cytoplasmic reaction in indirect immunofluorescence after staining with anti-G-MuLV antiserum × 2,000

TABLE II

Correlation of Transplantability with Membrane and Cytoplasmic Indirect Immunofluorescence Test

| Immunofluorescence | Transplantability | |
|---|---|---|
| | L/T* | % |
| membrane + cytoplasm + | 1/28 | 3.5 |
| membrane − cytoplasm + | 36/55 | 65 |
| membrane − cytoplasm − | 89/91 | 98 |

* L, lymphoma takes; T, total lymphoma transplanted.

cytotoxicity, and complement fixation tests. Assays for the identification of a possible RaLV by the focus-forming assay (20) were negative.

## DISCUSSION

The normal immunologic response of an animal to a specific antigen can be altered under a variety of conditions. A large number of reports dealing with this subject indicate its interest for both tumor and transplantation biology.

When antigen is administered in early life, before the acquisition of immuno-competence, the animal is rendered tolerant and will not react to that specific antigen when exposed to it later in life (21). When antigen in high dosage is given to an adult animal, a state of partial immunologic paralysis may ensue (22). Finally, when animals receiving a tumor homograft are preinjected with killed tumor tissue the growth of the tumor is markedly enhanced (23, 24). This effect can be passively transferred to new hosts with serum (23) or immunoglobulins (25) of pretreated recipients. The mechanism of tumor enhancement has long been debated and alternately attributed to either (a) blocking by antibodies of the development of cellular immunity in the regional lymph nodes (24, 26, 27), or to (b) some unknown physiological alteration in the tumor induced by its contact with the specific antibodies (23).

TABLE III

*Correlation of G-MuLV Antigens, Transplantability, and Anti-G-MuLV Sera Titers in Indirect Immunofluorescence Tests*

| Recipient | Transplant | | | | Sera (mean)‡ |
| | Origin | Antigenicity | | Takes* | |
| | | MF§ | CF‖ | | |
|---|---|---|---|---|---|
| Normal control | GLV-lymphoma | + | + | 0/11 | 43.1 |
| Conditioned at birth | GLV-lymphoma | + | + | 9/10 | 64.0 |
| Normal—tr. gener. 1 | Lymphoma—L₁ | − | + | 2/2 | $2.0/457.3$ |
| | | − | − | 7/7 | $587.4$ |
| Normal—tr. gener. 2 | Lymphoma—L₂ | − | + | 4/6 | $258.6/359.5$ |
| | | − | + | 21/21 | $250.6$ |
| Normal—tr. gener. 3 | Lymphoma—L₃ | − | + | 4/8 | $55.2/152.5$ |
| | | − | − | 23/23 | $181.2$ |
| Normal—tr. gener. 4 | Lymphoma—L₄ | − | + | 6/8 | $50.6/49.0$ |
| | | − | − | 16/16 | $48.4$ |
| Normal—tr. gener. 5 | Lymphoma—L₅ | − | − | 2/2 | $64.0/39.0$ |
| | | − | − | 11/11 | $36.0$ |
| Normal—tr. gener. 6 | Lymphoma—L₆ | − | − | 1/1 | $0.0/40.0$ |
| | | − | − | 8/8 | $50.0$ |

\* Takes, lymphoma takes/total lymphoma transplanted.
‡ Means, mean reciprocal titers.
§ MF, membrane immunofluorescence.
‖ CF, cytoplasmic immunofluorescence.

The physical form in which antigen is administered was shown to be of essential importance (28–30). Living cells as well as antigens in a particulate form are immunogenic and able to sensitize adult immunocompetent animals. However, similar recipients do not react to deaggregated antigens which induce a state of complete and prolonged immunologic unresponsiveness to subsequent challenge of the aggregated antigen (28–31).

In the present work, newborn rats were injected with deaggregated G-MuLV antigen and challenged later in life with grafts of lymphoma cells expressing the full complement of G-MuLV antigens. The cells grew progressively killing the host in contrast to their rejection which occurred consistently in normal-non-conditioned control animals. Similar results were obtained by Kobayashi et al. (32), who reported that rats injected at birth with live Friend, Gross, or Rauscher virus were tolerant to transplants of each particular tumor.

Before attempting to classify this situation into one of the categories previously mentioned, an additional feature must be taken into account. The grafted lymphoma cells able to grow in the apparently unresponsive-conditioned hosts incurred the loss of membrane antigens, which was readily detectable by immunofluorescence tests. If we assume that these changes occurred under immune pressures from the host, it appears that the conditioned rats although unable to reject the grafted lymphoma cells did respond in a way that induced the loss of cellular surface antigens.

This situation is therefore unlike both immunologic tolerance (28) and immunologic paralysis (22) where the lack of any responses are assumed. The absence of a complete immunologic tolerance as classically described was also reported by Oldstone et al. (33) to occur in AKR mice, which despite the persistence throughout their life of vertically acquired Gross virus are still able to form specific anti-G-MuLV antibodies. There are also difficulties in explaining the failure of graft rejection in the conditioned rats by immunologic enhancement when this is conceived as a blockade of antigenic sites by specific antibodies raised by the host (24, 26, 34). As pointed out by Medawar (28), immunologic enhancement being mediated through antibody depends on the success of immunization and not on its failure. However, enhancement in the interpretation of Kaliss (23) represents some "physiological" alteration in the tumor, induced by its contact with antiserum which insures its survival despite the hostile responses of the host. An alternative to this concept, possibly applicable to the present situation, may be that the immune response of the host forces a clonal selection among the grafted tumor cells.

We have selected the term immunologic tolerance and qualified it as partial and specific to describe the immune status of rats injected at birth with soluble G-MuLV antigen. These animals are tolerant to progressive tumor growth, however, the tolerance is only partial since they are still able to induce permanent antigenic changes in the grafted lymphomas. The changes result in the emergence of a new population of lymphoma cells that are better equipped, by their

loss of specific surface antigens, to survive in the hostile environment of an immunocompetent host. In further transplant generations, in normal-nonconditioned immunocompetent recipients, the lymphoma cells protected by their lack of surface antigens grew progressively, however, further antigenic changes occurred, this time expressed by the loss of the internal G-MuLV antigens.

This was a finding of particular interest, not previously reported in other systems, i.e., that the loss of antigens occurred progressively in the course of serial transplantation and resulted in a temporary disjunction between surface and internal antigenic components. Concerning other cases of antigenic disjunction an example may be provided by the expressions of RNA viral envelope, group specific antigens, and oncogene which appear independently (35). However, even in this case they all are quite as likely to be expressed at the same time.[2] In the present experiments antigenic losses were paralleled by progressive increase in transplantation takes, which rose from 3.5% in cells with positive membrane and cytoplasmic antigens, to 65% in cells with dissociation of antigenic expression, and to 98% in cells with total absence of antigenic determinants. It is not clear at the present time if these antigenic deletions have occurred by repression of antigen synthesis or by immune selection of lymphoma cells devoid of antigenic expression. Similarly, we do not know if these antigenic losses are still reversible as in our earlier experiments (5) where antigenic expression returned after explantation in vitro.

The unusually high titers of anti-G-MuLV antibodies detected in sera of rats bearing transplants of membrane-negative lymphoma cells were rather unexpected. Even more so were sera titers that continued to rise above normal in animals with both membrane and cytoplasmic G-MuLV-negative grafts. This was in sharp contrast with much lower anti-G-MuLV antibody titers in rats bearing lymphoma transplants showing the presence of both membrane and cytoplasmic antigens which were regularly rejected. A possible explanation for this apparent inconsistency may be the fact that lymphoma grafts lacking surface antigenic determinants grew to huge proportions and were carried for long intervals, in contrast to lymphoma grafts expressing G-MuLV antigens which were readily rejected. If very small, perhaps undetectable, amounts of antigens were still present on the former cells then the large number of cells and long duration of grafts may be accountable for the high rise in antibody titers. Another possible explanation would be that the antibodies were in response to different cell surface antigens (36) associated with GLV or perhaps with RaLV membrane antigens,[2] although assays for the identification of RaLV have remained negative.

Notwithstanding, in later transplant generations, when both surface and internal antigens had already been absent for one to three passages, the antibody titers of tumor-bearing animals consistently fell to baseline levels. There are

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[2] R. J. Huebner, personal communication.

presently numerous transplantable tumors available in our laboratories. These tumors which have been around for many years and are the result of numerous successive transplantations have two features in common: lack of any viral antigenic expression and a high rate of transplantability.

It seems reasonable to suggest, in light of the experiments reported here, that most of these tumors were originally virus induced and initially displayed viral-associated antigens. Attempts to transplant such tumors must have failed in all but those recipients which displayed either partial tolerance acquired at birth or temporary immunologic unresponsiveness induced by various agents during adult life. After the first transplant was thus established, immunological selection of antigen-negative clones of cells or immunological repression of antigen production made subsequently possible the successful serial grafting of these tumors. This system, together with recent evidence from work with Burkitt lymphoma cells in tissue cultures (37) and thymusless mice (6) may suggest that human tumor cells have undergone a similar antigenic evolution.

## SUMMARY

Gross virus-induced lymphoma cells express strong virus-associated (Gross murine leukemia virus [G-MuLV]) antigens and are consistently rejected when grafted in normal adult syngeneic rats. By contrast, similar grafts are tolerated and allowed to grow progressively by rats that have been injected at birth with deaggregated G-MuLV antigens. However, the tolerance induced by this procedure is only partial as the grafted lymphoma cells lose their G-MuLV membrane antigens. These cells showing an antigenic disjunction, with negative membrane and positive cytoplasmic G-MuLV antigenic expression, become transplantable in normal-nonconditioned adult recipients. By further grafting, the expression of cytoplasmic G-MuLV antigens is similarly lost while the lymphoma cells substantially increase their transplantability, rate of growth, and capacity for metastasis.

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