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IMMUNE REACTIVITY OF THE TUMOR-RESISTANT X/Gf MOUSE

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Parameters of immune reactivity of the tumor-resistant X/Gf mouse have been studied. X/Gf mice show better survival than do control strains following i.p. inoculation with graded doses of YAC lymphoma cells. The natural killer cell activity of X/Gf mice was higher than that of control mice, and this elevated NK activity was inherited by (C57BL/6 × X/Gf)F_1 mice. Differences in NK activity of fresh spleen cells from X/Gf, C57BL/6, and (C57BL/6 × X/Gf)F_1 mice were not reflected in their ability to lyse *in vitro*-derived, cloned, transformed cell lines from X/Gf or C57BL/6 mice in an 8 h ^{51}Cr assay; however, the X/Gf-derived malignant cells were more sensitive to lysis than the C57BL/6-derived cells. In order to test whether X/Gf mice demonstrated any exceptional ability to recognize and destroy altered autologous cells, experiments were performed with TNP-modified syngeneic cells. While X/Gf and B10.A effector cells appeared to respond with similar magnitude, hapten-modified X/Gf cells were stronger stimulator cells and more sensitive targets than control strain cells. The ability of X/Gf mice to reject H-2 compatible and incompatible skin grafts is normal, as judged by a comparison of the rejection times with control strains. Allogeneic mixed lymphocyte reactivity, T-cell-mediated lympholysis, delayed-type hypersensitivity reactions, and *in vivo* phagocytosis of inert particles are of comparable magnitude in X/Gf and control strain mice.

Inbred X/Gf mice, established by Goldfeder (Staats, 1980) have a very low incidence of spontaneous tumors (Goldfeder, 1974) and have been reported to be resistant to tumor induction by chemical carcinogens (Goldfeder *et al.*, 1966; Goldfeder, 1972, 1974), X-irradiation (Goldfeder, 1962, 1972, 1974; Goldfeder *et al.*, 1966) and combined treatment with X-irradiation and urethan (Goldfeder *et al.*, 1966; Goldfeder, 1972). We have demonstrated that the tumor resistance of the X/Gf mice is not based on the resistance of isolated cells from these animals to neoplastic transformation, but rather is attributable to an *in vivo* homeostatic mechanism which prevents tumor growth (Nahas *et al.*, 1983). Experiments performed to elucidate possible mechanisms of tumor resistance in the X/Gf mice have been along two lines, immunological and biochemical. Studies by Stern and Goldfeder (1971) demonstrated that X-irradiation had an equivalent effect on antibody production in X/Gf mice and in control strains. In the presence of growing transplantable tumors, *in vivo* phagocytosis of chromium-labelled foreign erythrocytes was less markedly suppressed in these mice than in control strains (Stern and Goldfeder, 1969). Goldfeder reported that X/Gf mice were resistant to hepatoma induction by *N*-2-fluorenylacetamide (Goldfeder, 1974). Weisburger *et al.* examined the possible difference in metabolism of *N*-2-fluorenylacetamide and *N*-hydroxy-*N*-2-fluorenylacedamide between X/Gf and control mice (Grantham *et al.*, 1976; Reddy *et al.*, 1980) and found no biochemical differences to which the resistance of X/Gf mice to the oncogenic effect of this carcinogen could be attributed. Both these groups have suggested that the resistance of X/Gf mice to oncogenesis may be attributable to differences in immunological reactivity between these mice and other strains. We have initiated a study of the immune reactivity of X/Gf mice in order to assess whether their apparent resistance to oncogenesis can be attributed to immunological parameters.

MATERIAL AND METHODS

Mice

The X/Gf (H-2^a) mice from which our colony originated were a gift from Dr. Anna Goldfeder (New York University) in 1972. Since that time we have maintained and expanded an inbred colony by sister-brother matings. C57BL/6J (H-2^b), B10.A (H-2^a) and A/J (H-2^a) mice of both sexes were purchased as weanlings from the animal breeding facility of the Weizmann Institute of Science, Rehovot, Israel. (C57BL/6 × X/Gf)F_1 mice were bred in our laboratory. All experiments were performed on 8- to 15-week-old mice.

Skin graft rejection

Full-depth skin grafts were performed on female mice according to Garvey *et al.* (1977). On day 8 after grafting, coverings were removed under light ether anesthesia, and all animals were observed daily thereafter to determine graft rejection time.

Mixed lymphocyte cultures

Cultures were performed in plastic test tubes (Corning No. 25200) in 1 ml MEM supplemented with 10% FCS-D, 0.15 mM L-asparagine, 5×10^{-5} M 2-mercaptoethanol, penicillin and streptomycin. Each culture contained 1×10^6 responder lymph-node cells (X/Gf, A/J, or C57BL) and 1×10^6 X-irradiated (1,000 R) stimulator spleen cells (X/Gf, A/J, or C57BL) in 1 ml medium. Irradiation of stimulator cells was performed by a Phillips MG-101 constant potential X-ray unit. Cultures were maintained in a water-saturated atmosphere of 5% CO_2 in air. At daily intervals, triplicate cultures were labelled with 1 μC ^{3}H-thimidine (2 Ci/mmole) for 24 h and harvested on GF/C filters (Whatman). Scintillation counting was performed in PPO-POPOP-toluene and monitored by a Packard-Tricarb scintillation counter.

Abbreviations: BSS, balanced salt solution; CS-D, Heat-inactivated calf serum; DNFB, dinitrofluorobenzene; DTH, delayed-type hypersensitivity; FCS-D, heat-inactivated fetal calf serum; MEM, Eagle's minimal essential medium; MHC, major histocompatibility complex; MLC, mixed leukocyte culture; NK, natural killer; PHA, phytohemagglutinin; TNBS, trinitrobenzene sulfonic acid; TNP, trinitrophenol.

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Allogeneic T-cell-mediated cytotoxicity

Mixed lymphocyte cultures for the generation of T-cytotoxic cells were performed in 100 × 14 mm plastic tissue-culture tubes (NUNC) (Denmark): 3×10^6 responder spleen cells from X/Gf, A/J, or B10.A female mice were incubated with either 6×10^5 or 1.5×10^5 EL_4 stimulator cells in 3 ml MEM plus 10% CS-D supplemented with 5×10^{-5} M 2-mercaptoethanol, thus giving responder-to-stimulator ratios of 20:4 and 20:1, respectively. On day 5, mixed lymphocyte cultures were harvested. Viable cells were counted and distributed as effector cells (6×10^4, 20×10^4 or 60×10^4) into the wells of U-bottomed 96-well microtest plates (NUNC) and incubated for an additional 4 h with 2×10^4 ^{51}Cr-labelled EL_4 target cells in a total volume of 150 µl. ^{51}Cr labelling of target cells was performed as previously reported (Walters and Claman, 1975). The assay was terminated by centrifugation of the microtiter plates at 200 *g* for 10 min, then 100 µl of supernatant medium were removed from each micro-well and counted in a Packard Auto-Gamma scintillation counter. Control cultures included chromium-labelled EL_4 cells incubated alone for spontaneous ^{51}Cr release and thrice frozen and thawed labelled EL_4 cells for maximal ^{51}Cr release. Percentage specific cytotoxicity was calculated as:

$$\frac{\text{Experimental release} - \text{spontaneous release}}{\text{Maximal release} - \text{spontaneous release}} \times 100$$

Delayed-type hypersensitivity (DTH)

Mice were sensitized with 2,4-dinitrofluorobenzene (Fluka) (Switzerland) (DNFB) in acetone-olive oil (4:1) solvent according to the procedure of Walters and Claman (1975) and, 4 d later, challenged by painting the dorsal side of the right ear with 20 µl of various concentrations of DNFB in acetone-olive oil. Control mice were sensitized and challenged with the solvent alone. Ear thickness of the experimental and control groups was measured daily with a micrometer. The ear swelling index was calculated as:

$$\frac{\text{Average ear thickness of experimental group}}{\text{Average ear thickness of control group}}$$

Phagocytosis in vivo

Mice were injected i.v. with 0.2 ml of a suspension of carbon (a 1:4 dilution of a suspension of Pelikan ink C 11/1431a). At timed intervals thereafter, individual mice were bled from the retro-orbital plexus, and 25 µl of blood were diluted in 2 ml of a 0.1% solution of Na_2CO_3 and read in a spectrophotometer at 675 m. Standard curves for dilution of carbon were made for each experiment by making two-fold serial dilutions of Pelikan ink in 0.1% Na_2CO_3, starting with a 1:400 dilution. The amount of carbon in each blood sample was determined, and the rate of clearance of carbon from the blood was established by plotting the log of blood carbon readings against the time after carbon injection. Straight lines were drawn through the resulting points by calculating linear regression by means of least squares. Correlation coefficients were calculated for these lines and the slopes of the lines were also calculated.

In vitro *growth of tumor lines and tumor graft rejection*

YAC and its subline YAC_1 are NK-sensitive (H-2^a) T-cell lymphomas. The YAC_1 subline is particularly sensitive to NK activity. EL_4 is a T-cell lymphoma of C57BL origin; P815, a DBA_2 mastocytoma; and YBA, a CBA-derived lymphoma. All tumor lines were grown in suspension culture in MEM plus 10% calf serum in 60-mm Petri dishes. Cultures were passaged twice a week. Clones of chemically induced, malignantly transformed embryonic fibroblasts from X/Gf and C57BL/6J mice, induced in our laboratory, were maintained *in vitro* in MEM plus 10% calf serum, and passaged twice weekly (Nahas *et al.*, 1983).

Mice of appropriate strains were inoculated i.p. with graded doses of YAC tumor cells and observed daily for ascites development and mortality.

In vitro *assay for NK activity*

NK activity was measured against ^{51}Cr-labelled tumor cells in 4- to 9 h assays *in vitro*. Effector spleen cell preparation, target cell labelling and assay conditions were the same as those described above for T-cell-mediated cytotoxicity. Briefly, pooled effector spleen cells collected from two or three mice were added to ^{51}Cr-labelled target cells in U-shaped microtiter wells at an effector-(2×10^6) to target-cell (2×10^4) ratio of 100:1. Plates were incubated at 37° C in 5% CO_2 in air and then centrifuged at 200 *g* for 10 min. Supernates of 100 µl were removed, the amount of ^{51}Cr release was determined, and the percentage specific cytotoxicity calculated.

Cell-mediated cytotoxicity to TNP-modified MHC identical cells

Spleen cell suspensions were prepared from the spleens of X/Gf and B10.A mice. Erythrocytes were lysed with 0.83% ammonium chloride in tris-HCl buffer, then the remaining cells were washed three times with cold Hanks' BSS and suspended in RPMI containing 10% fetal calf serum, 1.5×10^{-4} M L-asparagine, 1.5×10^{-5} M mercaptoethanol, penicillin and streptomycin. The spleen-cell suspensions were divided into two. One aliquot was used as responder cells, and the other, used as target cells, was irradiated (1,000 R) and treated with 1 mM TNBS, pH 7.2 in saline, for 10 min at 37° C with intermittent shaking. The target cells were then washed three times with

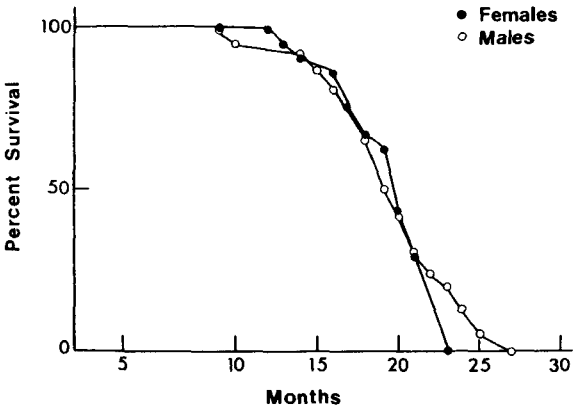

FIGURE 1 – Mortality of X/Gf mice. Survival curves are based on 21 female and 45 male X/Gf mice.

30 ml Hanks' BSS. Viable cells were counted with trypan blue. One-way-mixed leukocyte cultures (MLC) for the generation of cytotoxic effector cells were established by mixing 3×10^6 untreated spleen cells with 6×10^5 target cells in 3 ml medium. Controls consisted of spleen cells incubated under identical conditions without the addition of stimulator cells. Cultures were incubated for 6 days, viable cells were counted, and cytotoxicity assays were performed against TNP-modified B10.A or X/Gf blasts. Blasts were spleen cells (2×10^6) incubated for 36 h with 5 μg/ml PHA in a final volume of 3 ml, then treated with TNBS as above and labelled with ^{51}Cr. The cytotoxicity assay was performed as for allogeneic T-cell cytotoxicity at an effector-to-target cell ratio of 30:1.

RESULTS

Life span and mortality of X/Gf mice

Goldfeder has reported that the life span of X/Gf mice in her laboratory is approximately 2 years (Goldfeder *et al.*, 1966). In order to evaluate and compare our colony to the original one maintained by Goldfeder, progeny of the third generation of breeding in our laboratory were set aside for the established of life-span curves. Mice were segregated according to sex (21 females and 45 males) and caged 5-8 mice per cage with free access to water and food. Throughout their natural lifespan the mice were maintained with the same cage partners, and no regrouping was performed. The mean life span of our colony for both males and females was 19 months, with a maximum of 23 months for females and 27 months for males (Fig. 1).

Tumor graft rejection

The ability of X/Gf mice to reject tumor grafts was compared to that of control strains. These experiments were performed with YAC, an A/J-derived T-cell lymphoma which grows in H-2^a hosts. A/J, B10.A and X/Gf mice were injected i.p. with graded doses of YAC and observed for ascites formation and mortality (Fig. 2). While all the mice succumbed to the lethal growth of the YAC lymphoma at all cell doses tested (1×10^4-1×10^6), X/Gf mice were more resistant, as defined by maximum and 50% survival points, than were the A/J and B10.A mice. Even though YAC is syngeneic to A/J, these last two strains succumbed with similar mortality curves.

Natural killer cells

X/Gf mice are more resistant than control strains to the growth of transplanted YAC lymphoma cells (Fig. 2) and *in vitro*-derived carcinogen-induced sarcomas (Nahas *et al.*, 1983). This resistance is hereditary in that (C57BL/6 × X/Gf)F_1 mice survive inocula of *in vitro*-derived carcinogen-induced sarcomas of C57BL/6 origin at cell doses which are lethal to the C57BL/6 parental mice; moreover, their patterns of survival against X/Gf-derived sarcomas resemble those of X/Gf mice (Nahas *et al.*, 1983).

Since the YAC lymphoma is susceptible to the lytic activity of NK cells, experiments were performed with X/Gf and control strain mice to determine their natural killer cell activity and examine the role of natural killer cells in their tumor resistance. In experiments performed with YAC_1, a highly NK-susceptible subline of

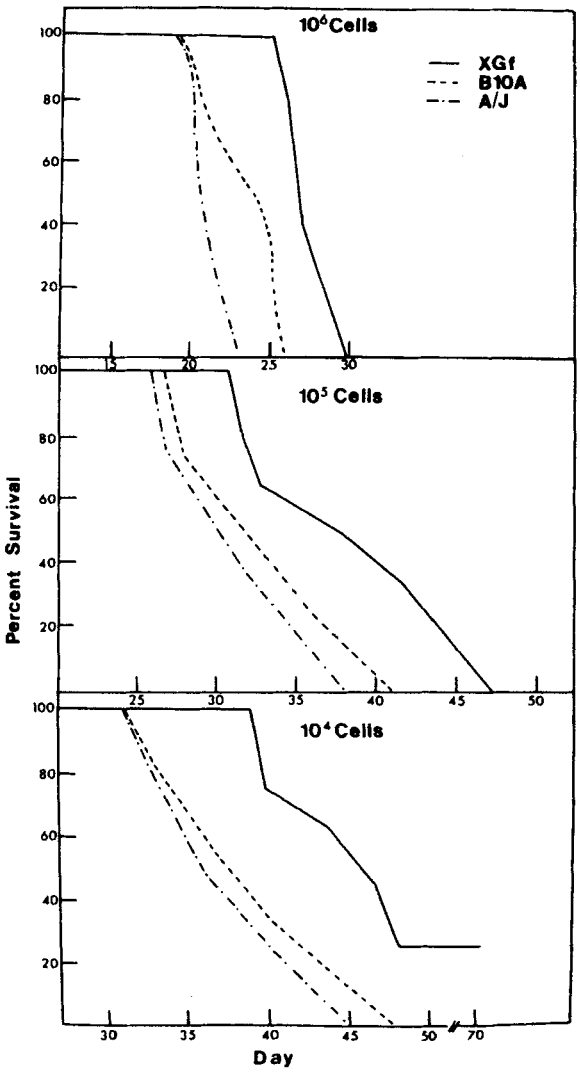

FIGURE 2 – Mortality of X/Gf, B10.A and A/J mice after i.p. inoculation with YAC lymphoma. Groups of eight female X/Gf, B10.A, or A/J mice aged 2-2½ months, inoculated i.p. with 10^4-10^6 YAC lymphoma cells, were examined daily for ascites growth and mortality.

the YAC lymphoma, YBA, a weakly sensitive NK target, and two NK-resistant targets, P815 and EL_4, X/Gf spleen cells demonstrated decidedly higher NK activity than did C57BL/6 or B10.A controls (Table I).

Since X/Gf mice demonstrate a high level of NK activity, which may be related to their ability to reject tumor grafts, comparisons were made between the NK activity of X/Gf, C57BL/6 and (C57BL/6 × X/Gf)F_1 spleen cells toward the YAC_1 lymphoma and toward six *in vitro*-derived carcinogen-induced sarcomas, (three of X/Gf origin and three of C57BL/6 origin) (Nahas *et al.*, 1983) (Table II). The X/Gf mice demonstrate higher NK activity toward YAC_1 than did C57BL/6 mice. (C57BL/6 × X/Gf)F_1 mice show NK activity which is intermediate between that of the two parental strains.

These differences in NK activity were not, however, reflected in a differential lysis by X/Gf, C57BL/6, or

TABLE I – NATURAL KILLER CELL ACTIVITY

Spleen cell donor	Percentage specific cytotoxicity against			
	YAC_1	YBA	P815	EL_4
X/Gf	77	12.5	0	0
	56	—	0	0
	76	12	0	0
C57BL/6	48	10.9	0	0
	39	—	0	0
	47	9.9	0	0
B10.A	41	9.9	0	0
	24	—	0	0
	40	9.7	0	0

Percentage specific cytotoxicity at an effector:target ratio of 100:1 after a 4 h incubation. Results of three separate experiments.

$(C57BL/6 \times X/Gf)F_1$ spleen cells of any of the six *in vitro*-derived carcinogen-induced transformed clones. It does appear, however, that all three X/Gf-derived clones are more susceptible to lysis, regardless of the strain of origin of the effector cells, than are the C57BL/6 clones. Similar conclusions as to the susceptibility of X/Gf cells to cell-mediated lysis were obtained in experiments on T-cell cytotoxicity to hapten-modified syngeneic cells (see below).

T-cell cytotoxicity to TNP-modified syngeneic cells

The capacity of X/Gf spleen cells to generate T-cytotoxic cells lytic for hapten-modified H-2 identical cells was examined *in vitro* and compared to that of B10.A spleen cells. These experiments were performed by *in vitro* sensitization of X/Gf and B10.A spleen cells, each to irradiated X/Gf or B10.A TNP-modified spleen cells. Subsequently, effector cells from each of the four sensitization combinations were tested for their lytic activity against TNP-modified X/Gf and B10.A PHA-induced blasts in a 4-h ^{51}Cr release assay. This series of experiments provided a means of comparing (1) the relative ability of X/Gf spleen cells to mount a T-cytolytic response to modified H-2 compatible cells, (2) the relative stimulatory efficiency of TNP-modified X/Gf cells, and (3) the relative sensitivity to lysis of TNP-modified PHA-blasts of X/Gf origin (Table III). For the sake of comparison, the results of these experiments have been expressed as the percentage of a particular response which was considered to be 100%. Comparison A relates all other responses to the fully homologous system of X/Gf responders, stimulators, and targets. It appears from this comparison that the responses of X/Gf and B10.A cytolytic T cells are alike when cultures with equivalent stimulator and target cells are compared. Comparison B relates all the results obtained with a specific target to the response of X/Gf spleen cells with X/Gf stimulator cells to the same target. This comparison emphasizes the finding that, whereas the lytic responses of X/Gf and B10.A are equal under comparable conditions of stimulator and target, X/Gf-TNP spleen cells are stronger stimulator cells than are B10.A-TNP spleen cells. In comparison C, the lytic activity against X/Gf-TNP target cells was considered to be 100% for each of the four different responder-stimulator combinations. This comparison emphasizes the finding that X/Gf-TNP target cells are consistently more sensitive to lysis (i.e., are better targets) than are the B10.A-TNP target cells.

TABLE II – SENSITIVITY OF CARCINOGEN-INDUCED *IN VITRO*-DERIVED CLONED TRANSFORMED CELL LINES TO LYSIS BY NK CELLS

Spleen cell donor	Percentage specific cytotoxicity against							
	YAC_1	P815	C57BL/6-derived clones			X/Gf-derived clones		
			A	B	C	A	B	C
X/Gf	60±2	0	24±6	32±1	24±2	45± 7	41± 2	36± 0
C57BL/6	38±1	0	24±5	31±7	21±4	39± 8	36± 6	37± 7
$(C57BL/6 \times X/Gf)F_1$	51±1	0	24±6	27±3	27±3	42±12	43±15	44±15

Percentage specific cytotoxicity ± SD at an effector:target ratio of 100:1 after an 8 h incubation. The results from a representative experiment are the average for two individual mice from each strain. Clones A and B from each strain were induced in separate *in vitro* experiments with dimethylbenzanthracene and clones C from each strain were induced with benzpyrene (Nahas *et al.*, 1983).

TABLE III – COMPARISON OF *IN VITRO*-GENERATED LYTIC REACTIVITY TO HAPTEN-MODIFIED CELLS

Comparison	Target (PHA-blasts)	Responder anti-stimulator			
		X/Gf *a* X/Gf TNP	B10.A *a* B10.A TNP	X/Gf *a* B10.A TNP	B10.A *a* X/Gf TNP
A	X/Gf TNP	100	79± 8.1	83±16	105± 9.3
	B10.A TNP	71±10	58±14	62±15.5	76±15.5
B	X/Gf TNP	100	79± 8.1	83±16	105± 9.3
	B10.A TNP	100	84±25	85±18	106±10
C	X/Gf TNP	100	100	100	100
	B10.A TNP	71±10	71±14	64±13	68± 3.5

These results are the average of six separate experiments. – Comparison A: Results are calculated as a percentage of the response of X/Gf spleen cells sensitized with TNP-modified X/Gf spleen cells and tested against TNP-modified X/Gf blasts. Comparison B: Results are calculated as a percentage of the response of X/Gf spleen cells sensitized to TNP-modified X/Gf spleen cells and tested against either TNP-modified X/Gf or B10.A PHA blasts, each of these being considered 100% for all responses with the same target. Comparison C: The lytic reactivity of each responder-stimulator combination is calculated relative to its own response to X/Gf-TNP PHA blasts.

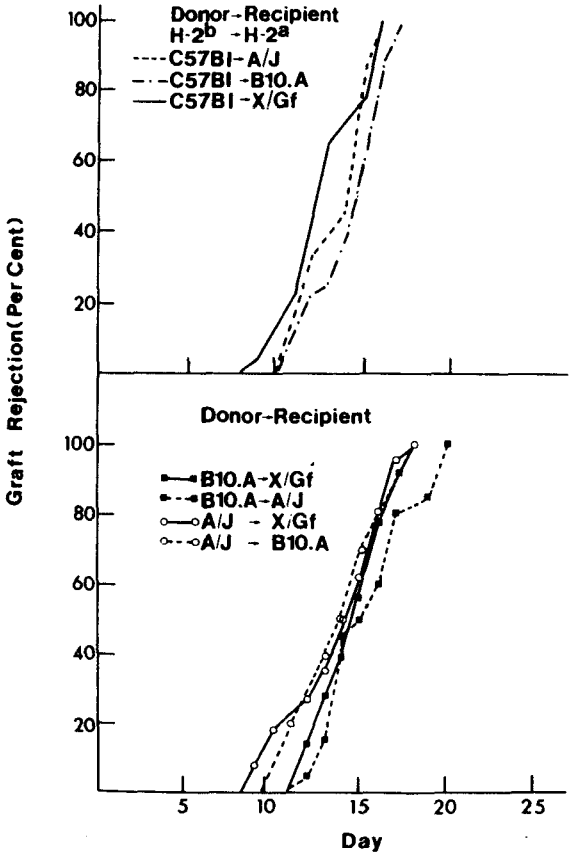

FIGURE 3 – Skin graft rejection across major and minor histocompatibility barriers. Donor and recipient mice of all strains were 8- to 10-week-old females. The data were obtained from the following number of mice per strain combination (donor → recipient): C57BL → A/J = 13; C57BL → B10.A = 21; C57BL → X/Gf = 23; B10.A → X/Gf = 28; B10.A → A/J = 20; A/J → X/Gf = 26; A/J → B10.A = 10.

Skin graft rejection

The capacity of X/Gf (H-2^a) (Staats, 1980; Goldfeder, 1974) mice to reject normal skin grafts was studied. Autografts and skin grafts across major histocompatibility barrier (H-2) and minor histocompatibility barriers (within the same H-2) were performed. In all cases, autografts were maintained for at least 30 days, at which time all experiments were terminated. The X/Gf mouse's capacity to reject skin grafts of C57BL/6J (H-2^b) origin was compared to the capacity of two other H-2^a strains, A/J and B10.A, to reject C57BL/6J skin grafts. X/Gf, A/J, and B10.A mice showed comparable mean and maximal rejection times of H-2 incompatible skin after a primary skin graft (Fig. 3). To determine the quality of skin graft rejection

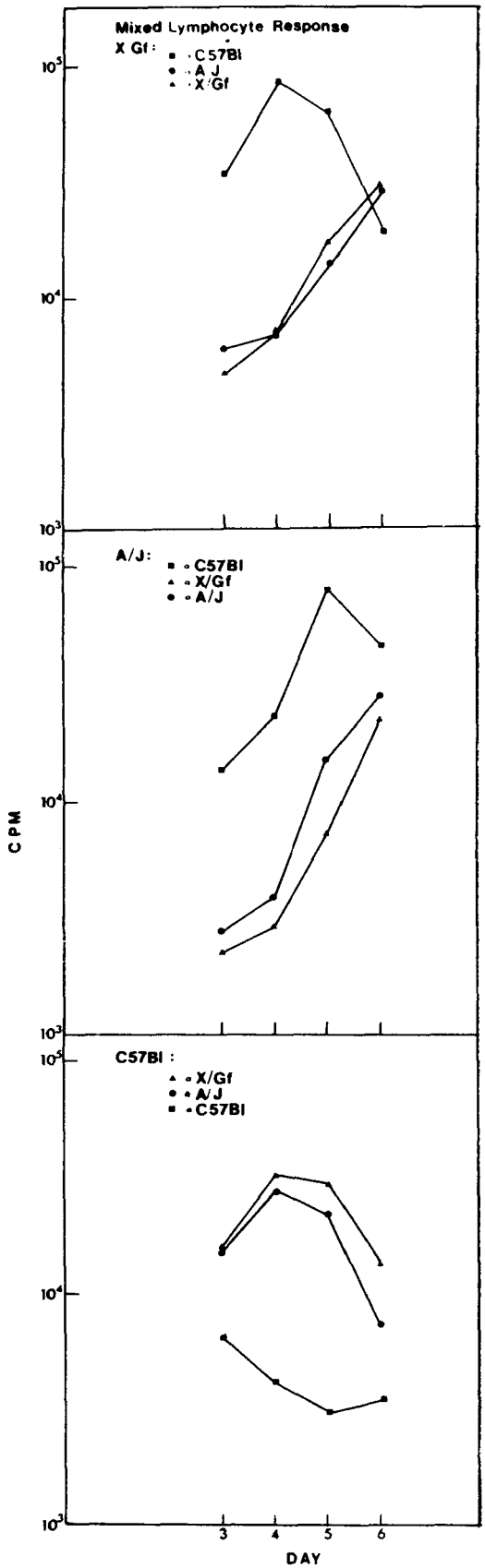

FIGURE 4 – Mixed lymphocyte reactivity of X/Gf, A/J and C57BL/6J lymph node cells. The results come from one representative experiment. Upper panel: the response of 1 × 10^6 X/Gf lymph node cells to 1 × 10^6 irradiated spleen cells from C57BL/6J, A/J, or X/Gf mice. Middle panel: the response of 1 × 10^6 A/J lymph node cells to 1 × 10^6 irradiated spleen cells from C57BL/6J, X/Gf or A/J mice. Lower panel: the response of 1 × 10^6 C57BL/6J lymph node cells to 1 × 10^6 irradiated spleen cells from X/Gf, A/J, or C57BL/6J mice.

across minor histocompatibility barriers, we grafted X/Gf mice with A/J or B10.A skin, A/J with B10.A skin, and B10.A with A/J skin. As can be seen in Figure 2, the kinetics of rejection of A/J and B10.A skin grafts were the same for X/Gf mice and for B10.A and A/J mice, respectively.

Mixed lymphocyte reactivity

The ability of lymph node cells from X/Gf (H-2^a) and A/J mice (H-2^a) to recognize and respond in mixed lymphocyte cultures to stimulation by irradiated spleen cells from C57BL/6J (H-2^b) or from each other was monitored. The response of C57BL/6J to these two H-2^a strains was also measured. As can be seen from Figure 4, the MLC responses of the A/J and X/Gf lymph node cells to H-2^b stimulation are similar in magnitude, while both of these strains fail to respond to each other. C57BL/6J lymph node cells respond to X/Gf and A/J with identical magnitude and kinetics. The high incorporation of ^{3}H-thymidine in unstimulated X/Gf cultures is not a characteristic which can be related solely to the cells of this strain, since cultures of A/J cells demonstrate similar kinetics of ^{3}H-thymidine incorporation. The increase in thymidine incorporation observed in cultures of X/Gf or A/J lymph node cells exposed to either irradiated X/Gf or A/J spleen cells occurred in cultures of allogeneic, syngeneic and autologous mixtures.

T-cell-mediated cytotoxicity

The ability of X/Gf spleen cells to undergo allogeneic stimulation in mixed lymphocyte cultures *in vitro* and to respond with the production of specific T-cytotoxic cells was examined. EL_4 lymphoma cells were used both as stimulators in the mixed lymphocyte cultures and as targets in the cell-mediated lympholysis assay. The X/Gf anti-EL_4 (H-2^a anti H-2^b) response was compared to A/J and B10.A anti EL_4 responses in similar cultures performed in parallel. As can be seen in Table IV, the degree of cell-mediated lysis developed at two different responder-to-stimulator ratios in the mixed lymphocyte culture and at all three effector-to-target ratios in the cytotoxicity assay is the same in all three mouse strains.

Delayed-type hypersensitivity (DTH)

The DTH responses to DNFB of X/Gf and C57BL/6J mice were compared. The magnitude and time course of the responses of the two strains of mice are comparable (Fig. 5). The experiments with DTH to various challenging doses of DNFB (0.1%-0.5% DNFB in 20 μl acetone-olive-oil) also demonstrate that the X/Gf and C57BL/6J do not differ in dose-response (Fig. 5).

TABLE IV – *IN VITRO*-DERIVED T-CELL-MEDIATED ALLOGENEIC CYTOTOXICITY[1]

	R:S[2] = 20:4			R:S[2] = 20:1		
E:T[3] / Responding strain	30:1	10:1	3:1	30:1	10:1	3:1
X/Gf	89	77	56	73	75	61
A/J	82	68	58	86	76	60
B10.A	82	70	60	80	74	60

[1]Results are the average of two separate experiments. – [2]R:S = responder: stimulator ratio. – [3]Effector:target ratio in chromium release assay.

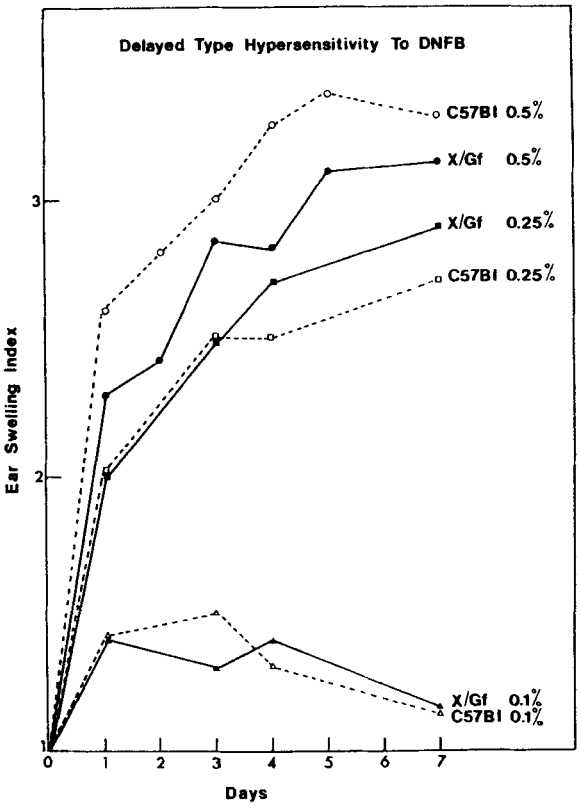

FIGURE 5 – Delayed-type hypersensitivity to 2,4,dinitrofluorobenzene. Four days after sensitization with DNFB, mice were challenged by ear pinna painting with 20 μl of 0.1%, 0.25%, or 0.5% DNFB in acetone-olive-oil (4:1) solvent. All points are based on the results of six experimental mice and six control mice per dose per strain.

Phagocytosis of inert particles in vivo

Stern and Goldfeder (1969) reported a high level of *in vivo* phagocytosis of ^{51}Cr-labelled xenogeneic eryth-

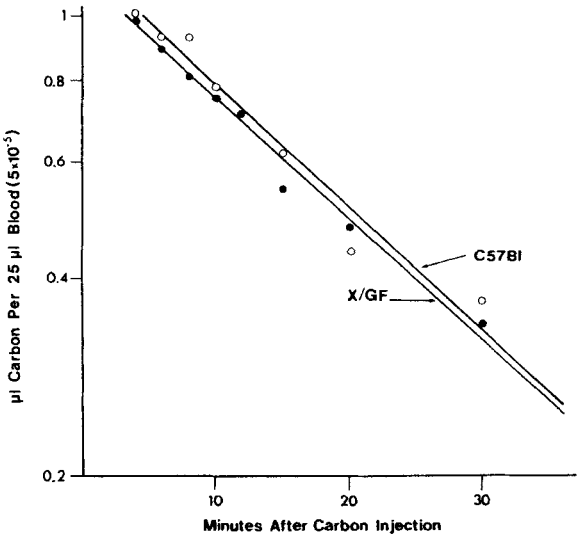

FIGURE 6 – *In vivo* clearance of colloidal carbon. ●—● = X/Gf; ○—○ = C57BL. Results are the average values from eight individual 3- to 4-month-old female mice per strain.

rocytes in tumor-bearing X/Gf mice. Healthy, non-tumor-bearing X/Gf and C57BL/6J mice were examined for their ability to clear intravenously injected colloidal carbon from the circulation (Fig. 6). The slopes of the regression lines for C57BL and X/Gf are identical with linear correlation coefficients of 0.98 and 0.99, respectively, thus showing that the reticuloendothelial systems of these two strains show identical abilities to clear foreign particles from the circulation.

DISCUSSION

X/Gf mice have been reported by Goldfeder to be a unique model of tumor-resistant animals (Staats, 1980). They demonstrate a low level of spontaneous tumors and are resistant to tumor induction by chemical carcinogens (Goldfeder 1972, 1974; Goldfeder *et al.*, 1966) oncogenic viruses (Goldfeder 1962, 1972, 1974; Goldfeder *et al.*, 1966, 1971). X-irradiation (Goldfeder 1962, 1972, 1974; Goldfeder *et al.*, 1966), and combined irradiation and carcinogen treatment (Goldfeder 1972; Goldfeder *et al.*, 1966). In a previous report, we have demonstrated that embryonic fibroblasts of X/Gf origin undergo malignant transformation *in vitro* when treated with the chemical carcinogens dimethyl-benzanthracene or benzopyrene (Nahas *et al.*, 1983). The frequency of transformation *in vitro* and optimum carcinogenic doses of both carcinogens were the same for the X/Gf and C57BL/6 fibroblasts. Studies on the *in vivo* growth of these *in vitro*-transformed fibroblasts from both strains in syngeneic and (C57BL/6 × X/Gf)F_1 mice demonstrated that the X/Gf and F_1 mice were decidedly more resistant to tumor growth than were the C57BL/6 mice. These experiments led to the conclusion that the very low level of tumors observed in the X/Gf mouse cannot be attributed to a resistance of X/Gf cells to malignant transformation, but rather is related to a homeostatic mechanism which prevents tumor growth in these mice. Similar conclusions as to the basis for the tumor resistance of X/Gf mice were drawn by Weisburger and colleagues (Grantham *et al.*, 1976; Reddy *et al.*, 1980) in their studies of the metabolism of the carcinogens 2FAA and N-OH-2FAA, to which X/Gf mice are resistant (Goldfeder, 1974).

In the present report, various parameters of immune reactivity of X/Gf mice have been examined to determine if aspects of the immune response to these mice might be connected to their tumor resistance. Of the parameters tested, reactivities comparable to those of control strains were found in skin graft rejection across major and minor histocompatibility barriers, allogeneic mixed lymphocyte cultures and cell-mediated lympholysis, delayed-type hypersensitivity reactions to dinitrofluorobenzene, and phagocytosis of inert particles *in vivo*. We have previously reported the resistance of X/Gf mice to the growth of *in vitro*-obtained carcinogen-induced syngeneic sarcomas (Nahas *et al.*, 1983). This *in vivo* resistance was heritable and, whereas C57BL/6 mice succumbed to similarly induced C57BL/6 sarcomas, the F_1 mice were resistant to the growth of sarcomas derived from X/Gf and from C57BL/6 (Nahas *et al.*, 1983). In the present series of experiments, YAC lymphoma cells of A/J origin were injected in graded doses i.p. into X/Gf and control mice. X/Gf mice showed better survival against YAC, which grows in H-2^a mice, than did mice of control H-2^a strains. Since the YAC lymphoma is sensitive to lysis by NK cells, experiments were performed to determine whether X/Gf mice demonstrate high levels of NK activity. X/Gf mice show significantly higher NK activity than do C57BL/6 and B10.A mice. The (C57BL/6 × X/Gf)F_1 mice demonstrate NK activity almost as high as that of the X/Gf parental mice.

These differences in NK activity of fresh spleen cells from X/Gf, C57BL/6, and (C57BL/6 × X/Gf)F_1 were not reflected in their ability to lyse *in vitro*-derived carcinogen-induced clones of transformed cell lines of X/Gf and C57BL/6 origin in a 8 h ^{51}Cr release assay. Thus, the resistance of F_1 and X/Gf mice to the growth of these clones cannot be attributed solely to their NK activity. However, it was noted in this series of experiments that all three X/Gf-derived clones were more sensitive to lysis by fresh spleen cells, regardless of effector cell origin, than were the C57BL-derived clones.

Tumor cells may present themselves to the body's immune system as modified autologous antigens. The production of H-2 restricted cytotoxic T cells to such altered autologous cells may be effective in tumor surveillance and rejection. Experiments were performed to compare the ability of splenic T cells from X/Gf and B10.A mice to respond to TNP-modified H-2 identical stimulator and target cells. These experiments were designed to compare (1) the capacity of X/Gf and B10.A spleen cells to develop a cytotoxic response, (2) the stimulatory ability of modified X/Gf and B10.A cells, and (3) their susceptibility to lysis, by examining all possible combinations of X/Gf and B10.A responder, stimulator, and target cells. The results of these experiments have shown that, whereas X/Gf and B10.A cytotoxic responses are comparable when the stimulator and target cells are kept constant, hapten-modified X/Gf spleen cells provide both better stimulation and more susceptible targets than do hapten-modified B10.A cells. While it may be premature to discuss the mediators of the immune phenomena described in this mouse strain, we should like to speculate that perhaps production of or sensitivity to interferon may provide a connection between our observations of high NK activity and high immunostimulatory ability. Interferon has been shown to elevate NK activity (Djeu *et al.*, 1979) and to induce Ia/DR expression on cells, thus enhancing their capacity to function as antigen-presenting cells (Steeg *et al.*, 1982). The properties of X/Gf cells which make them stronger stimulators and more susceptible targets to H-2-restricted cytotoxic T cells are at present under study.

The X/Gf mouse provides a valuable model for the continued study of resistance to cancer. The eventual understanding of the mechanism by which the cells of this mouse strain are both better stimulators and better targets of immune responses may provide a model for the manipulation of immune responses in other strains and species.

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