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Suppression of Chemical (DEN) Carcinogenesis in SWR/J Mice by Goat Antibodies Against Endogenous Murine Leukemia Viruses[1] (40285)

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Prevention of spontaneous leukemia in AKR mice has been successful following passive immunization with IgGs raised against endogenous ecotropic murine leukemia viruses (MuLVs), specifically the radiation leukemia virus (RadLV) (1, 2). Also, passive immunity against 3-methylcholanthrene-induced sarcomas in weanling C3H/f mice was achieved using anti-RadLV IgG. Price, et al. (3, 4) have shown that Fischer rat embryo cell clones required preinfection with MuLVs in order to be transformed by chemical carcinogens, and that the requirement of viral infection and replication could be fulfilled by both ecotropic (RLV) and xenotropic (AT124 and H5031) MuLVs; inhibition of viral replication by specific antiviral antibodies effectively blocked cell transformation (5). Thus it appears that the expression of endogenous MuLVs is a major determining factor in the chain of reactions following carcinogen treatment both *in vivo* and *in vitro*.

The carcinogenic effect of nitrosamines in inbred mice is well documented (6–8). Diethylnitrosamine (DEN) treatment of SWR/J mice resulted in a high incidence of lung adenomas and adenocarcinomas (72% versus 23% in untreated controls) 29 weeks after treatment (9). Since endogenous MuLV expression in inbred strains of mice generally increases with age (10) and upon chemical carcinogen treatment (11), we decided to test the DEN-SWR/J system as a model for determining the involvement, if any, of endogenous MuLVs in chemical carcinogenesis in vivo.

SWR/J mice lack both infectious ecotropic and xenotropic MuLVs but express the group-specific antigen (p30) in both spleens and thymuses (10). In the following communication we report that lung-tumorigenesis induced by diethylnitrosamine (DEN) in SWR/J mice is significantly delayed by treatments with antiviral antibodies against both RadLV and AT124.

Materials and methods. Antiviral antibodies. Goat IgGs raised against RadLV (Pool #3 NIH C5682) and AT124 (Pool #1 NIH C4928) were obtained from the Laboratory of RNA Tumor Viruses, NCI, Bethesda, MD 20014. These IgG preparations had neutralizing antibody titers of 1:800–1:1600 based on 70–100% inhibition of 60–70 AKR-XC plaques or 50–60 MSV (AKR) foci on SC-1 cells (12).

Mice and treatments. Twenty 8-week-old female SWR/J mice were pre- and post-treated with each goat anti RadLV and goat anti-AT124 IgG, and DEN according to the following schedule:

Day 0 (7-week-old)	0.1 ml anti-AT124 IgG	0.1 ml anti RadLV IgG
Day 4	0.1 ml anti-AT124 IgG	0.15 ml anti RadLV IgG
Day 7 (10 AM)	0.1 ml anti-AT124 IgG	0.15 ml anti RadLV IgG
Day 7 (5 PM)	DEN (90 mg/kg)	DEN (90 mg/kg)
Day 10	0.1 ml anti-AT124 IgG	0.2 ml anti RadLV IgG
Day 14	0.1 ml anti-AT124 IgG	0.2 ml anti RadLV IgG

Two groups of twenty control mice each received only the DEN treatment. DEN was freshly prepared in trioctanoin (Eastman Kodak) at a concentration of 10 mg/ml on each day of treatment. For each intraperitoneal injection the dose was 90 mg/kg (9).

The mice were set aside for tumor development and killed only when moribund. At necropsy lung nodules were counted and the lungs weighed to obtain a measure of tumor sizes. Lungs together with all other visceral organs were processed for histopathological evaluation according to standard procedures.

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While these experiments were under way, another group of 15 SWR/J mice received normal goat IgGs (Microbiological Associates, Bethesda) and DEN as per schedule used for anti-RadLV IgGs.

Statistical evaluation of data. All graphics and statistical analyses were done on a Tektronix microcomputer (Model 4051). Means, standard errors (S.E.) and analysis of variance were done according to Winner (13). F-tests were performed with 95% confidence intervals. The observed latency periods of AT124- and RadLV-IgGs treated groups of mice were compared with the corresponding untreated controls. The data on the normal goat IgG-treated group of mice was compared separately with all the other groups.

Results and discussion. DEN treated SWR/J mice passively immunized with goat anti-AT124 IgG survived up to 60 weeks following carcinogen treatment. Fifteen of 20 treated mice died from histologically confirmed lung tumors. In this group, 50% mortality because of lung tumors occurred at 54 weeks post-treatment. Thirteen of 20 untreated control mice died with multiple lung tumors and 32 weeks after treatment and the 50% mortality occurred by the 29th week (Fig. 1); seven mice had pneumonia.

Anti RadLV IgG immunized mice survived up to 64 weeks after DEN treatment. Eighteen of 20 mice developed histologically confirmed lung tumors by 64 weeks, with the 50% mortality incidence from lung tumors occurring at 58 weeks. Control mice for this group survived only to 38 weeks with a 60% (12/20) lung tumor incidence (Fig. 2) (Table 1). Five mice in this group died prematurely by a water bottle accident and the remaining three suffered from pneumonia. Similarly, three mice receiving normal goat serum died from injection accidents.

Eight of 12 normal goat IgG treated mice

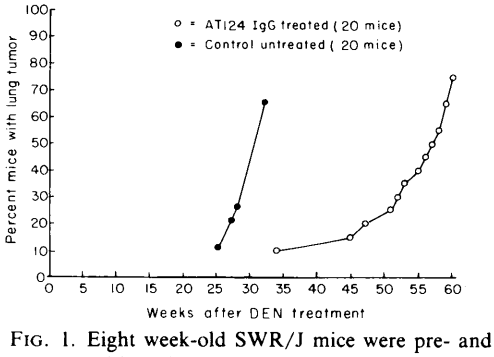

FIG. 1. Eight week-old SWR/J mice were pre- and posttreated with anti AT124 goat IgG and DEN as per schedule (see Materials and Methods). The mice were sacrificed when moribund and lungs and other viscera processed for histologic evaluation. Anti AT124 IgG treated mice (○——○) exhibited statistically significant (Table I) prolongation of their survival times when compared to untreated controls (●——●).

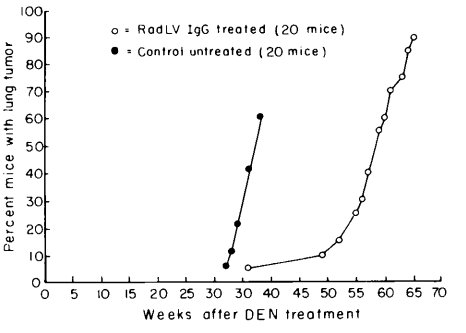

FIG. 2. Eight week-old SWR/J mice were given multiple doses of anti RadLV goat IgG and DEN (see Materials and Methods for details). Control mice received only DEN treatments. Anti RadLV IgG treated mice (○——○) showed a significantly prolonged tumor latency period (Table I) when compared to untreated control mice (●——●).

TABLE I. EFFECTS OF ANTIVIRAL ANTIBODIES ON LUNG TUMORIGENESIS IN DEN TREATED SWR/J MICE.

Immunization[a]	Lung tumor incidence	Mean survival period in weeks (±SE)[b]	Analysis on variance, *F* value[c]
None	13/20	29.8 (±1.83)	4.23[d]
Anti-AT124 goat IgG	15/20	52.0 (±1.70)	77.9[e,f]
None	12/20	35.7 (±1.57)	4.20[d]
Anti-RadLV goat IgG	18/20	57.33 (±1.28)	112.3[e,f]
Normal goat IgG	8/12	34.87 (±2.23)	[g]

[a] Procedure for immunization is given in materials and methods.

[b] Standard error in parenthesis.

[c] *F*-tests were done according to Winner (10).

[d] Fα critical value.

[e] *F* value in comparison with untreated control mice.

[f] Level of significance <0.0001 when compared to untreated controls.

[g] Normal IgG treated group when compared with other groups; the *F*-values for the different groups were: 38.46 (AT124 IgG), 3.15 (untreated controls), 70.22 (RadLV IgG) and 0.047 (untreated controls).

developed lung tumors by 40 weeks after DEN treatment. The mean survival period was significantly different from antiviral IgG treated groups.

The distribution pattern of lung weights of IgG treated and untreated mice is depicted in Fig. 3. Although immunized mice died with lung tumors after a long latency period, they had larger lung tumors (Fig. 3); obviously, their tumors had more time to grow than in control mice which died early. All lung tumors were either alveolar adenomas or adenocarcinomas as described previously (9).

If indeed the presence of endogenous MuLVs is required for *in vivo* cell transformation by chemical carcinogens virus suppression should either prevent or prolong the process of chemical carcinogenesis. The present data clearly indicate a very significant prolongation of the survival period of DEN treated mice probably because of a slowed tumor growth in the antiviral IgG treated groups (Fig. 4). Antiviral IgG treated mice survived to an expected average life-span of female SWR/J mice (9). Normal goat IgG had no beneficial effect on the survival time of DEN-treated SWR/J mice.

The data on lung tumor weights suggest that the antiviral IgGs had apparently very

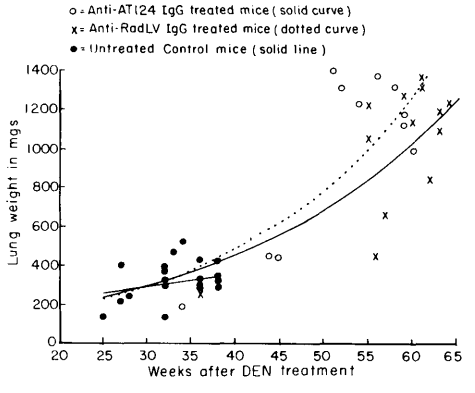

FIG. 3. Eight week-old SWR/J mice were given anti AT124 or anti RadLV IgG (see Materials and Methods for details) and DEN. Control mice received only DEN treatments. Wet lung weights of these mice were obtained at necropsy. Antiviral IgG treated mice developed larger lung tumors after a significantly prolonged latency period. The lung-weight distribution curves for AT124 (solid curve), RadLV (dotted curve) and untreated controls (solid line) were obtained and plotted using a graphic computer (Tektronix-4051).

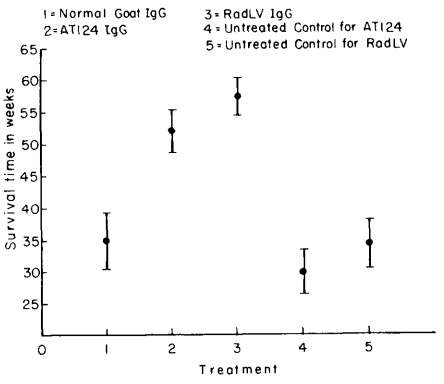

FIG. 4. Effect of antiviral IgGs on the latency period of DEN-induced lung tumors in SWR/J mice. The mean survival times after carcinogen was plotted against each treatment group. The bars (⊢●⊣) represent limits of variation. Both AT124- and RadLV IgG treated groups reveal a longer tumor latency period when compared to the control groups.

little effect, if any, on the growth rate of the tumors. Obviously by the time tumors appeared in most mice, the heterologous goat IgGs had long been eliminated.

Goat antiserum against MuLV gp71 as well as FeLV was shown to prevent oncornavirus induced sarcomas in cats (14). Thus it seems that the effectiveness of anti AT124 as well as RadLV IgGs against DEN carcinogenesis in SWR/J mice might be explained by the major homology between the two classes of MuLVs. Although the mechanism of the observed suppression of carcinogenesis is not clearly established, the data presented here tend to definitely indicate a viral involvement in chemical carcinogenesis. Presumably the antiviral IgGs partly suppress and delay the manifestations of chemical lung carcinogenesis in SWR/J mice.

Summary. We pre- and posttreated SWR/J mice given 90 mg/kg of DEN with goat anti RadLV and AT124 IgGs and studied their effects on the induction and latency of lung tumors. The results of these experiments tend to indicate a role of MuLVs in the etiology of the chemically induced lung tumors of SWR/J mice. The rate of tumor occurrence was greatly reduced in IgG treated mice and their survival time was significantly prolonged over nontreated mice. These findings require consideration of both ecotropic and xenotropic virus classes or their structural protein as cofactors in the chemi-

cally induced lung carcinogenesis process. Similar conclusions were drawn previously by others in an *in vitro* chemical transformation system (5).

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