# Aging Increases Susceptibility of Mouse Skin to DMBA Carcinogenesis Independent of General Immune Status

Abstract. *The dependence of chemical carcinogenesis on age of target tissue was studied by repeated DMBA-treatment of mouse skin grafted from old and young syngeneic donors to young recipients. Carcinomas developed on 39 percent (16/41) of grafts from old donors and on 12 percent (5/41) of grafts from young donors. The incidence of nonskin lymphoid tumors was highest in male recipients bearing grafts from old donors.*

A single application of dimethylbenz-[$\alpha$]anthracene (DMBA) caused a higher incidence of benign papillomas on skin grafts from old donors than on grafts from young donors (*1*). After the papillomas regressed, we studied the development of skin carcinoma following repeated application of DMBA.

Inbred BALB/c mice (2 months old) were given syngeneic skin grafts from old and young donors, 14 and 2 months old, respectively. Then DMBA was applied on the grafted skin, with 25 $\mu$g in 20 $\mu$l of acetone once 12 months after grafting and three times 17 months after grafting (Fig. 1). All animals were observed 7 days a week and killed when they became ill. Tumor, lung, liver, spleen, kidney, mesenteric and peripheral lymph nodes, thymus, and thyroid were taken for microscopy. Minced tissue (0.5 ml) from skin carcinomas and papillomas was transplanted intraperitoneally into adult BALB/c mice, each tumor mince being transferred to three recipients.

One application of DMBA resulted in the development of carcinomas on 4 out of 52 skin grafts from old donors and on 1 out of 49 skin grafts from young donors, whereas the number of papillomas was 30 and 12 respectively, as reported previously (*1*). Three applications of DMBA after regression of nearly all papillomas 5 months later boosted the number of carcinomas on the surviving 41 recipients of old skin grafts to 16, and the number on the 41 surviving recipients of younger skin grafts to 5 ($< P < .001$, chi square). After this final treatment both groups now had 12 mice with papilloma, but without carcinomas (Fig. 1). Intraperitoneal grafting of minced tissue from five skin carcinomas resulted in takes within 2 months in all cases, whereas no tumors were found after 6 months in mice having received tissue from five papillomas.

Male mice treated with DMBA and bearing grafts from old donors had more lymphoid tumors ($0.1 < P < .02$) than DMBA-treated male mice bearing grafts from young donors (Table 1). No such difference was found with the females. Twenty tumors were type B reticulosarcomas, and the remaining ten were lymphatic leukemias.

Skin grafts from old and young donor mice were of equal size at the conclusion of the experiment.

The importance of immunodeficiency for the effect of many carcinogens has been amply demonstrated. Co-occurrence of decreased immune reactivity and high incidence of tumors in old animals (*2*) suggests that deterioration of the general immune status is important for the increasing risk of developing spontaneous neoplasms with increasing age. However, abrogation of spontaneous tumor development in old animals by administration of immune competent cells from young donors has not met with success so far (*3*). Age-dependent change in hormonal status has also been suggested as being of general importance for neoplasia development in old animals (*4*).

Age-related change in each target cell, independent of the general immune and hormonal status, might also result in an increased susceptibility to exogenous carcinogens in organs, like skin, where cells are continually being replaced and thereby probably freed of most cells with mutational defects (*5*). This view was supported by the finding of a significantly increased incidence of "spontaneous" lymphoid tumors in syn-

Table 1. Survival time and incidence of nonskin lymphoid tumors in BALB/c mice bearing syngeneic DMBA-painted skin grafts. The mice that died before 14 months of age, when DMBA painting was done, are not included

| Source of skin graft | Treatment | Sex | No. | Survival time | | Lymphoid neoplasms | |
|---|---|---|---|---|---|---|---|
| | | | | Mean (months) | Range (months) | No. | Percent |
| Old donors | DMBA | F | 28 | 20 | (14–23) | 8 | 29 |
| | | M | 24 | 19 | (14–23) | 8 | 32 |
| Young donors | DMBA | F | 34 | 21 | (14–24) | 7 | 21 |
| | | M | 15 | 18 | (14–21) | 0 | 0 |
| Old and young donors | Acetone | F | 30 | 22 | (14–25) | 4 | 15 |
| | | M | 15 | 19 | (14–23) | 1 | 7 |

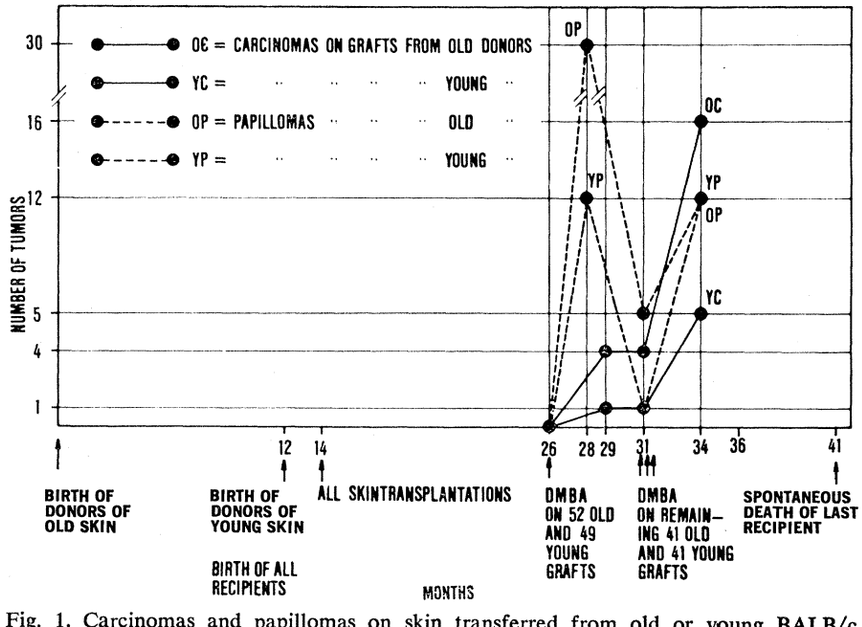


Fig. 1. Carcinomas and papillomas on skin transferred from old or young BALB/c mice to young syngeneic recipients.

geneic mouse recipients of lymphoid cells from old donors, whereas grafting of cells from young donors had no effect (*6*).

This work demonstrated that also development of carcinoma as a result of DMBA treatment occurs with a higher incidence on skin from old as compared to young donors. In that three applications were made only 14 days apart, an influence of hair-cycle (*7*) on tumor development appears unlikely. In the above experiments, age-dependent alterations in general immune reactivity and hormone secretion can also be disregarded as contributing factors because the recipient mice of the various test groups were of the same age. An age-dependent alteration of graft permeability to cellular and hormonal host factors that could influence skin carcinogenesis is conceivable. It seems more likely, however, that skin cells themselves become more susceptible to carcinogen with increasing age. Tumors other than skin tumors were most numerous in mice bearing skin grafts from old donors. This can hardly be a reflection of a different concentration of DMBA in the bodies of the mice, since old skin is believed to retain the same or more DMBA than young skin (*8*).

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**References and Notes**

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## Thymus Adenylate Cyclase Activity during Murine Leukemogenesis

Abstract. *Activity of thymus adenylate cyclase was more than three times higher in leukemic AKR mice than in nonleukemic AKR mice and CBA mice. Preleukemic AKR mice that had no evidence of leukemia but were expected to soon develop the disease exhibited similarly elevated activities of thymus adenylate cyclase.*

The postulate that adenosine 3,'5'-monophosphate (cyclic AMP) plays a role in the initiation of events leading to proliferation of lymphoid cells has been supported by observations that cyclic AMP increases (i) thymidine uptake by the nucleic acid fractions of spleen cells and thymocytes; (ii) uridine incorporation into RNA of peripheral lymphocytes; (iii) cell division of thymocytes in calcium deficient medium; and (iv) the number of antibody-forming cells after immunization (*1*). Smith *et al.* (*2*) reported that phytohemagglutinin, which stimulates the proliferation of lymphocytes, elevates intracellular concentrations of cyclic AMP and increases the activity of lymphocyte adenylate cyclase. Agents that have adjuvant effects on antibody formation also stimulate adenylate cyclase activity of spleen cells (*3*). Immunodeficient pituitary dwarf mice, with decreased thymic lymphopoiesis and inefficient splenic proliferative response to antigen, exhibit a defective lymphocyte adenylate cyclase that does not respond to epinephrine-induced stimulation (*4*).

In view of the role of cyclic AMP in lymphoid cell proliferation, the question was raised whether changes in cyclic AMP metabolism of thymic lymphocytes occurred during the development of leukemia in AKR mice. AKR mice have a high incidence of lymphoid leukemia that primarily affects the thymus and frequently involves other lymphoid tissues (*5*). The causative agent is a vertically transmitted oncogenic virus (Gross virus) (*6*) that apparently finds a suitable microenvironment in the AKR thymus for the induction of neoplastic transformation of lymphoid cells (*7*). This report describes our studies on adenylate cyclase of thymus cells during the development of leukemia in AKR mice.

Determination of adenylate cyclase activity in the 10,000*g* sediment fraction of thymus cell sonicates was performed with a modification (*8*) of the method of White and Zenser (*9*) and was based on the conversion of adenosine [$\alpha$-$^{32}$P]triphosphate ([$\alpha$-$^{32}$P]ATP) into cyclic [$^{32}$P]AMP. The 0.1-ml assay mixture contained 1 m*M* [$\alpha$-$^{32}$P]ATP (2 to 3 $\times$ $10^6$ count/min), 5 m*M* $MgCl_2$, 50 m*M* tris(hydroxymethyl)aminomethane (tris) HCl (*p*H 7.6), 0.1 m*M* cyclic [$^3$H]AMP (2 $\times$ $10^4$ count/min), 10 m*M* theophylline, an ATP generating system consisting of 30 m*M* creatine phosphate and 30 $\mu$g of creatine kinase, and 25 $\mu$l of the 10,000*g* sediment of thymus tissue sonicate. Assays were performed at 30°C for 1 to 2 hours, and radioactive cyclic AMP was recovered through chromatography on alumina columns as described (*8*). One

Table 1. Adenylate cyclase activity of thymus cells from AKR mice during stages of leukemia development and from normal CBA mice. Values are geometric means ± standard error.

| Group | Experiments (No.) | Age (days) | | Cells per thymus ($10^8$ cells) | Protein in 10,000*g* sediment of sonicate of $10^{11}$ cells (g) | Enzyme per gram of protein in 10,000*g* sediment (units) | Enzyme per $10^{11}$ cells (units) | Increase of activity with $10^{-5}$*M* epinephrine (%) |
|---|---|---|---|---|---|---|---|---|
| | | Mean | Range | | | | | |
| Nonleukemic AKR | 10 | 66 | 21–162 | 2.3 ± 0.3 | 0.58 ± 0.04 | 26 ± 4 | 15 ± 2 | 275 ± 27 |
| Preleukemic AKR | 10 | 231 | 200–268 | 1.1 ± 0.1 | 0.62 ± 0.08 | 80 ± 22 | 55 ± 18 | 190 ± 20 |
| Leukemic AKR | 5 | 224 | 200–252 | 6.1 ± 1.4 | 1.13 ± 0.17 | 88 ± 16 | 99 ± 23 | 100 ± 10 |
| Young CBA | 5 | 114 | 75–150 | 1.3 ± 0.1 | 0.49 ± 0.03 | 11 ± 2 | 5 ± 1 | 270 ± 33 |
| Old CBA | 3 | 241 | 228–253 | 0.6 ± 0.1 | 0.45 ± 0.05 | 11 ± 1 | 5 ± 1 | 110 ± 20 |