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Differences in DMBA-induced Mammary Neoplastic Responses in Two Lines of Sprague–Dawley Rats

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Abstract—*It has been reported that female Sprague–Dawley rats obtained from a U.S. source and studied in the U.S. gave a larger and more rapid mammary neoplastic response to radiation than did female Sprague–Dawley rats obtained from a Dutch source and studied in The Netherlands. To learn if the different mammary neoplastic responses of the two 'lines' of Sprague–Dawley rats are due to inherent differences between the lines of rats or due to differences in experimental conditions, two groups of rats from the American source and one group from the Dutch source were studied for their response to a chemical carcinogen, dimethylbenzanthracene (DMBA), at the same laboratory. When* 10 mg *of DMBA per* 100 g *body wt was given by stomach tube to* 28 *rats from the Dutch source,* 367 days *later approximately* 25% *of these rats had developed mammary carcinomas and approximately* 18% *had developed mammary fibroadenomas. When the same dose of DMBA was given to rats from the U.S. source,* 300 days *later* 90 *and* 100% *had developed mammary carcinomas and* 83 *and* 95% *had developed mammary fibroadenomas. Similar trends were found for the number of neoplasms per rat and the mean time of appearance of the neoplasms. It was concluded that there are inherent differences between Sprague–Dawley rats obtained in the U.S. and Sprague–Dawley rats obtained in The Netherlands in regard to their mammary neoplastic responses to DMBA, as well as in their responses to radiation. Genetic differences between the two lines were confirmed by establishing dissimilarities in the expression of erythrocyte antigens coded for by* RT1 *(major histocompatibility complex).*

INTRODUCTION

WHEN YOUNG, female Sprague–Dawley rats, obtained from the breeding colony of the REP Institutes|| TNO, Rijswijk, The Netherlands, were irradiated with X-rays or neutrons at TNO, mammary neoplasms did not begin to appear until about 12 months after irradiation [1, 2]. In contrast, when young, female Sprague–Dawley rats, obtained from ARS/Sprague–Dawley, Madison, WI, were irradiated with X-rays or neutrons at Brookhaven National Laboratory, Upton, New York, mammary neoplasms began to appear as soon as 2 months after irradiation [3, 4]. In The Netherlands the non-irradiated rats did not begin to develop mammary tumors until they were about 24 months of age, while in the U.S.A. spontaneous mammary tumors began at 9 months of age. The final cumulative spontaneous incidence of rats with mammary neoplasms was 30% in The Netherlands and 67% in the U.S.A. These observations strongly suggest a quantitative difference between the rats obtained from two different sources in regard to radiation-induced mammary neoplasia. In order to confirm this

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difference, Sprague-Dawley rats obtained from both the American source and the Dutch source should be studied under identical conditions at one laboratory. However, the study of radiation-induced mammary neoplasia is a lengthy procedure, requiring at least a 2-yr follow-up period.

Chemical carcinogen-induced mammary neoplasia studies can be done in a 1-yr period [5]. Also, it has been suggested [6] that rat strain sensitivity to chemical carcinogen-induced mammary neoplasia parallels that of radiation-induced mammary neoplasia. That is, the strain that is relatively sensitive to radiation-induced mammary neoplasia is apt to be also relatively sensitive to chemical carcinogen-induced mammary neoplasia. For these reasons, it was decided to study Sprague-Dawley rats obtained from both the American and the Dutch sources for their response to chemical carcinogen-induced mammary neoplasia in a single laboratory (Brookhaven) using dimethylbenzanthracene (DMBA) as the chemical carcinogen.

MATERIALS AND METHODS

Female Sprague-Dawley rats, 34 ± 3 days of age, were shipped from Rijswijk to Brookhaven. The source of these rats was the pathogen-free barrier-maintained Sprague-Dawley breeding colony in Rijswijk, which was initiated with germ-free stock obtained from the University of Ulm (Ulm, F.R.G.) in 1972. These rats were in their 7th generation of brother-sister mating at the time they were brought to Rijswijk. The stock had been introduced to Ulm by Prof. Dr O. Haferkamp in 1965 with breeding pairs obtained from Hoechst Pharmaceuticals, Frankfurt, F.R.G. The source of the rats maintained in Frankfurt at that time can no longer be ascertained. When the present studies were conducted, the Rijswijk Sprague-Dawley rats were in their 10th generation of inbreeding, resulting in an overall level of inbreeding of at least 17 generations. These rats, which are close to being an inbred strain, will be referred to as Rijswijk rats. When they were 56 ± 3 days of age they were given DMBA and studied for 367 days, at which time they were killed.

Outbred female Sprague-Dawley rats were obtained from ARS/Sprague-Dawley, Madison, WI, at 21 days of age, in two separate shipments. These will be referred to as BNL I and BNL II. The origin of these rats, according to Lindsey [7], is as follows:

> The precise origin of the so-called 'Sprague-Dawley rat' appears to be lost in uncertainty. The original stock was reportedly established about 1925 by Mr. Robert Worthington Dawley (1897–1949), a physical chemist at the University of Wisconsin. In naming the strain, he simply combined the maiden name (Sprague) of his first wife and his own name to form Sprague-Dawley. He subsequently established in Madison, Wisconsin the commercial firm known as Sprague-Dawley, Inc., dedicated exclusively to the advancement and sale of his rats. His original company continues today under the name of ARS/Sprague-Dawley.

Lindsey [7] also presented an excerpt from a letter dated 22 July 1946, from Sprague-Dawley, Inc. to Mr. Poiley at the NIH:

> Regarding the origin of the strain, this strain as developed by Mr. Dawley, started originally with a hybrid hooded male rat of exceptional size, and vigor, which genetically was half-white. He was mated to a white female and subsequently to his white female offspring for seven successive generations. The origin of this male is unknown. The original white female was of the Douredoure strain which was probably from Wistar. After his death, his white offspring were inbred in a number of different lines from which the best ten were combined. Selection was made to retain or acquire characteristics of high lactation, rapid growth, vigor, good temperament, and high resistance to arsenic trioxide.

When the ARS/Sprague-Dawley rats were 55 days of age they were given DMBA and studied for 300 days, at which time they were killed.

DMBA, purchased from Eastman Kodak Co., Rochester, NY, dissolved in sesame oil, 10 mg per ml, was given by stomach tube, at the rate of 10 mg per 100 g body wt, to the nearest 10 g and 0.1 ml.

All rats were maintained on commercial rat chow (Purina) and water *ad libitum* in temperature- (22 ± 2°C) and humidity-controlled (55 ± 10%) animal rooms under conditions of 7a.m.–7p.m. fluorescent light per day. Each rat was examined frequently, and when mammary tumors were noted by palpation and reached a size of 1–2 cm, the tumors were removed surgically under ether anesthesia and the rat returned to the experiment. Individual records were kept for each rat, and the time of appearance (days post-DMBA) and anatomic location of the mammary tumors were recorded. Mammary neoplasms that occurred in different quadrants in the same animal were recorded as separate neoplasms. If successive mammary neoplasms were noted in the same quadrant, they were considered to be different neoplasms only if they were of a different

histologic type. If they were of the same type, they were considered different neoplasms provided that at least 60 days had elapsed between removal of the first neoplasm and detection of the second neoplasm. All mammary neoplasms, after microscopic study, were given a pathologic classification of either carcinoma (CA) or fibroadenoma (FA), according to criteria published previously [8]. All mammary tumors in rats of the three separate groups were examined by the same investigator.

Animals representative of the Rijswijk (two females) and the ARS/Sprague-Dawley (four females) strains were tested by hemagglutination for the expression of three different erythrocyte antigens. The antigenic systems tested included the class I antigens of the major histocompatibility complex (*RT1.A*) and two additional erythrocyte antigens coded for by the *RT2* (*Ag-C*) and *RT3* (*Ag-D*) loci. All three of these genetic systems segregate as independent loci and the methodology employed for their detection has been described previously [9].

The BNL I study was completed before starting the BNL II study, which was started approximately 6 months before starting the Rijswijk study. In the BNL I study 30 rats were reserved as non-DMBA-treated control rats, and in the BNL II study 78 rats were reserved as non-DMBA-treated control rats.

RESULTS

A summary of all results is presented in Fig. 1 and Table 1. Toxic deaths, attributed to the DMBA treatment and occurring between 2 and 13 days after the treatment, were found in 0 of 30 rats in the BNL I study, 1 of 20 in the BNL II study and 9 of 37 in the Rijswijk study. At autopsy approximately 80% of the Rijswijk rats exhibited mammary cysts filled with milky fluid, while less than 10% of the BNL I and II rats developed such cysts.

Of the 30 non-DMBA-treated control rats in the BNL I study a single rat developed a single FA. In the BNL II study one of 78 control rats developed a single FA.

Essentially all of the DMBA-treated BNL I and BNL II rats developed one or more CA and one or more FA. In contrast, approximately 1/5 of the Rijswijk rats developed one or more CA and 1/7 developed one or more FA. Both CA and FA tumors developed sooner in BNL rats than in Rijswijk rats. The BNL rats developed many more CA and FA per rat than did the Rijswijk rats.

Historical data on survival and mammary tumor incidence in control Rijswijk and BNL Sprague-Dawley rats from earlier life-span studies [1, 2, 4] in our respective laboratories are presented in Table 2. The two Sprague-Dawley 'lines' had similar mean life-spans, but the Rijswijk rats had an overall tumor incidence of

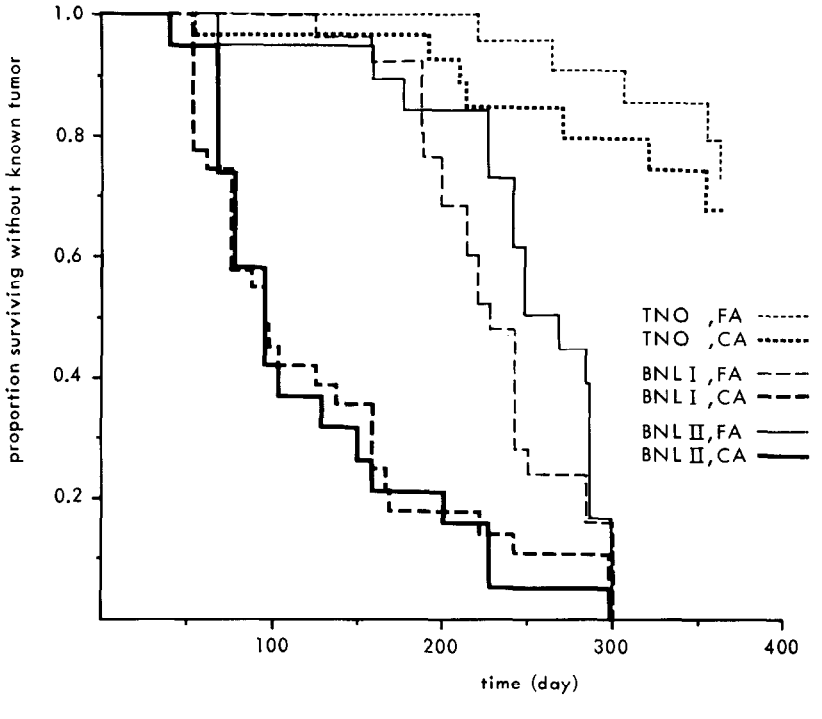

Fig. 1. Actuarial incidence of mammary carcinomas (CA) and fibroadenomas (FA) following DMBA administration in Sprague-Dawley rats from Rijswijk (TNO) and Brookhaven National Laboratory (BNL I and II).

Table 1. DMBA-induced mammary neoplasia in female Sprague–Dawley rats obtained from The Netherlands or the U.S.A. and studied at Brookhaven (BNL)

Study	Rijswijk DMBA-treated	BNL I DMBA-treated	BNL I Untreated controls	BNL II DMBA-treated	BNL II Untreated controls
Source of rats	The Netherlands	U.S.A.	U.S.A.	U.S.A.	U.S.A.
No. of rats	37	30	30	20	78
Toxic deaths (days 2–13)	9	0	-	1	-
Effective No. of rats studied	28	30	30	19	78
Length of study (days)	367	300	300	300	300
No. of rats surviving study (%)	16(57)	20(67)	30(100)	16(84)	78(100)
Days of study (mean ± S.E.)	310 ± 17	263 ± 14	300	295 ± 4	300
Rats with carcinomas	7	27	0	19	0
Rats with carcinomas [% (at risk %)]	25(34)	90(93)	0	100(100)	0
Days to first carcinoma (mean ± S.E.)	234 ± 41	116 ± 13	0	124 ± 16	0
Total No. of carcinomas	10	97	0	79	0
Carcinomas per rat (mean)	0.36	3.23	0	4.16	0
Days to all carcinomas (mean ± S.E.)	273 ± 34	194 ± 9	0	205 ± 9	0
Rats with fibroadenomas	5	25	1	18	1
Rats with fibroadenomas [% (at risk %)]	18(25)	83(96)	3(3)	95(95)	1(1)
Days to first fibroadenoma (mean ± S.E.)	306 ± 31	234 ± 10	300	251 ± 15	300
Total No. of fibroadenomas	7	174	1	86	1
Fibroadenomas per rat (mean)	0.25	5.80	0.03	4.53	0.01
Days to all fibroadenomas (mean ± S.E.)	323 ± 24	284 ± 3	300	282 ± 4	300

Table 2. Historical data from life-span studies on mammary tumor incidence in untreated control Sprague–Dawley rats from Rijswijk and Brookhaven

	Rijswijk*	BNL†
Historical controls (No. of rats)	30	167
Mean life-span (days ± S.E.)	732 ± 23	810 ± 13
No. of animals with tumor (%)	9 (30)	112 (67)
No. with fibroadenoma (%)	9 (30)	110 (66)
No. with carcinoma (%)	0	26 (16)
Mean time to first fibroadenoma (days ± S.E.)	743 ± 22	649 ± 15
Mean time to first carcinoma (days ± S.E.)	-	833 ± 25

*Data from ref. [2] and unpublished observations.
†Data from ref. [4].

Table 3. Serological testing of Rijswijk and ARS/Sprague–Dawley rats

	Antisera													
	Anti-RT1										Anti-RT2		Anti-RT3	
	a	b	c	d	f	g	h	k	l	u	a	b	a	b
Rijswijk rats	-	-	-	-	-	-	-	-	-	+	-	+	-	+
ARS/Sprague–Dawley rats	-	-	-	-	-	-	-	-	+	-	-	+	-	+

less than half that of BNL rats. In addition, spontaneous carcinomas were not found in the Rijswijk rats, whereas 16% of BNL rats developed such neoplasms. Also, fibroadenomas were detected in BNL rats approximately 150 days before such neoplasms were found in Rijswijk rats.

The results of the serological testing of representatives of the two strains are presented in Table 3. The two strains share the expression of the same *RT2* and *RT3* antigens but differ for *RT1.A*. Despite the description of the ARS/Sprague–Dawley as an outbred strain, all four females were serologically identical for each of the loci tested. This suggests that many generations of breeding within this closed colony may have produced a substantial reduction in the amount of genetic variability in this strain through

genetic drift. Other factors, e.g. mismatings, may also have contributed to the decreased genetic variability.

DISCUSSION

It seems clear that both the mammary carcinoma response and the mammary fibroadenoma response to DMBA was larger, and occurred sooner, in Sprague–Dawley rats obtained from ARS/Sprague–Dawley than in Sprague–Dawley rats obtained from the Rijswijk colony. This finding with a chemical carcinogen is in complete accord with earlier findings when the carcinogenic agent was either X-rays or neutrons [2, 4]. Thus the suggestion that rat strains that are relatively sensitive to chemical carcinogens are apt to be relatively sensitive to radiation, and vice versa [6], can now be extended to 'lines' of rat within a strain or stock.

The occurrence of spontaneous mammary tumors in the two 'lines' of Sprague–Dawley rats followed a similar pattern. In historical controls mammary fibroadenomas occurred earlier, and with a two-fold greater frequency in BNL rats than in Rijswijk rats, and spontaneous mammary carcinomas were found only in BNL rats.

In the study reported here outbred Sprague–Dawley rats from the American source and partially inbred Sprague–Dawley rats from the Dutch source were, for the first time, studied in a single laboratory. The American Sprague–Dawley rats gave a larger and more rapid mammary neoplastic response to DMBA than did the Dutch Sprague–Dawley rats within the time span of the experiment. Whether the Rijswijk rats would remain relatively free of tumors at a later time-point than that studied cannot be assessed based on the present data. Nonetheless, this finding indicates that the previous reports [1–4] that American rats studied in an American laboratory gave a larger and more rapid mammary neoplastic response to radiation than did the Dutch rats studied in The Netherlands was due to inherent differences in the two 'lines' of rats rather than differences in rat husbandry. In other words, the genetic control of the mammary neoplastic response to DMBA seems more likely to explain the differing responses in the two lines of Sprague–Dawley rats than do differences in the handling and care of the rats.

Sprague–Dawley rats from different sources may have little in common with each other besides their names and similarities in pelage, since many of the commercially available animals are outbred and have heterogeneous genetic backgrounds. In addition, the genetic makeup of the several available inbred lines may differ markedly since such lines are also derived from different stocks. Inbreeding fixes many (approximately 99.7%) genes [10] and many genes from the outbred stocks are lost during inbreeding; thus considerable genetic differences can be expected between strains and stocks, despite a common ancestry. For example, the two Sprague–Dawley lines used in this study differ at genes in the major histocompatibility complex (RT1): the Rijswijk Sprague–Dawley is $RT1.A^u$, $RT2^b$, $RT3^b$ whereas the ARS/Sprague–Dawley is $RT1.A^l$, $RT2^b$, $RT3^b$. As a further illustration, a Sprague–Dawley inbred line developed by the NIH and designated as strain NSD/N is $RT1.A^b$, $RT2^a$ and $RT3^a$ [9] but another inbred Sprague–Dawley strain (SDJ) maintained in Japan is $RT1.A^u$, $RT2^b$ and $RT3^b$ [Dr J. Yamada, Kyoto University, personal communication]. These examples merely serve to point out differences in selected genetic markers among rats designated as Sprague–Dawley and do not imply that the genetic control of the neoplastic response to DMBA or radiation lies within these gene systems. In fact, no information is available concerning the influence of specific gene systems, such as the major histocompatibility complex, on the induction of neoplasms in rats or at what levels of biological organization (e.g. endocrinological, immunological) such genetic control might operate. It is becoming evident, however, that the rat major histocompatibility complex is responsible for the control and regulation of a number of important immunologic functions, including resistance (or susceptibility) to certain transplantable tumors [9, 11]. Moreover, much has been learned about the role of specific gene loci in the mouse major histocompatibility (H-2) complex in determining mammary tumor development in that species [12]. Definitive studies to address the issue of genetic control of rat mammary tumor response will have to be performed in inbred congenic strains.

In summary, inherent differences exist between Sprague–Dawley rats obtained in the U.S. and The Netherlands with respect to their mammary neoplastic reponses to DMBA, and also in their responses to radiation. Further studies are needed to define the specific genetic basis for such differences, especially in light of the observed differences in the major histocompatibility haplotypes betwen the two 'lines'.

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