Short-term carcinogenicity testing of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in Eμ-pim-1 transgenic mice

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The usefulness of transgenic Eμ-pim-1 mice over-expressing the pim-1 oncogene in lymphoid tissues, as sensitive test organisms was studied in a short-term carcinogenicity study. The mice were fed standard diet Altromin 1314 supplemented either with 0.03% 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) for 7 months or with 0.03% 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) for 6 months. PhIP and IQ are heterocyclic amines formed during cooking of meat and fish and are mutagenic to bacteria and cultured mammalian cells. PhIP is a potent mouse lymphomagen, while IQ is a liver carcinogen and also causes lung tumors and tumors of the forestomach in mice. We found that transgenic Eμ-pim-1 mice are highly susceptible to PhIP induced lymphogenesis but do not respond to the IQ treatment. PhIP feeding of Eμ-pim-1 mice not only increased the total number of T-cell lymphomas but also decreased the latency time compared to either transgenic or wild-type controls. The effect was most pronounced in the treated female Eμ-pim-1 mice, which showed a higher incidence of PhIP induced T-cell lymphomas than transgenic males and a strongly reduced latency period after PhIP treatment compared to non-transgenic mice. Our results suggest that the transgenic Eμ-pim-1 mouse may be a useful model for short-term carcinogenicity screening of potential genotoxic carcinogens having the lymphoid system as target tissue. The carcinogen IQ which does not have the lymphoid system as a target was not recognized in this model.

Introduction

A highlight of the last two decades has been the discovery of new genes, i.e. proto-oncogenes and tumor suppressor genes that in their mutated forms are responsible for the development of human cancers. The introduction of these genes into mice has become a novel approach to the investigation of the multistep nature of the cancer process. This transgenic approach has provided access to perform studies on oncogene cooperativity in vivo but also may allow short-term in vivo carcinogenicity testing of chemicals. Furthermore, contemporary needs and trends in modern toxicology require the use of smaller numbers of laboratory animals and faster responsiveness to events that cause tumor formation.

Transgenic mice carrying an active oncogene present a useful model for assessing the tissue specific action of oncogenes because the biological function and expression pattern may predispose to a specific type of neoplastic growth. In general, the transgene does not directly provoke tumors but establishes a high predisposition since the emergence of a malignant clone will require several additional genetic changes in affected cells. As yet too few studies have been conducted to assess the usefulness of such transgenic mice in carcinogenicity testing. For review see (1).

So far over two dozen tumor types have been modelled in transgenic mice but only a couple of them have been tested in carcinogenicity studies (2). The carcinogens tested included both genotoxic (3-5) and non-genotoxic carcinogens (6).

The next decade should bring about the characterization of transgenic animal models that are more susceptible to carcinogenic agents leading to more rapid and precise categorization of genotoxic carcinogens and to a better understanding of the role of genetic events in the process that results in cancer caused by these agents. In this context the present study contributes to characterization and establishment of the usefulness of the transgenic Eμ-pim-1 mouse model in short-term carcinogenicity testing.

Eμ-pim-1 mice express the pim-1 oncogene at elevated levels in their lymphoid compartments and are predisposed to T-cell lymphomas (3). The spontaneous tumor incidence in Eμ-pim-1 transgenic mice is very low: ~10-20% of these transgenic mice will develop T-cell lymphoma after 34 weeks, which points to the potential usefulness of the Eμ-pim-1 mice in carcinogenicity testing (7). Previous short-term carcinogenicity assays in Eμ-pim-1 mice have been performed by treating neonatal mice intraperitoneally with a single dose of 60 mg/kg of the alkylating agent N-ethyl-N-nitosourea (ENU*), a potent direct acting mouse lymphomagen. All treated transgenic mice rapidly developed T-cell lymphomas, compared to only 20% of the control mice (3). In a subsequent study the dose–response to ENU induced lymphomagenesis was investigated, demonstrating at least 25-fold increased sensitivity of Eμ-pim-1 mice to ENU, compared to wild-type mice (5). In a more recent carcinogenicity study using Eμ-pim-1 mice, the results of four genotoxic procarcinogens: 2-acetylaminofluorene (AAF), n-nitrosodimethylamine (NDEA), 1,2-dichloroethane (1,2-DCE) and benzene (BEN) were evaluated (8). These compounds all require metabolic activation and, with the exception of benzene, are not mouse lymphomagens. Compounds were administered by gavage daily for 38-40 weeks and only small, but statistically significant, increases in the incidence of malignant lymphomas were seen in the high dose males treated with AAF, high and low dose females treated with NDEA, and high dose females treated with DCE.
Surprisingly, the mouse lymphomagen benzene did not cause a statistically significant increase in the incidence of malignant lymphomas in transgenic E|pim-l mice or non-transgenic controls.

The aim of the present study was to further evaluate the potential of E|pim-l mice as test animals for a short-term carcinogenicity bioassay. As test compounds a potent mouse lymphomagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and a mouse lung, liver and forestomach carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) were chosen. PhIP and IQ are heterocyclic amines, compounds formed during cooking of proteinaceous foods such as fish and beef (9-11). PhIP requires metabolic activation by cytochrome P-450-mediated N-hydroxylation and Phase II esterification to N-acetoxy derivatives (12-15). PhIP has been shown to be carcinogenic in mice and rats when administered in the diet, producing lymphomas in mice (16) and colon and mammary carcinomas in rats (17). IQ is activated to a mutagenic metabolite by microsomes (18) and is a liver, lung and forestomach carcinogen in mice (19) and a colon, small intestine, liver, Zymbal gland and clitorial gland carcinogen in rats (20). The present study was designed to determine whether the carcinogenic potential of PhIP and IQ will be enhanced by the pim oncogene expressed in lymphatic tissues of transgenic E|pim-l mice.

Materials and methods

Test compounds and diets

Synthetic 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) were obtained from the National Cancer Institute (USA). The pelleted diets Altromin 1314 with 0, 0.01, 0.02, 0.03 and 0.04% PhIP and 0.03% IQ were obtained from Altromin GmbH u., Co KG, Lage, Germany.

Animals, housing and clinical observations

In the dose range finding study with PhIP, 80 C57BL/6ByA female mice weighing 18.1 ± 0.8 g (SD), obtained from IPFA Credo, Lyon, France were used. The mice were housed at five mice/cage. In the main study with PhIP, 59 E|pim-l heterozygous transgenic mice and 60 non-transgenic littermates, both sexes, age 9-12 weeks were used. The initial body weight of non-transgenic littermates was 24.0 ± 1.5 g (males) and 20.2 ± 1.3 g (females) and of transgenic littermates 26.9 ± 1.7 g (males) and 22.6 ± 1.6 g (females). In the study with IQ, 60 E|pim-l heterozygous transgenic mice and 60 non-transgenic littermates were used. The initial body weight of non-transgenic littermates was 20.3 ± 1.4 g (males) and 16.6 ± 1.1 g (females) and of transgenic littermates 22.3 ± 1.9 g (males) and 18.2 ± 1.3 g (females). All E|pim-l transgenic mice and the non-transgenic littermates were obtained from the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. All mice in the main study with PhIP and IQ were housed one male/cage and one or two females/cage. All mice were kept under controlled environmental conditions (temperature 21 ± 1°C, the relative humidity 55 ± 5%, 12/12 h light/dark cycle, air changed 10 times/h) and had free access to feed and tap water. Feed and water intake and body weight were recorded once weekly. All mice were observed at least twice a day for any abnormalities in clinical appearance.

Experimental designs

In the dose range finding study with PhIP, the mice were randomized into four groups, 20 animals in each were fed diets containing 0, 0.01, 0.02 or 0.04% PhIP during 4 weeks and killed on day 29.

In the main study with PhIP, the mice were allocated to four groups based on genotype and body weight: the non-transgenic littermates in groups I and II, each comprising 15 males and 15 females; E|pim-l transgenic littermates in group III comprising 14 males and 15 females; and group IV comprising 15 males and 15 females. Groups I and III included a diet without PhIP (controls), groups II and IV a diet with 0.03% PhIP during 31 weeks, which was also the observation period. In the study with IQ the mice were allocated to four groups based on genotype and body weight: the non-transgenic littermates in groups I and II, and transgenic littermates in group III and IV, each comprising 15 males and females. Groups I and III received diet without IQ (controls), groups II and IV received diet with 0.03% IQ during 24 weeks.

The observation period for all groups was 40 weeks. In both studies, all mice that became moribund or showed pronounced clinical manifestations (respiratory distress, central nervous symptoms) were killed and autopsied. Selected organs were fixed in 4% neutral buffered formaldehyde, and paraffin-embedded sections, 4-6 µm thick, were stained with hematoxylin and eosin for histopathological examination. In addition, lymphoid tissues were snap frozen for immunohistochemistry.

Immunohistochemical examination

Cryostat sections (4 µm) were collected on clean gelatine-coated glass slides. The tissues were dried at room temperature overnight and fixed in acetone for 20 min. Sections were air-dried for 5 min, washed in 1×phosphate buffered saline (PBS) for 5 min and then washed in 1×PBS including 0.5% bovine serum albumin (BSA) for 5 min. This was followed by a 10 min incubation at room temperature with blocking serum, 2% BSA in PBS to aid the suppression of non-specific binding of IgG. All incubations were performed in a moisture chamber. Excess serum was poured off and the sections were incubated for 30 min with biotin conjugated hamster anti-mouse CD3e monoclonal antibody specific for a 25 kDa protein component (e-T3) of the antigen specific T-cell receptor (Cedarlane CL7203B, Lot 3021) diluted 1:20 in PBS/0.5% BSA. Sections were washed in 2×PBS/0.5% BSA, and after washing the sections were incubated with the ABC reagent (DAKO K 355) for 30 min, employed for the avidin-biotin staining technique. The binding was detected by a final incubation in 0.8% 3-amin-9-ethylcarbazole (Sigma A5754) including 0.1 M acetate buffer pH 5.2 and 3% H₂O₂ for 15 min. The preparations were counterstained with haematoxylin, mounted with aqueamount, and examined by light microscopy.

Statistical analysis

Data on body weight, relative feed and water consumption were analyzed by analysis of variance followed by Duncan’s test. Data on total number of lymphomas were analyzed by Fisher’s exact test. Kaplan-Meier survival curves were plotted for all treatment groups for both sexes and data on survival were analyzed using the lifetest procedure. All analyses were performed using Statistical Analysis System (SAS) software (SAS Institute Inc.). The effects were considered significant for P < 0.05.

Results

Dose range finding study with PhIP

This study was performed in order to find the optimal dose of PhIP that would not cause toxic effects. The mean body weight of the mice fed 0.04% PhIP (group IV) was statistically significantly lower (P < 0.01) from the first week and throughout the study, and the feed intake in this group was statistically significantly lower (P < 0.01) in the first week, when compared with the control group. Since the body weight and feed intake of mice given 0.01% and 0.02% PhIP were not statistically significantly different from those in the control group the PhIP level in the diet in the main study was chosen to be 0.03%.

Main study with PhIP

The data on body weight are shown in Figures 1 and 2. The body weight of the transgenic littermates for both sexes was higher than that of the non-transgenic littermates from the start to the end of the experiment. This difference was statistically significant for group III compared to I (controls) for males in weeks 0-13, 15, 22, 25, 26, and for females throughout the study, and for group IV compared to II in both sexes throughout the study. The initial mean body weight in groups I and II, and groups III and IV were not statistically significantly different for males and females respectively. The PhIP feeding decreased the body weight in non-transgenic and transgenic littermates compared to their respective untreated controls. The body weight in group II was statistically significantly lower than in group I: for males from the first week and throughout the study thereafter and for females with the exception of weeks 1, 3–6, 8–10 and 13. The body weight in group IV was statistically significantly lower compared to...
Short-term carcinogenicity testing of PhIP and IQ in transgenic mice

Table I. Total number of mice with lymphoma in non-transgenic wild-type and transgenic pim-1 littermates on a diet with 0% or 0.03% PhIP.

<table>
<thead>
<tr>
<th>Group</th>
<th>0.03% PhIP</th>
<th>Sex</th>
<th>No. of mice</th>
<th>No. of mice at termination*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. WT</td>
<td>+</td>
<td>M</td>
<td>15</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>F</td>
<td>15</td>
<td>0 (0)</td>
</tr>
<tr>
<td>II. WT</td>
<td>+</td>
<td>M</td>
<td>15</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>F</td>
<td>15</td>
<td>4 (27)</td>
</tr>
<tr>
<td>III. TR</td>
<td>-</td>
<td>M</td>
<td>14</td>
<td>1 (7)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>F</td>
<td>15</td>
<td>3 (20)</td>
</tr>
<tr>
<td>IV. TR</td>
<td>+</td>
<td>M</td>
<td>15</td>
<td>4 (27)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>F</td>
<td>15</td>
<td>12 (80)</td>
</tr>
</tbody>
</table>

WT, non-transgenic wild-type littermates; TR, transgenic littermates.
*Day 218.

$p < 0.01$ females from group IV compared to females from group III.
$cP < 0.01$ females from group IV compared to females from group II.

Results

The average intake of PhIP during the study was as follows: non-transgenic littersmates: 46.8 mg/kg/day (males) and 47.7 mg/kg/day (females), and transgenic littersmates 44.0 mg/kg/day (males) and 49.6 mg/kg/day (females).

The total number of mice that had developed lymphomas is shown in Table I. There was no statistically significant difference in the total number of spontaneous lymphomas in Eg-pim-1 males and females (group III) compared with non-transgenic controls (group I). PhIP feeding increased the total number of Eg-pim-1 mice (group IV) with lymphomas compared to the transgenic controls (group III). However, the increase in total number of mice with lymphomas was statistically significant for females only. Furthermore, transgenic Eg-pim-1 females fed PhIP also had a statistically significantly higher incidence of lymphomas when compared to PhIP fed non-transgenic littersmates.

Lymphoma onset and incidence are shown in Figures 3 and 4. For males, the statistically significant difference in survival of lymphoma-free mice was only seen between groups II and...
IV (P < 0.05). For females, a statistically significant difference in survival of lymphoma-free mice was seen between groups I and II (P < 0.05), III and IV (P < 0.01) and II and IV (P < 0.01). However, no statistically significant difference in survival was seen between groups I and III.

At autopsy enlargement of thymus, spleen and lymph nodes were seen in affected animals. Infiltrations of liver, kidneys and ovaries were sometimes also grossly apparent. Microscopically, the lymphoblastic lymphomas consisted of a homogeneous population of large size lymphoid cells with a moderate amount of cytoplasm. Mitotic figures were frequently seen. Both lymphatic and non-lymphatic organs were infiltrated by lymphoblasts. The type of lymphoblastic cells observed in lymphoid organs was confirmed by immunohistochemistry for T-cell markers (data not shown).

**Main study with IQ**

The IQ dose of 0.03% was chosen based on a previous report (19). The data on body weight are shown in Figures 5 and 6. The body weight of the transgenic littermates of both sexes was statistically significantly higher (P < 0.05) than of the non-transgenic littermates from the start and throughout the study. The initial mean body weight in groups I and II, and groups III and IV were not significantly different (P > 0.05) for males and females respectively. From week 3 and throughout the study, the body weight of non-transgenic littermates of both sexes fed 0.03% IQ (group II) was statistically significantly lower (P < 0.05) compared with that of the respective controls (group I). The body weight of transgenic Eµ-pim-1 males fed 0.03% IQ (group IV) was statistically significantly lower (P < 0.05) compared with that of the controls (group III) from week 2 and throughout the study. The body weight of transgenic Eµ-pim-1 females fed IQ was statistically significant lower (P < 0.05) when compared with that of the controls in weeks 20, 21, 25, 26, 28, 30, 33, 35 and 38.

The average relative intake of IQ during the study was in non-transgenic littermates: 49.9 mg/kg/day (males) and 53.5 mg/kg/day (females), and in transgenic littermates 44.8 mg/kg/day (males) and 50.2 mg/kg/day (females). The statistically significantly difference in IQ intake was recorded only for males (group II versus group IV, P < 0.05).

The total number of mice that had developed lymphomas is shown in Table II. There was no statistically significant difference in the total number of spontaneous lymphomas between the transgenic Eµ-pim-1 and non-transgenic littermates of both sexes. IQ feeding did not statistically significantly increase lymphoma incidence in transgenic Eµ-pim-1 or non-transgenic littermates of both sexes.

**Discussion**

The usefulness of transgenic mice for short-term *in vivo* carcinogenicity testing remains to be established. The studies of Breuer *et al.* (5) pointed out that Eµ-pim-1 transgenic

![Fig. 5. Mean body weight of male non-transgenic wild-type (group I control: — — — — and group II 0.03% IQ: — — —) and transgenic Eµ-pim-1 littermates (group III control: — — — and group IV 0.03% IQ: — — —).](image)

![Fig. 6. Mean body weight of female non-transgenic wild-type (group I control: — — — — and group II 0.03% IQ: — — —) and transgenic Eµ-pim-1 littermates (group III control: — — — and group IV 0.03% IQ: — — —).](image)

### Table II. Total number of mice with lymphoma in non-transgenic wild-type and transgenic Eµ-pim-1 littermates on a diet with 0% or 0.03% IQ

<table>
<thead>
<tr>
<th>Group</th>
<th>IQ</th>
<th>Sex</th>
<th>No. of mice</th>
<th>No. of mice at termination*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>With lymphoma</td>
</tr>
<tr>
<td>I. WT</td>
<td>-</td>
<td>M</td>
<td>15</td>
<td>0 (0)</td>
</tr>
<tr>
<td>II. WT</td>
<td>+</td>
<td>M</td>
<td>15</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>F</td>
<td>15</td>
<td>1 (7)</td>
</tr>
<tr>
<td>III. TR</td>
<td>-</td>
<td>M</td>
<td>15</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>F</td>
<td>15</td>
<td>2 (13)</td>
</tr>
<tr>
<td>IV. TR</td>
<td>+</td>
<td>M</td>
<td>15</td>
<td>1 (7)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>F</td>
<td>15</td>
<td>4 (27)</td>
</tr>
</tbody>
</table>

WT, non-transgenic wild-type littermates; TR, transgenic littermates.

*Day 280.*
pim-l transgenic mice developed clonal T-cell lymphomas

Before 7 months of age, whereas none of the age matched

in the diet has been reported to cause 15-20% reduction in

The mechanism by which overexpression of the pim-1 gene

body weight in CD1 mice (16). Therefore we first performed

and on the strain of mice used. The 0.04% PhIP

result with the fourth carcinogen benzene, a potent mouse

mediates the enhanced susceptibility to

administration was by gavage at two dose levels also employed

mice, bearing the pim-1 gene supplemented with an upstream

mice for benzo[a]pyrene

especially in male mice. Since both B[a]P and PhIP require

metabolic enzymes involved.

administration by gavage was for 13 weeks at a dose regimen of 13 mg/kg, three times weekly. As a result, a

short-term carcinogenicity assay with benzo[a]pyrene (Kroese et al., submitted). Administration by gavage was for 13 weeks at a dose regimen of 13 mg/kg, three times weekly. As a result, a high sensitivity of the E[i]-pim-1 mice for benzo[a]pyrene (B[a]P) was observed with a rapid induction of lymphomas, especially in male mice. Since both B[a]P and PhIP require metabolic activation, the sex differences observed in the tumorigenic response of the E[i]-pim-1 mice may well be due to sex-related differences in the levels or activity of the metabolic enzymes involved.

In a previous reported study (8) the potential utility of E[i]-pim-1 model was investigated by examining its response to four genotoxic procarcinogens, three of which (2-AAF, 1,2-DCE and NDEA) are known to not be mouse lymphomagens. Administration was by gavage at two dose levels also employed in the chronic rodent bioassays performed with these compounds. Upon termination at 38-40 weeks, small increases in the incidence of lymphomas were observed for 2-AAF, 1,2-DCE and NDEA, which were statistically significant primarily for one of the sexes tested at the highest dose. Ironically, the result with the fourth carcinogen benzene, a potent mouse lymphomagen, was negative. Whether this lack of responsiveness is due to the different (genotoxic) properties of benzene, namely clastogenic and not mutagenic in Salmonella, or its predominant mechanism of action involved in lymphoma induction (initiation or promotion), is as yet unclear. Also it cannot be excluded that the duration of the study was too short to pick up possible (weaker) carcinogenic effects at longer latency times.

The results from the main study demonstrate that PhIP in the dose of 0.03% is a rapid and potent lymphomagen for E[i]-pim-1 mice. Especially transgenic E[i]-pim-1 females showed a higher PhIP induced lymphoma incidence than treated wild-type mice or untreated E[i]-pim-1 mice and also a strongly reduced latency period for lymphoma induction after PhIP treatment. However, it cannot be excluded that the more potent response to PhIP recorded in E[i]-pim-1 females compared to males could be due to their higher PhIP intake. Our results on the effect of PhIP in E[i]-pim-1 mice are comparable with the high frequency of lymphoma induction by ENU in this strain of transgenic mice reported by Breuer et al. (5). However, the study of Breuer et al. (5) did not specify sex differences in the responsiveness of E[i]-pim-1 mice to ENU. In our study, lymphomas were found in four of 15 (27%) non-transgenic C57BL/6ByA females fed 0.03% PhIP for 218 days. This incidence is slightly higher than we had expected when comparing our study design with the study of Esumi et al. (16) and could be due to strain differences. In the Esumi-study CDF1 mice were given a diet containing 0.04% PhIP throughout a study period of 579 days, and the first lymphoma was detected on day 236 in a female mouse fed PhIP. At the end of that study 68% (26 of 38) of the female and 31% (11 of 35) of the male mice had lymphomas.

E[i]-pim-1 mice have also recently been studied in a short-term carcinogenicity assay with benzo[a]pyrene (Kroese et al., submitted). Administration by gavage was for 13 weeks at a dose regimen of 13 mg/kg, three times weekly. As a result, a high sensitivity of the E[i]-pim-1 mice for benzo[a]pyrene (B[a]P) was observed with a rapid induction of lymphomas, especially in male mice. Since both B[a]P and PhIP require metabolic activation, the sex differences observed in the tumorigenic response of the E[i]-pim-1 mice may well be due to sex-related differences in the levels or activity of the metabolic enzymes involved.

We further evaluated the potential utility of the E[i]-pim-1 model by testing the rodent carcinogen PhIP, which also is a mouse lymphomagen but requires metabolic activation. The mutagenic and carcinogenic potential of the heterocyclic amine PhIP in rodents is well described in the literature (16-1,17,21-1,22). The onset of PhIP induced lymphomas in wild-type mice in these studies has been demonstrated to depend on the dose of PhIP and on the strain of mice used. The 0.04% PhIP in the diet has been reported to cause 15-20% reduction in

Fig. 7. Onset and incidence of lymphoma in male non-transgenic wild-type (group I control: O and group II 0.03% IQ: □) and transgenic E[i]-pim-1 littermates (group III control: V and group IV 0.03% IQ: *).

Fig. 8. Onset and incidence of lymphoma in female non-transgenic wild-type (group I control: O and group II 0.03% IQ: □) and transgenic E[i]-pim-1 littermates (group III control: V and group IV 0.03% IQ: *).
lymphomagenesis is still unclear. Studies with either overexpressing transgenic pim-1 or pim-1 deficient (knockout) mice indicate that the pim-1 gene, encoding a serine/threonine kinase, plays a role in the response to specific interleukin growth factors in some types of hematopoietic cells (23). In another study by Möröy et al. (24) Eμ-pim-1 mice were backcrossed to C57BL/6 mice homozygous for a lpr mutation, which causes a lymphoproliferative syndrome at 26–30 weeks of age. The observed strongly accelerated lymphoproliferation and dramatic enlargement of lymph nodes in the double transgenics, were considered indicative for a possible role of the pim-1 gene through inhibition of apoptosis.

Limitation in the usefulness of Eμ-pim-1 mice model for carcinogenicity testing has become apparent by the results of Storer et al. (8) and by our IQ feeding study. There was no statistically significant difference in the total number of spontaneous lymphomas or in the lymphoma incidence between transgenic Eμ-pim-1 mice and non-transgenic littermates in both sexes. The few lung and liver tumors found after 280 days in the IQ-dosed wild-type female mice and the forestomach papilloma in the IQ-dosed transgenic male mouse (Table II) are in accordance with the literature. In a carcinogenicity study with CDF1 mice fed 0.03% IQ in the diet up to 675 days, Ohgaki et al. (19) found that the target organs for IQ in mice were liver, forestomach and lung, and that female mice on IQ diet were more susceptible to the induction of liver tumors than male mice. Our results, therefore, suggest that the effects of a relatively strong genotoxic carcinogen such as IQ are not affected by transgenes relevant for and transcriptionally targeted to the lymphoid tissues.

Eμ-pim-1 mice predisposed to lymphoma may provide an important tool in understanding genetic nature of predisposition to this type of cancer. Lymphoma resistance gene loci might possibly be mapped by using appropriate crosses of tumor susceptible strains with a series of test strains as it has been done with genetic mapping of a pulmonary adenoma resistance locus (Parl) in mouse (25).

In conclusion, we found that the Em-pim-1 transgenic mouse model is a highly sensitive short-term in vivo system for PhIP-induced lymphomagenesis, but fails to respond to a genotoxic carcinogen (IQ) that does not target the lymphoid cells. This is in line with results from other investigators (8) and clearly limits the general usefulness of this model for carcinogenicity testing. The suitability of other transgenic mouse models, however, is at present still the subject of investigation. In this respect, the preliminary results with for instance transgenic mice with a gene through inhibition of apoptosis.

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References


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