ABSTRACT

Mutator phenotypes, a common and largely unexplained attribute of human cancer, might be better understood in mouse tumors containing reporter genes for accurate mutation enumeration and analysis. Previous work on peritoneal plasmacytomases (PCTs) in mice suggested that PCTs have a mutator phenotype caused by M-cdc-related chromosomal translocations and/or phagocyte-induced mutagenesis due to chronic inflammation. To investigate this hypothesis, we generated PCTs that harbored the transgenic shuttle vector, pUR288, with a lacZ reporter gene for the assessment of mutations in vivo. PCTs exhibited a 5.5 times higher mutant frequency in lacZ (40±5 x 10^-5) than in normal B cells (7.36 ± 0.77 x 10^-5), demonstrating that the tumors exhibit the phenotype of increased mutability. Studies on lacZ mutant frequency in serially transplanted PCTs and phagocyte-induced lacZ mutations in B cells in vitro indicated that mutant levels in tumors are not determined by exogenous damage inflicted by inflammatory cells. In vitro studies with a newly developed transgenic model of inducible Myc expression (Tet-off/MYC) showed that deregulated Myc sensitizes B cells to chemically induced mutations, but does not cause, on its own, mutations in lacZ. These findings suggested that the hypermutability of PCT is governed mainly by intrinsic features of tumor cells, not by deregulated Myc or chronic inflammation.

INTRODUCTION

The cause and significance of cancer-associated mutator phenotypes present a controversial, unresolved problem in cancer research. One school of thought postulates that mutator phenotypes are a common and requisite feature of neoplasia. Those who hold this view propose that tumor initiation and progression require a variety of specific genetic changes, necessitating a mutagenic pace that exceeds the spontaneous mutation rate (1, 2). The increased level of genomic instability (e.g., microsatellite instability, loss of heterozygosity) within a wide spectrum of tumors (3) and the high frequency of silent (unselected) mutations in genes, such as the one encoding p53, have supported this view (4). An alternative school of thought believes that oncogenesis is an essentially evolutionary process driven by cellular selection, considered to be capable of causing cancer in the presence of normal (spontaneous, background) mutation rates (5). The holders of this view argue that the mutator phenotype hypothesis is largely a product of overgeneralization from rare genetic syndromes linked with raised mutation rates and cancer predisposition; e.g., hereditary non-polypsis colon cancer and ataxia telangietasia. Furthermore, the growth of colorectal tumors and, presumably, many other common neoplasms begins with a normal mutation rate (6).

Mouse models of human cancer are suitable for studying the relationship between general mutagenesis and oncogenesis under genetically controlled conditions. Among the many advantages of the mouse is the ability to assess mutations in vivo using transgenic shuttle vectors with a bacterial reporter gene. Several transgenic reporter systems for measuring mutation frequency in vivo have been developed (7–11). Two widely used systems rely on the phage λ vector, ALIZ (12, 13), or the plasmid vector, pUR288 (14). Both vectors contain nontranscribed, neutral reporter genes (lacI in case of ALIZ and lacZ in case of pUR288) that have been incorporated into the mouse genome in which mutations occur. The reporter genes are excised from mouse genomic DNA and transferred to Escherichia coli, affording efficient selection, enumeration, and analysis of mutations. Both mutagenesis assays, ALIZ/lacI and pUR288/lacZ, are validated and thought to accurately reflect the mutant frequency (MF) in the overall genome (15, 16). One notable difference, however, is that the ALIZ/lacI assay detects primarily point mutations and small deletions in lacI, whereas the pUR288/lacZ assay additionally detects large deletions in lacZ and intra- and interchromosomal rearrangements of lacZ with mouse genomic sequences.

Elevated mutagenesis may facilitate the development of plasmacytomases (PCTs), malignant tumors of terminally differentiated B lymphocytes. Peritoneal plasmacytomagenesis in mice is dependent on a number of pathogenetic factors (reviewed in Ref. 17), of which at least two have the potential to increase general mutagenesis in B cells and plasma cells. The first one is the chronic inflammatory, granulomatous tissue that is formed in response to i.p. injections with pristane, which are required to induce PCT in genetically susceptible BALB/c (C) mice. The mutagenic principle in the pristane granuloma is the resident population of inflammatory cells, mainly macrophages and neutrophils, which are able to mutagenize bystander B/plasma cells; e.g., by releasing oxygen intermediates into the extracellular milieu (18–20). The second factor is the chromosomal translocations that result in the deregulated expression of the oncogene c-Myc. Virtually all pristane-induced PCT carry Myc-activating translocations, most commonly T(12;15). Deregulated expression of Myc in consequence of T(12;15) translocation may cause increased mutagenesis by a variety of mechanisms, including (a) uncoupling of DNA replication from cell cycle progression (21, 22), (b) down-regulation of genes involved in DNA repair (e.g., members of the GADD family; Ref. 23) or antimitageneus (e.g., BCL2; Ref. 24), (c) interference with DNA mismatch repair (25), and (d) disruption of redox homeostasis (26–28). The involvement of pro-mutagenic factors in PCT development, such as chronic inflammation and deregulated Myc, provided the rationale for the hypothesis that PCTs exhibit a phenotype of increased mutability (mutator phenotype).

Here we used the pUR288/lacZ mutagenesis assay to investigate whether PCTs have a mutator phenotype, and, if so, whether this phenotype may be caused by chronic inflammation and/or deregulated Myc. We thus backcrossed the pUR288 transgene from PCT-resistant C57BL/6 (B6) mice onto PCT-susceptible C mice and induced PCTs in the newly generated C.pUR288 congenics. We found that primary and transplanted PCTs exhibited the mutator phenotype of 5.5-fold...
elevated MFIs in *lacZ* compared with the background levels in normal B cells. To elucidate potential mechanisms of hypermutability, we designed *in vitro* model systems for assessing the mutagenic impact of inflammatory cells and activated Myc on primary B cells. The latter involved a new transgenic mouse model of inducible Myc expression, Tet-off/MYC. Although the mutation-enhancing effect of inflammatory cells and deregulated Myc was readily demonstrated, the aggregate of our findings suggested that the mutator phenotype of PCTs is mainly determined by intrinsic features of the tumor cells, presumably including mutator genes whose nature remains to be identified.

**MATERIALS AND METHODS**

**Generation of C.pUR288 Mice.** Strain C.pUR288 was generated by transferring the pUR288 transgene residing on chromosome (Chr) 3 (14) from C57BL/6 (B6) to BALB/cAnPt (C) using speed backcrossing. The original B6 mice carrying pUR288 (line 60) were developed by Dr. Jan Vijg, University of Texas, San Antonio. These mice harbor two virtually identical copies of the pUR288 transgene on Chr 3 and 4. Both copies consist of approximately 10 individual pUR288 genes. The chromosomal integration site of the transgene residing on Chr 4 happens to be in a region that contains allelomorphic variants of tumor modifier genes that confer susceptibility to PCTs in strain C but resistance to PCTs in strain B6 (29). To avoid the risk of replacing the susceptibility alleles of C with resistance alleles from B6, the transgene on Chr 4 was not transferred onto C (Supplementary Fig. 1). The intentional loss of susceptibility alleles of C with resistance alleles from B6, the transgene on Chr 4 was not transferred onto C (Supplementary Fig. 1). The intentional loss of susceptibility alleles of C with resistance alleles from B6, the transgene on Chr 4 was not transferred onto C (Supplementary Fig. 1). The intentional loss of susceptibility alleles of C with resistance alleles from B6, the transgene on Chr 4 was not transferred onto C (Supplementary Fig. 1). The intentional loss of susceptibility alleles of C with resistance alleles from B6, the transgene on Chr 4 was not transferred onto C (Supplementary Fig. 1).

**Mutagenesis in B Cells with Inducible Myc Expression.** To develop a system of inducible Myc expression in primary B cells, B6 mice harboring the Tet-off/MYC transgene were developed (Fig. 3A). The transgene consisted of an *Mlu-PvuI* fragment obtained from construct, pmyc-tet (BC266), described in greater detail elsewhere (33). The Tet-off/MYC contains the tetracycline-controlled transactivator, tTA, a minimal MYC promoter, and the protein-encoding portion of the human MYC gene (exons 2 and 3). The expression of MYC is activated on withdrawal of the tetracycline analogue, doxycycline (Tet-off). Tet-off/MYC mice were crossed with B6.pUR288 mice to develop a system in which mutagenesis in primary B cells can be studied under conditions of inducible Myc expression. B220<sup>+</sup> splenocytes from Tet-off/MYC-pUR288 bispeciﬁnic mice were fractionated by MACS (Miltenyi Biotec) using B220 (CD45R) V5<sup>+</sup> columns. Cell recovery relative to total splenocytes was ~90%. The purity of MACS-sorted B cells was ~90% by ﬂuorescence-activated cell sorting. The pUR288-harboring B cells were cocultured in the presence of doxycycline (MYC off) or absence of doxycycline (MYC on). Myc expression was monitored by Western blotting. In some experiments, 4-NOQ was added to induce heightened levels of mutagenesis compared with the pro-mutagenic environment of standard cell culture. Cells were harvested after 3 and 27 h to prepare genomic DNA for the pUR288 assay.

**RESULTS**

**C.pUR288 Mice Are Susceptible to PCTs and Are Suitable for Evaluations of MFs.** The genetic susceptibility to pristane-induced PCTs is a complex trait. Strain B6 and many other common inbred strains of mice are resistant to PCTs (34). In contrast, strain C is susceptible, which is caused, in part, by three susceptibility alleles on Chr 4 (35, 36). The transfer of genetic material from a PCT-resistant strain, such as B6, onto C involves the risk of contaminating the C genome with PCT resistance alleles, which may result in the decrease or even abrogation of PCT susceptibility. To evaluate whether the backcross of the pUR288 transgene from B6 onto C altered the susceptibility of C to PCTs, a tumor induction study with the newly generated C.pUR288 congenics was performed. Eight (40%) of 20 C.pUR288 mice developed PCTs by 260 days after the first injection.
of pristane (Fig. 1A, squares). Pristane-treated C mice showed a similar incidence [7 (35%) of 20] and onset of PCTs (Fig. 1A, circles). These findings indicated that strain C.pUR288 is as susceptible to PCTs as strain C.

Compromising the performance of the pUR288/lacZ mutagenesis assay is another risk when generating pUR288 congenics. The lacZ assay is dependent on the integrity of the pUR288 transgene, which can be altered by deletions and other genetic rearrangements that result in the loss of individual gene copies from the concatemer. Loss of copy number affects assay performance by reducing the efficiency with which reporter genes can be excised from mouse genomic DNA and transferred to E. coli. This problem was encountered in a previous study involving the B6 to C transfer of the pUR288 transgene, which is noted at backcross generations N_4 and N_5 (37). To avoid similar problems during the B6 to C transfer of the pUR288 transgene, the performance of the pUR288/lacZ assay was monitored at each backcross generation (N_1–N_8) to select breeders with a properly functioning mutagenesis assay. The average plasmid rescue efficiency at generation N_2 (used for the tumor induction study described above) was ±9.0 × 10^7/µg DNA, ~50% lower than in parental B6 mice. The drop in rescue efficiency was expected because, unlike B6 mice, C mice contained only one copy of pUR288. Despite the reduction in assay performance, strain C.pUR288 was suitable for determining MF in PCTs using the lacZ reporter gene assay.

**Elevated MFs in Primary PCTs.** The determination of the MF in transgenic reporter genes in tumors and corresponding normal and premalignant tissues provides important information about genomic stability control during oncogenesis. To evaluate whether pristane-induced PCTs exhibit genomic instability, the MF in lacZ was compared with that in B cells and peritoneal granulomas. This comparison was meaningful because B-lymphocytes are the precursors of PCTs and the inflammatory granuloma is the tissue site in which PCTs was meaningful because B-lymphocytes are the precursors of PCTs and the inflammatory granuloma is the tissue site in which PCTs develops (Fig. 1B). The mean MF in primary PCTs (40.3 × 10^−5; n = 5) was 5.5 times higher than the mean MF in MACS-sorted B220⁺ splenocytes (B cells; 7.36 × 10^−5). This indicated that PCTs have a phenotype of moderate hypermutability. The MF in B220⁺ splenocytes was 2.2 times higher than in B220⁻ splenocytes (non-B cells), consistent with a previous result using ALIZ (38). The relatively high MF of splenic B cells may be related to their high proliferative activity in vivo, considering that increased proliferation facilitates mutagenesis (39). The MF in the granulomas (17.3 × 10^−5) was 2.3 times lower than the MF in the PCTs, but 3.7-fold higher compared with the mesentry (4.71 × 10^−5). The mesentry, a fatty tissue, was included as a control for the granulomas because it serves as the matrix for granuloma development in pristane-treated mice. The increased MF in the granulomas relative to the mesentry suggested that resident granuloma cells (mainly phagocytes, such as macrophages and neutrophils) accumulate somatic mutations by self-inflicted mutagenesis, possibly by releasing reactive oxygen intermediates. It further suggested that the granulomas provide a mutagenic environment for infiltrating B cells, which may become targets of phagocyte-mediated oxidative damage (Fig. 1B, top). However, the contribution of B cells to the elevated MF in the granulomas remained unclear, because mutations in B cells were not distinguished from mutations in granuloma cells (both cell types carried the lacZ reporter gene).

**Mimicking the Mutagenic Effect of the Inflammatory Granuloma in Vitro.** To measure mutations in B cells exposed to inflammatory granuloma cells, we coincubated LPS-stimulated B220⁺ splenocytes from C.pUR288 mice in vitro with pristane-elicited PECs (macrophages and neutrophils) from normal C mice. Mutations in the B cells (positive for lacZ) were enumerated, whereas mutations in PECs (negative for lacZ) were not (Fig. 2A). The coincidence of B cells and PECs for 24 h at a ratio of 1:2 resulted in a MF of 20.1 × 10^−5 in the B cells, a 3.2-fold increase compared with B cells without PECs (6.2 × 10^−5). Activation of the oxidative burst in PECs (four applications of 50 nM TPA spaced 6 h apart) resulted in a small additional increase in the MF to 23.2 × 10^−5, but this was not significant. Treatment of B cells with TPA in the absence of PECs caused an ~1.5-fold increase in MF (10.3 × 10^−5) relative to background levels in untreated B cells (Fig. 2B). These results were consistent with previous ones using the ALIZ assay (20). The magnitude of PEC-induced mutagenesis was impressive compared with that induced by the powerful UV-mimetic mutagen, 4-NQO, which generates oxidative stress and DNA adduct formation through intracellular redox cycling (40). TPA-stimulated PECs and 25 nM 4-NQO were roughly equipotent (Fig. 2C). The addition of the antioxidant catalase...
Fig. 2. Phagocytes mutagenize neighboring B cells in vitro. A, cytomytology of cocultured LPS blasts and peritoneal exudate cells (PECs). The blasts harbored the shuttle vector pUR288, but the PECs did not. PECs consisted mainly of neutrophils (arrow pointing right) and macrophages (arrow pointing down). LPS blast included large activated cells (arrow pointing up) frequently exhibiting mitotic figures (arrow pointing left; H&E ×40). B, mean lacZ mutant frequency (MF) in LPS blasts after cocultivation with PECs either stimulated with TPA (column 4) or left nonstimulated (column 3). TPA-treated blasts (column 2) and untreated blasts (column 1) were included as controls. Vertical lines above the columns, SD, of mean MF. The Ps were calculated in two-sided t tests. See legend to Fig. 1C for definition of Mutants, Total lacZ, Exp., MF, and SD. C. 4-nitroquinoline-1-oxide (4-NQO)-induced mutations in lacZ in LPS blasts. Cells were treated for 24 h in the presence of the indicated concentrations of 4-NQO. Asterisks above the columns, significant increases (P < 0.05) in MF relative to the background value in the untreated control (y2 test). MF calculation was based on a total of 784 mutants (range, 40 to 299 mutants) scored among 2.1 × 106 wild-type colonies (range, 2.64 × 105 to 5.59 × 106). D, inhibition of PEC-mediated mutagenesis in B cells by the antioxidants, catalase [C (column 3)] or catalase plus pyruvate [C + P (column 4)]. Above the columns, residual lacZ MF (%) relative to TPA-stimulated PECs without antioxidants (100%, column 1). MF calculation was based on a total of 179 mutants (range, 20 to 76) scored among 8.07 × 105 wild-type colonies (range, 122 × 103 to 272 × 103). E, genetic defects in oxidative burst leading to diminished mutagenic potential of PECs. PECs deficient in p47phox, an essential component of active NADPH oxidase, are unable to produce superoxide (O2·−) in the NADPH oxidase reaction. O2·− is further reduced to the highly reactive hydroxyl radical (not shown) and reacts with nitric oxide (NO) to form peroxynitrite (ONOO·−), which is highly toxic compared with O2·− and NO (68). Peroxynitrite is also a potent genotoxin (69). PECs with a partial deficiency in glucose-6-phosphate dehydrogenase (G6PD) due to the hypomorphic Gpdx−/− allele produce insufficient amounts of NADPH, which is used, among many other enzymes, by NADPH oxidase and the inducible nitric oxide synthase (iNOS). Thus, although G6PD-deficient PECs have a partial but compound defect in generating mutagenic oxygen and nitrogen species, p47phox−/− null PECs have a complete defect in one pathway of reactive oxygen intermediate production. F, partial loss of mutagenic potency in p47phox−/− null PECs (P) and G6PD-deficient PECs (G) relative to normal PECs (C) obtained from C mice. The experiment enumerated 682 mutants (range, 35 to 224) in a total of 6.22 × 106 colonies (range, 3.84 × 105 to 2.21 × 106).

(2 units/μl) reduced the mutagenic potency of PECs by ~75% (Fig. 2D, column 3), but no further reduction in MF was achieved by adding 1 mM pyruvate on top of the catalase (Fig. 2D, column 4). PECs obtained from mice with genetic defects in the oxidative burst (Fig. 2E) were less efficient in causing mutations in the lacZ reporter gene than were normal PECs (Fig. 2F). Total loss of NADPH oxidase function (phox−/− null mice; Ref. 31) resulted in a drop of mutant levels by 35% on TPA stimulation (Fig. 2F, column 4) and 41% without TPA stimulation (Fig. 2F, column 2). Partial deficiency in glucose-6-phosphate dehydrogenase (G6PD) caused a reduction in MF by 51% in TPA-stimulated PECs (Fig. 2F, column 5) and 49% in unstimulated PECs (Fig. 2F, column 3). These findings agreed with evidence that PEC-induced mutagenesis is complex and involves both oxidative and nonoxidative mechanisms (41).

Mimicking the Mutagenic Effect of Deregulated Myc In Vitro. Peritoneal PCTs carry chromosomal translocations that result in the deregulated expression of Myc, which may promote neoplastic plasma cell transformation by facilitating genomic instability in B cells (42–44). To assess the potential role of deregulated Myc as a catalyst of B-cell mutagenesis, a transgenic mouse model of conditional Myc expression was developed. The model, designated Tet-off/MYC, uses the tetracycline-responsive regulatory system of gene expression (45) to overexpress a human Myc gene in all tissues, including B cells, on withdrawal of the tetracycline analogue, doxycycline (Fig. 3A). Fig. 3B (top) shows the time course of Myc protein expression in Tet-off/Myc-pUR288 bitransgenic B cells on withdrawal of doxycycline. The increase in Myc reached a maximum after ~3 h and lasted for 27 h. A similar peak of Myc expression at 2–4 h was also observed in SV40 large T-transformed Tet-off/Myc-pUR288 fibroblasts used as controls (Fig. 3B, bottom).

To evaluate mutagenesis in B cells under conditions of inducible Myc expression, MACS-sorted LPS-stimulated bitransgenic B220+ splenocytes were cultured in the presence or absence of doxycycline before preparing genomic DNA for the lacZ mutagenesis assay. Some cell cultures were exposed to oxidative cell culture stress and others were treated with 4-NQO to generate additional genotoxic stress (Fig. 3C). Treatment with 4-NQO resulted in a significantly higher MF in Myc-overexpressing B cells (MYC on) compared with normal B cells (MYC off) at 27 h (88.6 × 10−5 versus 41.8 × 10−5), indicating that deregulated Myc can sensitize B cells to undergo elevated mutagenesis in the presence of a strong mutagen. The difference was much smaller at 3 h (20.8 × 10−5 versus 18.6 × 10−5), suggesting that the mutation-enhancing effect of overexpressed Myc was dependent on time (cell division) to fix the mutations. Under conditions of relatively
planted (G 1–G 3) four primary PCTs (G 0) into pristane-primed C mice. The ability (13, 46–vivo propagation of primary tumors leads to run-away genomic instability (13, 46–vivo propagation of primary tumors leads to run-away genomic instability. Furthermore, the mutation-enhancing ability of Myc and Prkdc, on its own, is not a mutator gene in B cells.

Myc-dependent changes in lacZ mutant levels in fibroblasts were strikingly similar to those observed in B cells (Fig. 3D). In the presence of 4-NQO, the MF was higher (2.4-fold) in Myc-overexpressing fibroblasts relative to normal fibroblasts (Fig. 3D, compare columns 4 and 2). In the absence of 4-NQO, the MF in Myc-overexpressing fibroblasts was lower (by 18%) than in normal fibroblasts (compare columns 3 and 1). The matching results in two different cell types (B cells and fibroblasts) indicated that deregulated Myc might act as a catalyst of chemically induced mutagenesis in many tissues. Furthermore, the mutation-enhancing ability of Myc might be fully compensated when genotoxic stress is low.

Mutant Levels and Patterns of Mutations in Transplanted PCTs. Transgenic reporter genes are useful to evaluate whether in vivo propagation of primary tumors leads to run-away genomic instability (13, 46–49). To investigate this possibility, we serially transplanted (G 1–G 4) four primary PCTs (G 0) into pristane-primed C mice and harvested cells 4–6 weeks posttransplantation. Pristane treatment of recipient mice was required for the outgrowth of transfused tumor cells, which are as dependent on the microenvironment of the pristane granuloma as are primary PCTs (50). The MF in G 1 to G 3 PCTs was lower relative to normal B cells: 11.9 × 10^{-5} versus 14.7 × 10^{-5} at 3 h and 17.2 × 10^{-5} versus 20.0 × 10^{-5} at 27 h. This result demonstrated that overexpressed Myc, on its own, is not a mutator gene in B cells.

Fig. 3. Myc sensitizes B cells to chemically induced mutagenesis. A, scheme of the Tet-off/MYC transgene. The transactivator, tTA, is driven by the constitutively active, minimal cytomegalovirus (CMV) promoter and, thus, is always produced. In presence of doxycycline (small gray circles binding to tTA), tTA cannot dock onto the tetO7-TP element, which includes the Myc promoter. On withdrawal of doxycycline, tTA binds to tetO7-TP, which results in the expression of Myc mRNA and protein (33, 70). B, time course of Myc protein expression on withdrawal of doxycycline in LPS blasts (top) and a fibroblast line (bottom) transformed by the SV40 large-T antigen. Shown are Western blots of lysates of B-lymphoblasts developed with the horseradish peroxidase-conjugated antibodies 9E10 (1:100) or lysates of fibroblasts developed with the polyclonal antibody A-14 (1: 500). Both antibodies were from Santa Cruz Biotechnology. Actin, which was used as loading control, was detected with an antibody from Sigma. C and D, lacZ mutant frequency (MF) in B cells and fibroblasts overexpressing Myc (on) or not overexpressing Myc (off). Cells were treated with 50 nm 4-nitroquinoline-1-oxide (4-NQO) for the indicated time (+) or left untreated (–). The MF in the fibroblasts are given in relative units, using the background MF in untreated cells as the reference point (100%). The background MF was 1.7 × 10^{-5}, which was unusually high. The reason for this is not known but is possibly related to the origin of the cell line, which was derived from primary C.pUR288 fibroblasts by transformation in vitro with large T antigen from SV40 virus (using plasmid, pSV40-T Ag), followed by transfection with the Tet-off/MYC expression vector (LipofectAMINE, Boehringer Mannheim) and selection in 100 μg/ml hygromycin. The background MF of other cell lines obtained by this protocol was even higher (results not shown). Large T antigen is a potent mutator gene in mice (71). See legend to Fig. 1C for definition of Mutants, Total lacZ, and MF.

DISCUSSION

The present study describes a mutator phenotype in a mouse model of neoplastic plasma cell development that is tightly associated with chronic inflammation and activated Myc. The mutator phenotype of...

MUTATOR PHENOTYPE IN MOUSE PCTs

Analysis of mutational patterns in transgenic reporter genes can provide important mechanistic insights into tumor-associated mutator phenotypes. The pUR288/lacZ assay affords the opportunity to distinguish two important classes of mutations by simple restriction analysis of lacZ mutants. Point mutations are identified by the wild-type pattern of pUR288, whereas rearrangements (R) are identified by a deviant pattern. Increases in R mutations, which include large deletions that extend into the mouse genome, deserve particular attention, because R mutations may be underrepresented in the pUR288/lacZ assay. R mutations are probably at a selective disadvantage whereas point mutations are not (51). In lacZ mutants obtained from PCTs, R mutations were clearly the dominant category of mutations [132 (72.5%) of 182; Fig. 4B]. R mutations were less prevalent in B cells exposed to PECs [44 (63.7%) of 69], in B cells from the Tet-off/MYC experiment [490 (54.5%) of 868], and in control tissues [145 (51%) of 284]. The prevalence of R mutations in normal tissues of C.pUR288 mice (51%) was higher than that reported for B6.pUR288 mice (42–45%: Refs. 14, 52), which may reflect genotypic differences in genomic stability control between strains C and B6. One of the genes that may contribute to the apparent proclivity of C mice to undergo R mutations is the weak allele of Pkdc encoding the catalytic subunit of the DNA double-strand break repair protein, DNA-PK, a key player in DNA double-strand break repair (53). The weak efficiency allele of DNA-PK has recently been shown to be associated with delayed genomic instability in B cells (54) and increased lymphoma susceptibility (55).

DISCUSSION

The present study describes a mutator phenotype in a mouse model of neoplastic plasma cell development that is tightly associated with chronic inflammation and activated Myc. The mutator phenotype of...
peritoneal PCT was characterized by a 5.5-fold increase in the MF of a transgenic lacZ reporter gene relative to the MF in normal B cells, and a preponderance of rearrangement mutations of lacZ (~75%) in the tumor cells. To better understand this mutator phenotype, we evaluated the mutagenic consequences of inflammation and the deregulated Myc expression in B cells with the help of two dedicated in vitro model systems. The first model used the coinoculation of B cells and PECs to mimic the mutagenic environment of the inflammatory granuloma. It showed that macrophages and neutrophils are able to mutagenize neighboring B cells, in part by releasing reactive oxygen intermediates. The second model used the Tet/off-MYC transgenic of lacZ mutant levels in primary B cells. It demonstrated that overexpressed Myc in Tet/off-Myc-pUR288 bitransgenic B cells exposed to oxidative cell culture stress (59, 60) in the absence of 4-NQO was not accompanied by elevated mutagenesis indicated that deregulated Myc can function as a mutator gene (reviewed in Refs. 43, 44). Some mechanisms by which Myc induces genetic instability and increased mutagenesis are currently emerging (21, 22, 24, 26–28). However, our finding that overexpressed Myc in Tet/off-Myc-pUR288 B cells exposed to oxidative cell culture stress (59, 60) in the absence of 4-NQO was not accompanied by elevated mutagenesis indicated that deregulated Myc is not a mutator gene per se. In fact, the lacZ mutant levels in Myc-overexpressing B cells were lower than in the controls: 11.9 × 10⁻⁵ versus 14.7 × 10⁻⁵ after 3 h of cell culture and 17.2 × 10⁻⁵ versus 20.0 × 10⁻⁵ after 27 h. Lack of DNA damage was not the underlying reason, because normal B cells in cell culture (Myc off) showed a clear upward trend in lacZ MF over time: 9.66 × 10⁻⁵ at 0 h, 14.7 × 10⁻⁵ at 3 h, and 20.0 × 10⁻⁵ at 27 h. These results indicated that the impact of deregulated Myc on MF in B cells is variable and dependent on the type and intensity of the mutagenic attack. The pro-mutagenic potential of Myc may be fully restrained under conditions of moderate oxidative stress, possibly because of Myc-dependent adaptive responses that involve the up-regulation of key components of oxidative stress control, such as thioredoxin peroxidase, glutathione transferase, and lactate dehydrogenase. Lactate dehydrogenase is clearly activated in mouse plasma cell tumors (Supplementary Fig. 2,5, leading to increased production of pyruvate, a scavenger of oxygen radicals (23, 61). The ability of Myc to positively regulate DNA repair genes (62), including NBS1 (63) involved in DNA double-strand break repair, may further contribute to harnessing the detrimental effects of Myc on the genome. These considerations suggest that although Myc may be involved in the PCT mutator phenotype, Myc is unlikely to be the sole cause for this phenotype.
In conclusion, mouse PCTs exhibit the increased mutability of a lacZ reporter gene, which is associated with, but presumably not caused by, chronic inflammation and activated Myc. The elevated lacZ mutant levels can be added to previously reported manifestations of genomic instability in PCTs, e.g., cytogenetic aberrations (64) including Myc-activating chromosomal translocations (65), activation of intracisternal A particles (66), and DNA mutations due to deficiencies in transcription-coupled nucleotide excision repair (67) and DNA double-strand break repair (53, 54). Thus far, none of these changes has been elucidated with regard to the underlying molecular mechanism, and the relationship to deregulated Myc has also remained unclear. On the other hand, it has been shown that Myc-dependent mouse B-cell and plasma cell tumors can be associated with different Myc mutant phenotypes. For example, Burkitt-like lymphomas of λ-MyC-pUR288 bitransgenic mice exhibited the same propensity to rearrangement mutations in lacZ as PCTs did, but demonstrated, in contrast to PCTs, a very small increase in lacZ MF. These findings illustrate the persistent difficulty of distinguishing mutant phenotypes associated with Myc from mutant phenotypes caused by Myc. Our newly developed model of mutation assessment in pUR288 transgenic mice and pUR288-Tet-off/MYC bitransgenic mice may be useful to elucidate this distinction.

ACKNOWLEDGMENTS

We thank Dr. Jan Vigj, University of Texas at San Antonio, San Antonio, TX, for providing pUR288-transgenic B6 mice; Drs. Stephen M. Holland and Harry L. Malech, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, for the kind gift of p47phox null mice; Drs. Martin Döllé, University of Texas at San Antonio, and Michael Boerrigter, Leven, Inc., for providing pUR288-transgenic B6 mice; Drs. Stephen M. Holland and Jackson B. L. Cbl-2 is an apoptotic target suppressed by both c-Myc and E2F-1. Oncogene, 20: 6983–6993, 2001.


44. Soucek, L., and Evan, G. Myc is this the oncogene from Hell? Cancer Cell, 1: 406–408, 2002.


Moderate Hypermutablety of a Transgenic lacZ Reporter Gene in Myc-Dependent Inflammation-Induced Plasma Cell Tumors in Mice


Cancer Res 2004;64:530-537.