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# Inflammation, Oxidative DNA Damage, and Carcinogenesis

## by J. G. Lewis* and D. O. Adams*

**Inflammation has long been associated with carcinogenesis, especially in the promotion phase. The mechanism of action of the potent inflammatory agent and skin promoter 12-tetradecanoyl phorbol-13-acetate (TPA) is unknown. It is thought that TPA selectively enhances the growth of initiated cells, and during this process, initiated cells progress to the preneoplastic state and eventually to the malignant phenotype. Many studies support the multistep nature of carcinogenesis, and a significant amount of evidence indicates that more than one genetic event is necessary for neoplastic transformation. Selective growth stimulation of initiated cells by TPA does not explain how further genetic events may occur by chronic exposure to this nongenotoxic agent. We and others have proposed that TPA may work, in part, by inciting inflammation and stimulating inflammatory cells to release powerful oxidants which then induce DNA damage in epidermal cells. Macrophages cocultured with target cells and TPA induce oxidized thymine bases in the target cells. This process is inhibited by both catalase and inhibitors of lipoxygenases, suggesting the involvement of both $H_2O_2$ and oxidized lipid products. Furthermore, macrophage populations that release both $H_2O_2$ and metabolites of arachidonic acid (AA) are more efficient at inducing oxidative DNA damage in surrounding cells than populations which only release $H_2O_2$ or metabolites of AA. *In vivo* studies demonstrated that SENCAR mice, which are sensitive to promotion by TPA, have a more intense inflammatory reaction in skin than C57LB/6 mice, which are resistant to promotion by TPA. In addition, macrophages from SENCAR mice release more $H_2O_2$ and metabolites of AA, and induce more oxidative DNA damage in cocultured cells than macrophages from C57LB/6 mice. These data support the hypothesis that inflammation and the release of genotoxic oxidants may be one mechanism whereby initiated cells receive further genetic insults. They also further complicate risk assessment by suggesting that some environmental agents may work indirectly by subverting host systems to induce damage rather than maintaining homeostasis.**

## Introduction

Numerous observations in humans and animals have suggested a strong association between inflammation and carcinogenesis. As early as the late 1800s, Virchow published observations noting the appearance of tumors in sites of chronic irritation (*1*). For example, in humans there is a high rate of carcinoma of the colon in patients with the inflammatory bowel disease ulcerative colitis (*2*). In rats, the instillation of inflammatory peptides into the colon induces an inflammatory process similar to ulcerative colitis (*3*), and increased rates of carcinoma of the colon occur in animals coexposed to the colon carcinogen 1,2-dimethylhydrazine (*4*). Similarly, patients with chronic infections such as osteomyelitis or decubitous ulcers can develop very aggressive squamous cell carcinomas and soft tissue sarcomas in draining sinuses and ulcers (*5*). Inflammatory reactions to foreign bodies have also been linked to carcinogenesis. If flat sheets of plastic are placed subcutaneously in mice, sarcomas will develop at that site (*6*). If the material is ground to a powder or large holes drilled into the sheets, tumors do not develop, suggesting that it is not the material *per se* causing tumors, but the reaction of the host to foreign material (*6*).

The terms initiation and promotion were first employed in describing the induction of tumors in animals by chronically wounding skin that had been previously exposed to a subthreshold dose of tar (*7*). Later experiments demonstrated that croton oil, known as a blistering agent at the time, could efficiently promote tumors (*8,9*). Subsequently, 12-*O*-tetradecanoyl-13 acetate (TPA) was separated from croton oil and shown to be both the inflammatory and tumor promoting agent (*10*). Over time TPA has been shown to induce a vast array of changes in cells depending on the system investigated (*11*). Much of the subsequent work on the mechanism of action of TPA centered on its effects on cell replication and differentiation (*11*). Based on data from such studies, it has been proposed that TPA selectively enhances the replication of initiated cells, thereby increasing their representation in the skin (*11*). Subsequent exposure to TPA causes progression of some of these cells to form benign neoplasms, papillomas, and further exposure induces some of the papilloma cells to progress to the malignant phenotype.

Studies from several other laboratories have sug-

*Department of Pathology, Duke University Medical Center, Durham, NC 27710.

gested an additional and nonmutually exclusive mode of action for TPA that has relinked inflammation, tumor promotion, and carcinogenesis. When inflammatory cells, such as neutrophils and macrophages, are exposed to active phorbol esters, they release large quantities of reactive oxygen intermediates such as $O_2^-$ and $H_2O_2$ and oxidized products of arachidonic acid (AA) (*12–14*). This, in conjunction with the observation that reagent peroxides are active promoters in mouse skin (*15*), has suggested that phorbol esters may work, in part, by inciting inflammation and causing infiltrating inflammatory cells to release large quantities of oxidants which may then induce DNA damage in epidermal cells. We and several other groups have shown that leukocytes have the capacity to be genotoxic to surrounding cells. Leukocytes exposed to phorbol esters have been shown to suffer breaks in their own DNA, induce chromosomal aberrations, breaks, and oxidized thymine bases in the DNA of surrounding cells, and to be mutagenic to bacteria (*15–18,34*).

Presented here are results of studies in our laboratory on the ability of chronic inflammatory cells, macrophages, to induce oxidative DNA damage in surrounding cells, mechanisms by which this may occur, and correlations between inflammation, the release of oxidants from macrophages, and sensitivity to tumor promotion *in vivo*.

# Materials and Methods

***Animals.*** Inbred SENCAR mice were obtained from the Oak Ridge Research Institute, Oak Ridge, TN, and C57LB/6 mice were obtained from the Trudeau Institute, Lake Saranac, NY.

***Macrophage Culture.*** Murine peritoneal macrophages were elicited, obtained, purified, and cultured as previously described (*19*).

***Quantitation of Oxidized Thymine Bases.*** Oxidized thymine bases were quantified by base-acid degradation as previously described (*20*) and performed in this laboratory (*18*).

***Quantitation of $H_2O_2$.*** $H_2O_2$ was quantified by the horseradish peroxidase-catalyzed oxidation of phenol red as previously described (*21*).

***Separation and Quantitation of the Release of Arachidonate Metabolites.*** Arachidonic acid metabolism was quantified and the metabolites separated using reverse-phase HPLC as previously described (*22*).

***Evaluation of Inflammation in Skin.*** Inflammation responses in skin were quantified by measurement of edema formation by increases in skin sample weight, measurement of vascular permeability by the leakage of $^{125}$I-labeled albumin into the interstitium, and the infiltration of leukocytes by light microscopy. Detailed methods have been published elsewhere (*23*).

# Results

## Macrophage Activation

It is important to note that macrophages, unlike neutrophils, are not short-lived end cells. They are long-lived cells which, after leaving the marrow, continue to develop in response to various signals (*24*). As they develop or pass through various stages of activation, they acquire the capacity to perform certain functions and lose the capacity to perform others (Table 1). For example, resident macrophages have a very low capacity for the generation of $H_2O_2$, but possess a high capacity for the release of metabolites of arachidonic acid (Table 1). Conversely, fully activated macrophages elicited with agents such as bacille Calmette-Guérin (BCG), which have an enhanced capability for microbial and tumor cell killing, have a high capacity for the release of $H_2O_2$, but a very low capacity for arachidonate metabolism (Table 1). Inflammatory macrophages, elicited with sterile casein, have an intermediate capacity for both $H_2O_2$ generation and arachidonate metabolism (Table 1). In addition, different stimuli, such as TPA or the particulate zymosan, have different effects on $H_2O_2$ release and AA metabolism (*14*). TPA is a much better stimulant than zymosan for $H_2O_2$ generation, and zymosan is more efficient at stimulating AA metabolism than TPA (*14*).

## Induction of Oxidative DNA Damage by Macrophages: Role of $H_2O_2$

We first wanted to determine if macrophages had the capacity to induce oxidative damage in surrounding cells. Casein-elicited macrophages were cocultured with NIH 3T3 target cells with and without TPA. In order to quantify DNA damage of oxidative origin, we measured the formation of oxidized thymine bases (T*) in targets that were prelabeled with [$^3$H]-thymidine. T* were induced in targets that were exposed to both macrophages and TPA (Fig. 1). No T* were induced by TPA or macrophages alone (Fig. 1). The induction of T* and the production of $H_2O_2$ were dependent on the concentration of macrophages (Fig. 2). When targets were exposed to reagent $H_2O_2$ at concentrations actually produced by macrophages, T* were induced in a dose-dependent manner (Fig. 3). To determine if $H_2O_2$ was involved in the induction of T* by macrophages, the specific scavengers catalase and superoxide dismutase (SOD) were added to cocultures of macrophages, TPA, and targets. To allow for access of these large scavengers, exposures were conducted in suspension. Catalase completely inhibited the induction of T* by macrophages, whereas SOD significantly enhanced induction (Fig. 4). The enhancement of damage by SOD

**Table 1. Functional capacities of macrophages in different stages of activation.[a]**

| Eliciting agent | $H_2O_2$ | AA metabolism | Microbial killing | Tumor cell killing |
|---|---|---|---|---|
| Nothing | + − | + + + + | + − | − |
| Casein | + + + | + + | + | − |
| BCG | + + + + | + − | + + + + | + + + + |

[a] Data from (*14,24*).

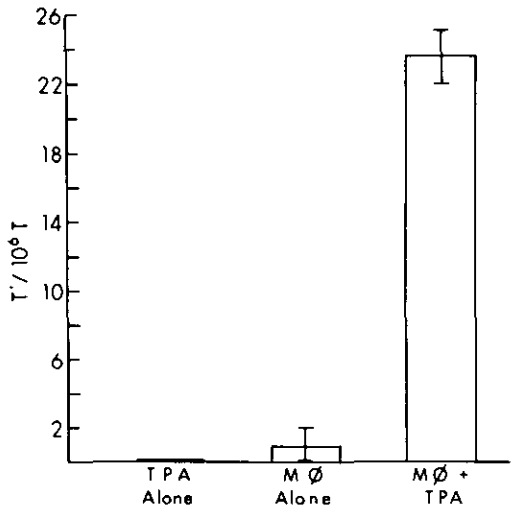


FIGURE 1. Induction of T* in the DNA of NIH 3T3 cells cocultured with inflammatory macrophages (Mϕ) stimulated with TPA. Casein-elicited peritoneal exudate cells were added to monolayers of 3T3 cells that had been prelabeled with [methyl-$^3$H]thymidine. The flasks were incubated for 60 min, and the nonadherent cells were washed off the target cells. Following adherence purification of the macrophages, serumless medium was added with and without TPA (100 ng/mL). The flasks were incubated for a further 60 min and T* was quantified as described in "Materials and Methods." Control and samples cultured with TPA alone received the same number of macrophages at 4°C immediately before precipitation of the macromolecules (mean of duplicate samples). Reprinted with permission of *Cancer Research* (*18*).

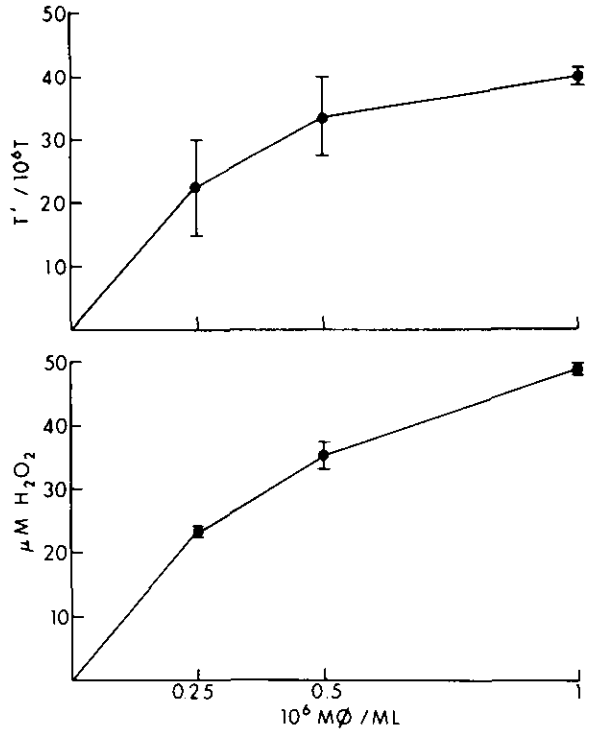


FIGURE 2. Relationship between the density of macrophages (Mϕ), the concentration of $H_2O_2$ in the culture medium, and the amount of T* induced in target cells. The curves connect the means at duplicate determinations. Points, individual determinations. Reprinted with permission of *Cancer Research* (*18*).

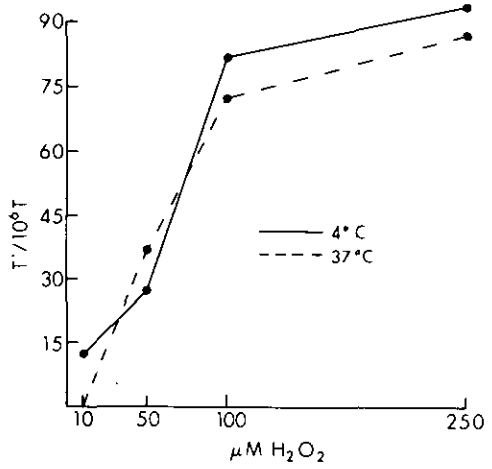


FIGURE 3. Induction of T* in the DNA of NIH 3T3 cells by reagent $H_2O_2$. Prelabeled monolayers of target cells were exposed to varying concentrations of $H_2O_2$ for 60 min at 4° or 37°C. Following exposure, the cells were collected by trypsinization and centrifugation, and T* was quantified as described in "Materials and Methods" (mean of duplicate samples). Reprinted with permission of *Cancer Research* (*18*).

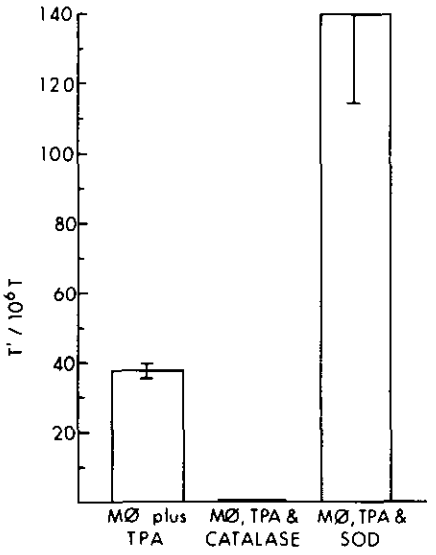


FIGURE 4. Effects of catalase and superoxide dismutase (SOD) on the induction of T* in cocultured target cells by inflammatory macrophages (Mϕ). Casein-elicited peritoneal exudates were separated from polymorphonuclear leukocytes by density gradient centrifugation. Prelabeled monolayers of 3T3 cells were trypsinized and cultured with the casein-elicited macrophages (106 macrophages/mL) in suspension, with and without catalase (100 units/mL) or SOD (100 units/mL) for 60 min at 37°C (mean of duplicate samples). Reprinted with permission of *Cancer Research* (*18*).

**Table 2. Effects of macrophage activation and type of stimulus on the induction of T*.[a]**

| Macrophage type | T* TPA | T* zymosan | T* zymosan + NDGA | T* zymosan + indomethocin |
|---|---|---|---|---|
| Resident | + + | + + + | − | + + + + |
| Casein | + + + + | + + + + + + | ND[b] | ND |
| BCG | + | ND | ND | ND |

[a] Data from (*14*).
[b] Not determined.

presumably occurred because SOD does not completely detoxify $O_2^-$ but converts it to $H_2O_2$, thus resulting in increased rates of $H_2O_2$ production.

## Role of AA Metabolism in the Induction of Oxidative DNA Damage

As Emerit and Cerutti have shown the possible involvement of AA metabolism in the clastogenic action of leukocytes (*17*), and Slaga et al. have reported that inhibitors of AA metabolism can inhibit or enhance the promotion of tumors by TPA *in vivo* (*25*), we next determined if AA metabolism had a role in the induction of oxidative DNA damage by macrophages. To do this we exploited the fact that different populations of macrophages have different capacities for the secretion of $H_2O_2$ or metabolites of AA (Table 1). We reasoned that the macrophage population most able to generate the relevant effector molecule would be the most efficient at inducing oxidative DNA damage. Surprisingly, BCG-elicited macrophages, which have the highest capacity for the generation of $H_2O_2$, induced the lowest levels of T* (Table 2). Resident cells, which have the lowest capacity for $H_2O_2$ release, induced more T* than the BCG-elicited cells (Table 2). Interestingly, the casein-elicited cells, which perform both functions fairly well (Table 1), were the most efficient cells at inducing T* (Table 2). Further support for the involvement of AA metabolism in the induction of oxidative DNA damage was the observation that zymosan, which is more efficient at stimulating AA metabolism than $H_2O_2$ release, was a better stimulant for the induction of T* than TPA, which is a better stimulant for $H_2O_2$ release than AA metabolism (*14*,*22*) (Table 2). We then tested the ability of inhibitors of lipoxygenases and cyclooxygenases to inhibit the induction of oxidative DNA damage by macrophages. Nordihydroquaiaretic acid (NDGA), an inhibitor of both lipooxygenases and cyclooxygenases, completely inhibited the induction of T* by resident macrophages stimulated with zymosan (Table 2). Indomethacin, a specific inhibitor of cyclooxygenases, significantly enhanced T* induction (Table 2).

If AA metabolism is involved in the induction of oxidant damage by macrophages, the studies showing that reagent $H_2O_2$ alone at the concentrations released by macrophages could induce similar amounts of T* as mac-

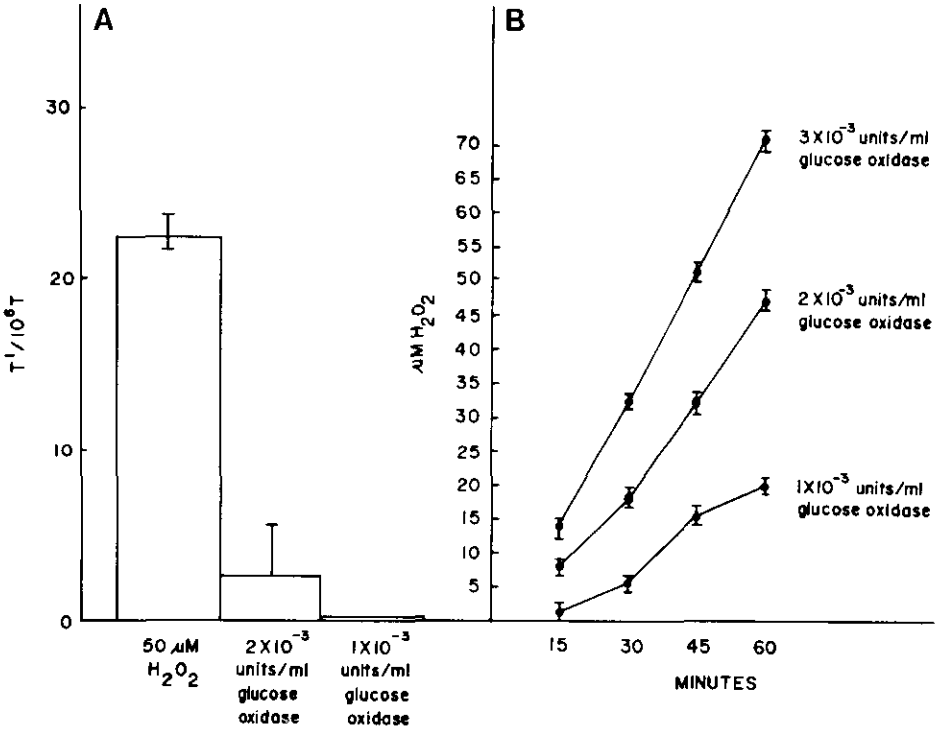


FIGURE 5. Comparison of the induction T* in target cells by a single bolus of reagent $H_2O_2$ or a flux generated by glucose oxidase. Target cells were prelabeled with [$^3$H]thymidine, exposed to 50 μM $H_2O_2$ or glucose oxidase for 60 min at 37°C and the amount of T' quantified (*A*) as described in "Materials and Methods." The amount of $H_2O_2$ produced over 60 min by different amounts of glucose oxidase was quantified as previously described (*20*). (*B*) Mean ± the range of duplicate samples. Reprinted with permission of *Carcinogenesis* (*14*).

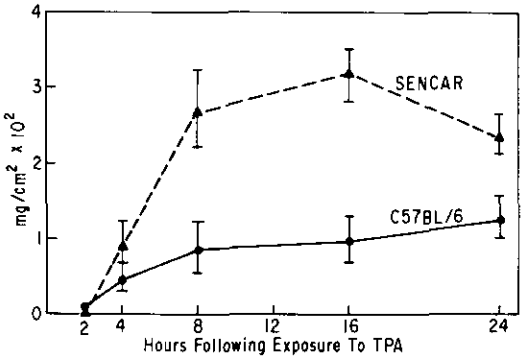


FIGURE 6. Edema formation in skin of C57LB/6 and SENCAR mice following application of 5 μg of TPA in 100 μL of acetone. Animals were exposed and at various times 1 $cm^2$ samples of skin were taken and weighed. Controls were animals exposed to acetone. Weights of control animals were subtracted from experimentals and the data are present as net increase in weight over time. Mean ± SEM of triplicate animals. Reprinted with permission of *Carcinogenesis* (*23*).

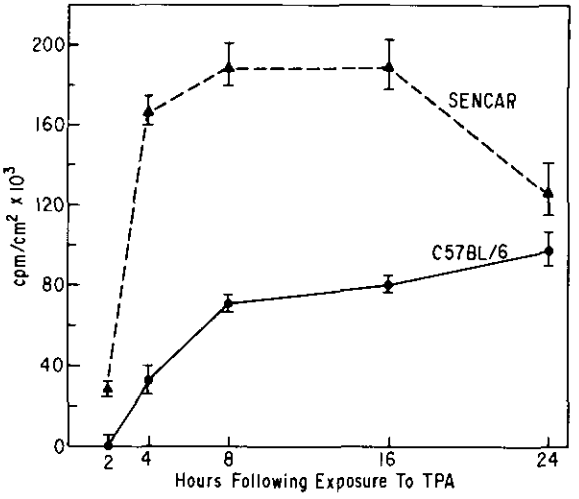


FIGURE 7. Increase in vascular permeability in the skin of C57LB/6 and SENCAR mice following exposure to TPA. Two hours before sacrifice animals were injected IV with 1.6 times $10^5$ cpm of $^{125}$I-labeled BSA. Several 1-$cm^2$ samples of skin were taken and counted in a beta counter. Controls were animals exposed to acetone. The radioactivity in controls was subtracted from that in experimental animals and data are presented as net increase in cpm over time. Mean ± SEM of triplicate animals. Similar results were obtained in three separate experiments. Reprinted with permission of *Carcinogenesis* (*23*).

rophages are paradoxical. To resolve this we tested the effects of exposure of targets to a flux of $H_2O_2$ generated over time by glucose oxidase versus the effects of the same concentration of reagent $H_2O_2$ given as a single bolus. When targets were exposed to a flux of $H_2O_2$, similar to that generated by macrophages, much less T* was induced by the flux than by a bolus of reagent $H_2O_2$ (Fig. 5).

## The Role of Inflammation in the Sensitivity to Phorbol Ester Tumor Promotion *In Vivo*

In order to investigate the possible role of inflammation in tumor promotion *in vivo*, we used the SENCAR/C57BL/6 mouse model. SENCAR mice have been selectively bred for their sensitivity to two-stage carcinogenesis in skin employing TPA as the promoter, whereas C57LB/6 mice are almost totally resistant (*26*). C57LB/6 mice are, however, sensitive to complete carcinogenesis by the initiating agent, and are sensitive to promotion by benzoyl peroxide, a free radical-generating compound (*26*). This indicates that the difference in tumor response in these strains is limited to the promotion phase, the action of phorbol esters, and that exogenously supplied radicals can overcome the resistance of the C57LB/6 mice.

When TPA was applied to the backs of SENCAR and C57LB/6 mice, there was a more intense inflammatory response in the skin of SENCAR mice as measured by increases in edema, vascular permeability, and infiltration of leukocytes (Figs. 6–8). Table 3 summarizes the observed dose and time effects. The major differences in the inflammatory response occurred during the first 48 hr following the first application of TPA. Massive inflammatory infiltrates were observed in the skin of SENCAR mice beginning at 16 hr following exposure to TPA and peaking at 24 hr (Fig. 8). No inflammation was observed in the skin of correspondingly treated C57LB/6 mice (Fig. 8). After the hyperplastic response in the epidermis had begun, the infiltration of leukocytes in SENCAR mice dramatically subsided to a slight chronic infiltration (Table 3). Hyperplasia occurred in the epidermis of both strains of animal, but was more intense in SENCAR animals (Table 3).

## Release of $H_2O_2$ and Metabolites of AA by Macrophages from SENCAR and C57LB/6 Mice

The studies cited above demonstrated that more inflammatory cells were present in the skin of mice sensitive to the promotion of skin tumors by TPA. We next determined if there were any differences in the amount of oxidants released by inflammatory cells from the two strains of mice. Macrophages from SENCAR mice released four times the amount of $H_2O_2$ per milligram of cell protein than similar cells obtained from C57LB/6 mice when phorbol dibutyrate (PDBU) was used as a stimulant (Fig. 9). In addition, the half maximal effective dose of TPA was 60% lower in SENCAR mice than in C57LB/6 mice. The differences between $H_2O_2$ release by macrophages from the two strains was much less when zymosan was the stimulant (Fig. 10). Similarly, macrophages from SENCAR mice released more AA metabolites than cells from C57LB/6 mice when TPA was used as a stimulant (Fig. 11). Conversely, macrophages from C57LB/6 mice released more metabolites of AA when zymosan was used as a stimulant (Fig. 12). When the individual metabolites were separated by

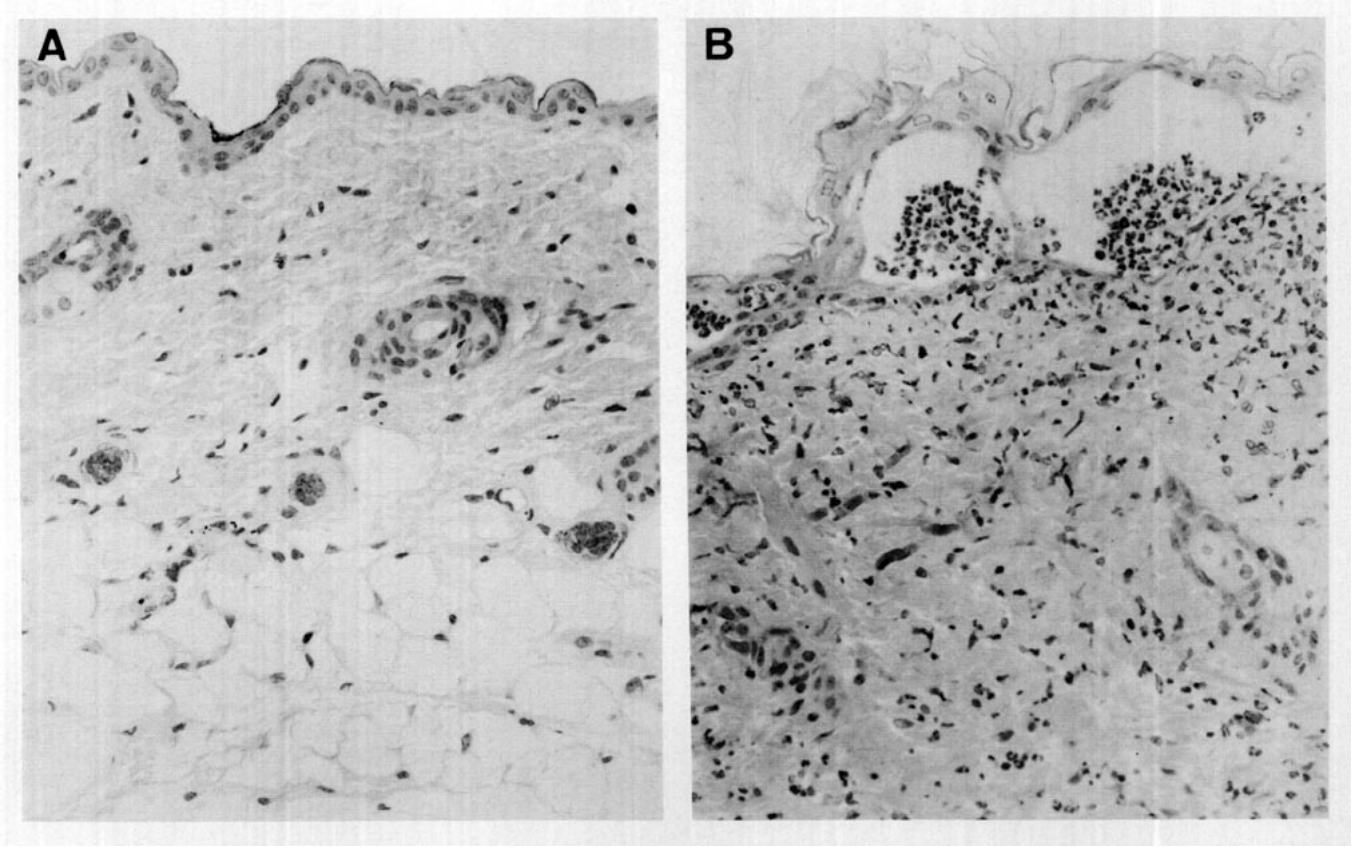


FIGURE 8. Photomicrographs of skin from (*A*) C57LB/6 mice and (*B*) SENCAR mice 24 hr after a single exposure to 5 μg of TPA. No toxic or inflammatory changes are present in the skin of C57LB/6 mice (*A*). In skin of SENCAR mice there is infiltration of acute inflammatory cells in the epidermis (*B*). Vacuolated keratinocytes are also present in the skin of SENCAR mice (*B*). × 250.

**Table 3. Effects of dose, time, and number of application of TPA on inflammation and hyperplasia in the skin of SENCAR and C57BL/6 mice.**

| Dose and number of exposures[a] | | SENCAR mice | C57BL/6 mice |
|---|---|---|---|
| 5 μg × 1 | Leukocytes[b] | + + | − |
| | Hyperplasia[b] | − | − |
| 2 μg × 1 | Leukocytes | + + + + | + − |
| | Hyperplasia | − | − |
| 5 μg × 1 | Leukocytes | + + + + | + |
| | Hyperplasia | − | − |
| 0.5 μg × 4 | Leukocytes | + | − |
| | Hyperplasia | + + | − |
| 2 μg × 4 | Leukocytes | + − | − |
| | Hyperplasia | + + + + | + + |
| 5 μg × 4 | Leukocytes | + | + |
| | Hyperplasia | + + + + | + + |

[a] Mice were exposed twice weekly and sacrificed 24 hr following the last exposure. Data from (*23*).
[b] Evaluated by light microscopy.

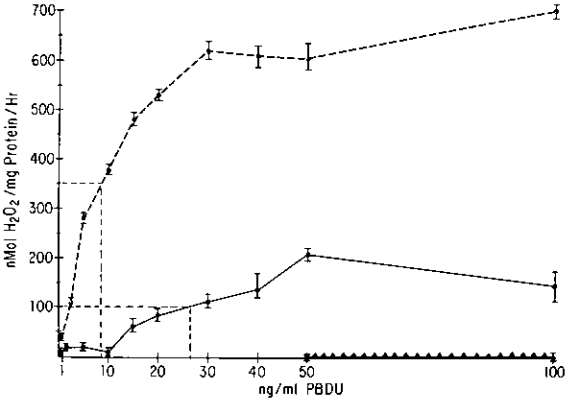


FIGURE 9. Release of $H_2O_2$ by resident and casein-elicited macrophages from SENCAR (- - -) and C57LB/6 (—) mice exposed to varying concentrations of PBDU. $H_2O_2$ release over 60 min was measured by the horseradish peroxidase-catalyzed oxidation of phenol red as described previously (*21*). Points, mean triplicate samples; bars, SE. Reprinted with permission of *Cancer Research* (*22*).

HPLC, very few differences in the pattern of metabolites released were noted (*14*).

Since macrophages from SENCAR mice release more of the oxidants and lipid oxidation products associated with genotoxicity in target cells than macrophages from C57LB/6 mice, we next determined if SENCAR macrophages would induce more oxidative DNA damage than macrophages from C57LB/6 mice. When casein-elicited macrophages from SENCAR and C57LB/6 mice were cocultured with P388 targets, the macrophages from SENCAR mice induced more T* than cells from C57LB/6 mice (Fig. 13). Interestingly, macrophages

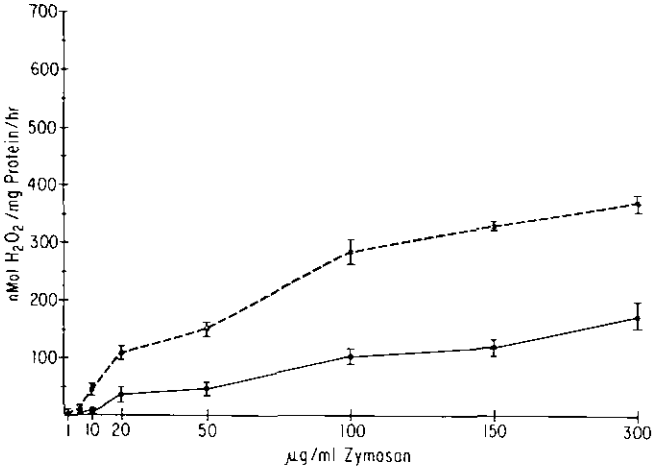


FIGURE 10. Release of $H_2O_2$ by casein-elicited macrophages from SENCAR (---) C57LB/6 (—) mice over 60 min in response to varying concentrations of zymosan. Points, mean of triplicate samples; bars, SE. Reprinted with permission of *Cancer Research* (*22*).

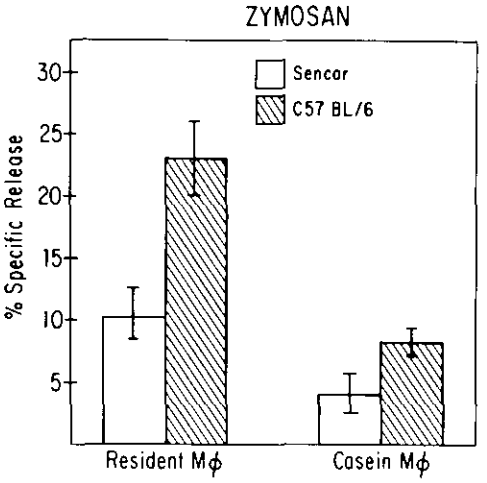


FIGURE 12. Release of metabolites of AA from resident and casein-elicited macrophages (Mϕ) from SENCAR and C57LB/6 mice exposed to zymosan (150 μg/mL). Columns, mean of duplicate samples; bars, range. Similar results were obtained in three separate experiments. Reprinted with permission of *Cancer Research* (*22*).

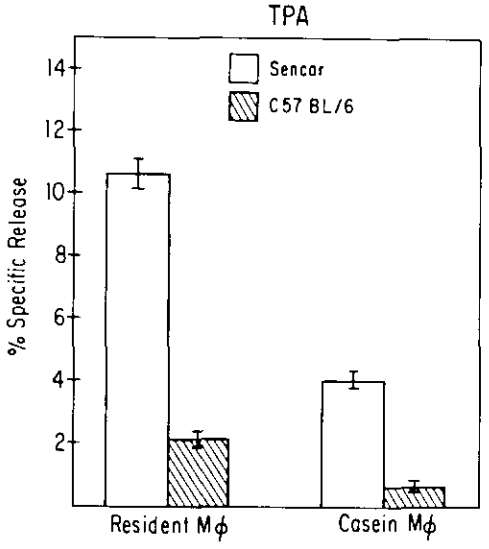


FIGURE 11. Release of metabolites of AA from resident and casein-elicited macrophages (Mϕ) from SENCAR and C57LB/6 mice in response to TPA. Macrophages were obtained, cultured, and prelabeled with [$^3$H]AA as previously described. Cells were exposed to TPA (100 ng/mL) for 60 min, and the amount of label released was determined by scintillation counting. Spontaneous release determined in control cultures was subtracted and the counts were normalized to the amount of label uptake, which was also determined in control cultures. Columns, mean duplicate samples; bars, range. Reprinted with permission of *Cancer Research* (*22*).

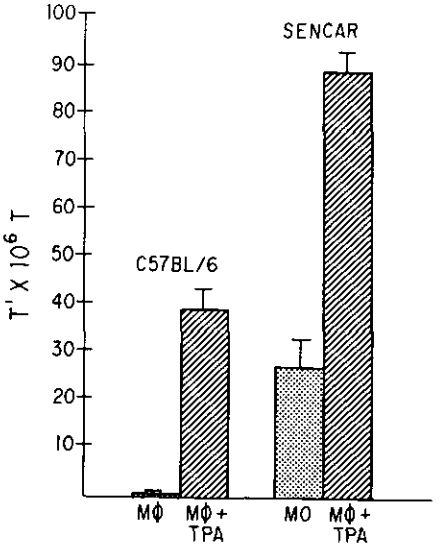


FIGURE 13. Induction of oxidized T* in the DNA of BP388 cells by casein-elicited macrophages from SENCAR and C57LB/6. Target cells were prelabeled with [$^3$H]-thymidine and cocultured with macrophages ± TPA for 60 min. T* was measured as previously described (*18,20*).

from SENCAR mice induced a significant amount of T* in the absence of TPA (Fig. 13).

## Discussion

The data presented here demonstrate that chronic inflammatory cells have the capacity to induce oxidative DNA damage in surrounding cells under ideal, controlled conditions *in vitro*. They further suggest that both $H_2O_2$ release and AA metabolism by the macrophages are necessary for damage to occur. *In vivo* studies demonstrated a strong relationship between the sensitivity to tumor promotion by TPA and the intensity of the inflammatory response in skin following exposure to TPA. Strong correlations were also demonstrated between enhanced sensitivity to TPA promotion and enhanced release of $H_2O_2$ and AA metabolites by macrophages. Taken together these data support the hypothesis that inflammation, the release of powerful oxidants by inflammatory cells, and the induction of DNA

damage by these oxidants, may play a role in the promotion or potentiation of carcinogenesis.

The multistep nature of carcinogenesis is supported in several organ systems, mathematical models, and in oncogene transformation studies using primary cultures of cells (*11,27*). In all of these systems, more than one genetic event is postulated as necessary for full transformation. This can be provided by repeated exposures to initiating agents, most of which are carcinogens, and mutagens which bind to DNA, or by transfection with at least two oncogenes (*27*). The manner in which further genetic events occur during exposure to TPA is not adequately explained by the selective proliferation hypothesis. Undoubtedly, selective proliferation of initiated cells occurs and is a necessary event. Many more initiated cells are present in the skin than those that form papillomas or carcinomas (*28*). The reason some initiated cells form papillomas, and the majority lie dormant, is not known. Thus, it may be that both selective replication and further genetic insults are necessary in some initiated cells.

Several recent studies suggest that inflammatory mechanisms, such as those presented here, may play an important role both in very early interactions with initiated cells and in the late stages during the final progression of cells to the malignant phenotype. Mezerine, a weak promoter, becomes a strong promoter if it is applied after a few applications of TPA (*29*). TPA can be applied before the initiating agent, and a significant amount of time can pass between exposure to TPA and the initiator (*30*). This suggests that one event evoked by TPA is rapid and possibly long lasting. The observation presented here that the inflammatory response quickly subsides during TPA administrations suggests that if genotoxic damage is to occur because of oxidants released from inflammatory cells, it must occur quickly. The memory effect of a limited number of TPA exposures is also consistent with a genotoxic insult early in TPA exposure. The observations that reagent peroxides are promoters, that a higher number of peroxide-promoted tumors are carcinomas, that peroxides are efficient converters of papillomas to carcinomas, and that acetic acid, a nonspecific toxic agent, can also convert papillomas to carcinomas (T. J. Slaga, personal communication) suggests that inflammation and the release of oxygen radicals induced by necrosis may also play an important role in the progression of premalignant cells to the malignant phenotype.

It is important to note that while inflammatory cells are an excellent source of reactive species of oxygen, they are not the only source. Fischer has shown the luminol-enhanced chemiluminescence occurs in epidermal cells exposed to TPA (*31*). Others have shown that cultured cells can be promoted *in vitro* by TPA in the absence of inflammatory cells (*32*). However, these latter studies also demonstrated that reactive oxygen intermediates were still necessary for TPA promotion, suggesting that oxygen radicals, regardless of source, are crucial to the action of TPA and the promotion process.

In terms of risk assessment in humans, the studies presented here represent a considerable complicating element. These data suggest that any comprehensive risk assessment plan must address not only the effects of xenobiotics on cells directly (i.e., induction of DNA adducts), but must consider how host systems such as inflammation can be subverted into causing damage rather than homeostasis. The human population is exposed constantly to a complex mixture of physical and chemical genotoxic agents. Even with the high capacity for DNA repair exhibited by longer-lived species such as man, it is not unreasonable to assume that many individuals have initiated cells in their bodies. The easiest task in risk assessment may be to identify those agents that adduct DNA, only to discover that agents that act indirectly on initiated cells are also a significant variable in the probability of tumor formation. To rationally add these variables into risk assessment schemes, we need a much better mechanistic understanding of the changes which occur in cells at all phases of carcinogenesis.

## REFERENCES


1. Virchow, R. Cellular Pathology, 7th edition. Translated by F. Chance. Robert M. Dewitt, New York, 1860, p. 554.
2. Goldgraber, M. B., Humphreys, E. M., Kirsner, J. B., and Palmer, W. L. Carcinoma and ulcerative colitis. A clinical pathologic study. II. Statistical analysis. Gastroenterology 34: 840–846 (1958).
3. Chester, J. F., Ross, J. S., Malt, R. A., and Weitzman, S. A. Acute colitits produced by chemotactic peptides in rats and mice. Am. J. Pathol. 121: 284–290 (1985).
4. Chester, J. F., Gaissert, H. A., Ross, J. S., Malt, R. A., and Weitzman, S. A. Augmentation of 1,2-dimethylhydrazine induced colon cancer by experimental colitis in mice: Role of dietary vitamin E. JNCI 76: 939–942 (1986).
5. Cruickshank, A. H., McConnell, E. M., and Miller, D. G. Malignancy in scars, chronic ulcers and sinuses. J. Clin. Pathol. 16: 573–580 (1963).
6. Brand, G. K. Cancer associated with asbestosis, schistosomiasis, foreign bodies and scars. In: Cancer, Vol. 1 (F. F. Becker, Ed.), Plenum Press, New York, 1982, pp. 661–692.
7. Friedwald, W. F., and Rous, P. The initiating and promoting elements in tumor production. An analysis of the effect of tar, benzpyrene, and methylcholanthrene on rabbit skin. J. Exp. Med. 80: 101–115 (1944).
8. Cherbuliez, E., Ehninger, E., and Bernhard, K. Researchers sur la graine de croton, le principe visicant. Helv. Chim. Acta 15. 658–670 (1932).
9. Mottram, J. G. Developing factor in experimental blastogenesis. J. Pathol. Bacteriol. 56: 181–190 (1944).
10. Boutwell, R. K. Some biological aspects of skin carcinogenesis. Prog. Exp. Tumor Res. 4: 207–250 (1964).
11. Heckler, E., Kunz, W., Fusenig, N. E., Marks, F., and Thielmann, H. W., Eds. Cocarcinogenesis and Biological Effects of Tumor Promoters. Raven Press, New York, 1982.
12. Goldstein, B. D., Witz, G., Amoruso, M., Stone, D. S., and Troll, W. Stimulation of human polymorphonuclear leukocyte superoxide anion radical production by tumor promoters. Cancer Lett. 11: 257–262 (1981)
13. Nathan, C. F., and Root, R. K. Hydrogen peroxide release from peritoneal macrophages. Dependence on sequential activation and triggering. J. Exp. Med. 146: 1648–1662 (1977).
14. Lewis, J. G., Hamilton, T. A., and Adams, D. O. The effect of macrophage development on the release of reactive oxygen intermediates and lipid oxidation products and their ability to induce oxidative DNA damage. Carcinogenesis 7: 813–88 (1986).

15. Slaga, T. J., Klein-Szanto, A. J. P., Triplett, L. L., and Yotti, L. P. Skin tumor promoting activity of benzoyl peroxide, a widely used free radical generating compound. Science 213: 1023–1025 (1981).
16. Birboim, H. C. DNA strand breakage in human leukocytes exposed to a tumor promoter, phorbol myristate acetate. Science 215: 1247–1249 (1982).
17. Emerit, I., and Cerutti, P. A. Tumor promoter phorbol-12-myristate-13-acetate induces chromosomal damage via indirect action. Nature 293: 144–146 (1981).
18. Lewis, J. G., and Adams, D. O. Induction of 5,6 saturated thymine bases in NIH 3T3 cells by phorbol ester-stimulated macrophages: Role of oxygen intermediates. Cancer Res. 45: 1270–1285 (1985).
19. Adams, D. O. Effector mechanism of cytolytically activated macrophages. I. Secretion of neutral proteases and the effect of protease inhibitors. J. Immunol. 124: 286–292 (1980).
20. Hariharan, P. V., and Cerutti, P. A. Excision of damage thymine residues from gamma irradiated poly (dA-dT) by crude extracts of *Escherichia coli*. Proc. Natl. Acad. Sci. (U.S.) 71: 3532–3536 (1974).
21. Pick, E., and Keizari, Y. A simple colormetric method for the measurement of hydrogen peroxide produced by cells in culture. J. Immunol. Methods 38: 161–165 (1980).
22. Lewis, J. G., and Adams, D. O. Enhanced release of hydrogen peroxide and metabolites of arachidonic acid by macrophages from SENCAR mice following stimulation with phorbol esters. Cancer Res. 46: 5696–5700 (1986).
23. Lewis, J. G., and Adams, D. O. Early inflammatory changes in the skin of SENCAR and C57BL/6 mice following expsoure to 12-O-tetradecanoylphorbol-13-acetate. Carcinogenesis 8: 889–898 (1987).
24. Adams, D. O., and Hamilton, T. A. The cell biology of macrophage activation. Annu. Rev. Immunol. 2: 283–318 (1984).
25. Slaga, T. J., Fischer, S. M., Weeks, C. E., Nelson, K., Mamrack, M., and Klein-Szanto, A. J. P. Specificity and mechanisms of promoter inhibitors in multistage carcinogenesis. In: Carcinogenesis, Vol 7 (E. Heckler, N. E. Fusenig, W. Kunz, F. Marks, and H. W. Theilmann, Eds.), Raven Press, New York, 1982, pp. 19–34.
26. Reiners, J. J., Neshow, S., and Slaga, T. J. Murine susceptibility to two-stage skin carcinogenesis is influenced by the agent used for promotion. Carcinogenesis 5: 301–307 (1984).
27. Land, H., Parada. L. F., and Weurberg, R. A. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 304: 596–602 (1983).
28. Miller, D. R., Viaje, A., Aldaz, C. M., Conti, C-J., and Slaga, T. J. Terminal differentiation resistant epidermal cells in mice undergoing two-stage carcinogenesis. Cancer Res. 47: 1935–1940 (1987).
29. Slaga, T. J., Fischer, S. M., Nelson, K., and Gleason, G. L. Studies on the mechanism of skin tumor promotion evidence for several stages in promotion. Proc. Natl. Acad. Sci. (U.S.) 77: 3659–3667 (1980).
30. Furstenberger, G., Kinzel, V., Schwartz, M., and Marks, F. Partial inversion of the initiation promotion sequence of multistage tumorigenesis in the skin of NMRI mice. Science 230: 76–78 (1985).
31. Fischer, S. M., Baldwin, J. K., and Adams, L. M. Effects of antipromoters and strain of mouse on tumor promoter-induced oxidants in murine epidermal cells. Carcinogenesis 7: 915–918 (1986).
32. Kennedy, A. R., Troll, W., and Little, J. B. Roles of free radicals in the initiation and promotion of radiation transformation *in vitro*. Carcinogenesis 5: 1213–1218 (1984).
33. Weitzman, S. A., and Stossel, T-P. Effects of oxygen radical scavengers and oxidants on phagocyte-induced mutagenesis. J. Immunol. 128: 2770–2772 (1982).