Inflammation, Chromosomal Instability, and Cancer: The Schistosomiasis Model

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Abstract

Evidence is accumulating in support of a role for reactive oxygen species in the etiology of cancer. Inflammatory cells, such as neutrophils, macrophages, and eosinophils, are an important endogenous source of oxygen radicals. Stimulation of these cells by tumor promoters or by foreign bodies (parasites, bacteria, etc.) causes the release of reactive oxygen species. Laboratory studies have shown that genetic damage and neoplastic transformation are induced in vitro in cells cocultured with activated inflammatory cells. We have recently begun to study the role of inflammatory reactions in inducing genetic damage in a human population. This paper describes our initial studies of Egyptian patients infected with Schistosoma haematobium. This infection induces chronic inflammation and irritation in the urinary bladder and is associated with increased cancer at this site. We describe a recently completed population study that shows that infected individuals have elevated levels of genetic damage in their bladders, as measured by the exfoliated cell micronucleus test. Treatment that kills the parasite also reduces the micronucleus frequencies. We also explore the hypothesis that altered sensitivity of clones of cells in these patients to reactive oxygen species could be a force that drives the development of neoplasia by facilitating clonal expansion. Evidence is presented for the possible involvement of loci on chromosome 11 in controlling the level of chromosomal breakage caused by oxidative damage. We have shown that bladder carcinoma cells are sensitive to micronuclear induction by promoting-activated neutrophils and that they can be protected from this damage by insertion of a normal chromosome 11. Further work is in progress to define the source of chromosomal breakage in schistosomiasis patients and to begin to develop an understanding of the host factors protecting bladder cells in these individuals from genetic damage.

Introduction

One of the hallmark activities of tumor promoters in animals is their ability to recruit inflammatory cells to an application site and stimulate a respiratory burst in these cells. The cells release ROS such as superoxide anion and hydrogen peroxide, as well as lipid oxidation products (1, 2). This activity may be critical to tumor promotion. There is a strong concordance between the capacity of tumor promoters to stimulate inflammatory cells to release ROS and their capacity to promote tumors (3). Furthermore, blocking the respiratory burst in animal models with agents such as retinoids, steroids, or antioxidants leads to the inhibition of tumor promotion (3).

Little is known about the forces driving tumor promotion and progression in humans. However, there is some suggestion that inflammatory processes may again play a role. Clinical studies have documented an association between inflammation and cancer for decades (2, 4—7). As shown in Table 1, this association has been documented an association between inflammation and cancer for decades (2, 4—7). As shown in Table 1, this association has been observed in many different tissues. The source of the inflammation is varied: in some cases it is not known, while in others viral, bacterial, or parasitic infections are implicated. However, in each case the inflammation is longstanding (chronic) and the cancer develops at the site of the inflammation. It is not known whether transient, recurrent inflammatory reactions are also associated with increased cancer risk. Nor is it known to what extent chemical and physical agents are acting in humans to increase tumorigenesis by stimulating inflammatory reactions. Many irritants thought to increase cancer development by stimulation of proliferation may also act by aggravating inflammation in the tissue and vice versa (4, 6, 8). It is often difficult to separate the two events since they commonly occur concurrently.

Interest in the role of inflammation in carcinogenesis has been revived recently. This renewed attention is largely due to accumulating evidence that stimulated inflammatory cells are capable of inducing genotoxic effects, such as DNA strand breaks (9, 10), sister chromatid exchanges (11), and mutation (12, 13), and of promoting neoplastic transformation (14, 15) in nearby cells. Evidence suggests that these effects may be oxidant mediated. DNA base modifications in cells cocultured with stimulated inflammatory cells (i.e., activated with TPA to produce a respiratory burst) are characteristic of hydroxyl radical injury (16). Oxidized DNA bases are also induced in vivo in mice receiving a topical exposure to TPA. Wei and Frenkel (17) reported the induction of 8-hydroxy-2'-deoxyguanosine, thymidine glycol, and 5-hydroxymethyl-2'-deoxyuridine in epidermal DNA of such mice and attribute this induction to TPA-induced infiltration and activation of inflammatory cells. The path by which this damage is induced in a cell is still unresolved. One possibility is that the hydrogen peroxide produced by activated inflammatory cells is reacting with chromatin-bound metal ions and generating hydroxy radicals via a Fenton-type reaction. An alternative explanation is that superoxide anions interact with nitric oxide (another product of stimulated inflammatory cells) and form a peroxynitrite radical that decomposes to hydroxyl radical and damages the DNA (18—20).

There are two other mechanisms by which inflammatory cells may increase genotoxic damage in a tissue. Inflammatory cells have been shown to participate in the metabolic activation of procarcinogens to DNA-damaging species (7, 21, 22). Neutrophils activate aromatic amines, aflatoxins, estrogens, phenols, and polycyclic aromatic hydrocarbons by oxidant-dependent mechanisms (7). Finally, several studies implicate inflammatory cells in the formation of carcinogenic nitrosamines (23). Leaf et al. (24) reported that immunostimulated rats are capable of nitratosing morpholine, probably from reactants arising from nitric oxide production by macrophages.

The main goal of our current research is to develop a better understanding of promotion in humans by focusing on the role of inflammation and dysregulated cell proliferation in cancer development. Our initial focus is on inflammatory reactions. We have approached this goal by devising in vivo and in vitro model systems. The in vivo approach has focused on studies of early cellular changes in the bladders of Egyptian patients infected with Schistosoma haematobium. This parasite infection induces chronic inflammation and irritation in the urinary bladder and is associated with increased cancer at this site (25—28). The in vitro studies are aimed at identifying host factors that elevate sensitivity of bladder cells to oxidative stress. This paper will describe our experience to date with these models.
Schistosomiasis Model

Schistosomiasis is a common parasitic disease that ranks second only to malaria as a global cause of morbidity and mortality (29). It occurs in 74 tropical and subtropical countries and affects about 10% of the world’s population (27, 28). The prevalence of this infection remains high despite the availability of efficacious anthelminthic drugs. Individuals become infected with the parasite at an early age by exposure to the free-swimming larval stage (termed cercariae) in contaminated water. Even when treated, they will often become reinfected, since they return to the same water source. Thus, the infection is long term with the progressive development of pathological changes, often throughout a lifetime.

Three major species infect man. In each case the infection is associated with an increase in cancer. Schistosoma mansoni infections are associated with the development of follicular lymphoma of the spleen (27, 30), Schistosoma japonicum infections with colon cancer (31), and S. haematobium infections with cancer of the urinary bladder (25-28). Although the evidence supporting the first two associations is somewhat limited, the involvement of S. haematobium infection in bladder cancer is more strongly supported. Elevated numbers of bladder carcinomas occur in geographic areas that are endemic for the parasite. In these regions, the majority of bladder cancers will occur in patients with schistosomal cystitis. Finally, carcinoma of schistosome-infected bladders have clinical features that are distinctive from those observed in bladder carcinomas in populations not at risk for schistosomal infection. The cancers develop 1 to 2 decades later and are predominantly squamous rather than transitional cell carcinoma (for reviews see Refs. 26 and 32).

Various hypotheses have been proposed to explain why S. haematobium infections predispose to bladder cancer (26, 32). It has been suggested that environmental carcinogens such as cigarette smoke or pesticides (many infected individuals are farmers) could play a role in the development of cancer by interacting synergistically with the infection (26, 33). Another more favored suggestion implicates urinary nitrosamines as initiating agents. In support of this hypothesis are data showing that N-nitroso compounds are elevated in the urine of infected individuals to levels greater than those observed in uninfected individuals (34—37). These N-nitroso compounds may come from exogenous sources (e.g., diet) or, alternatively, be formed endogenously through the action of bacteria or stimulated inflammatory cells, both commonly present in the bladder of these patients (36, 37). Further support for the involvement of nitrosamines in schistosomal bladder cancers comes from a recent study by Badawi et al. (37). These workers reported the presence of O^6^-MedG in bladder tissue DNA of Egyptian subjects with bladder carcinoma and suffering from schistosomal infections. O^6^-MedG was detected more frequently in the bladder DNA of these patients than in normal bladder tissue of European origin (93% of schistosomiasis samples compared with 33% of normal bladders) and at significantly higher levels. The formation and persistence of O^6^-MedG in DNA is correlated with the carcinogenic and mutagenic action of nitrosamines.

Another factor that may play a major role in bladder carcinogenesis in schistosomiasis patients is the presence of continuous physical irritation and inflammation produced by schistosomal eggs in the bladder mucosa. The adult S. haematobium worms inhabit the veins of the perivesical plexus, where the female lays eggs. Some eggs pass through the bladder mucosa and are excreted in the urine. Other eggs are trapped in the tissue. A chronic inflammatory reaction is initiated, with the invasion of histiocytes and other inflammatory cells into the bladder, the formation of granulomas, and eventually fibrosis. In addition, the eruption of the eggs through the mucosa stimulates reparative urothelial hyperplasia and cell turnover.

Several workers have proposed multistep and multifactorial models for bladder cancer development in schistosomiasis patients (26, 32, 36). These models attribute the initiation of carcinogenesis to low doses of nitrosamines or some other environmental carcinogen, with the infection supplying the proliferative stimulus to drive the expansion of clones of initiated cells. Although animal studies with this parasite infection have been difficult to perform, data obtained in a study by Hicks et al. (38) support such a model. These workers studied the effect of S. haematobium infections and low initiating doses of the bladder carcinogen N-buty1-N-(4-hydroxybutyl)nitrosamine on the development of urothelial neoplasia in the baboon. Their data showed that, although proliferative and inflammatory changes occurred in infected animals without carcinogen exposure, the nitrosamine treatment was necessary before early neoplastic changes (carcinoma in situ) became apparent. The carcinogen treatment alone was too low to produce changes in the tissue. This experiment was terminated after 2½ years, while the animals were still relatively immature, so the long-term effects of the treatments are not known. Also left unresolved is the mechanism by which the infection was contributing to the carcinogenic process. It is not known whether the inflammation itself was important or whether the infection was working through its effect on proliferation.

Involvement of Inflammation in Genetic Damage in Schistosomiasis Patients

Schistosomiasis is endemic in Egypt, particularly in rural communities. In 1990 we began working in a small village in the Fayyum, an oasis some 65 miles southwest of Cairo. This region is extensively irrigated by canals from the Nile and represents a major farming and fishing region in Egypt. Approximately 40% of the 10,000 inhabitants of the village of Shakhshouk are infected with the parasite.

The question that we posed was whether or not schistosomiasis patients would have elevated levels of chromosome damage in their bladders, and if so, whether these levels would be associated with the presence of the infection (39). Our study design involved collecting exfoliated urothelial cells from the urine of schistosomiasis patients before and after treatment with an anthelminthic drug. MN frequencies were determined in these cells and used as an estimate of genetic damage occurring in the bladder. Micronuclei are formed by damage to chromosomes or to the spindle apparatus in the basal cells of the tissue epithelium. When these cells divide, chromosomal fragments (or entire chromosomes lacking an attachment to the spindle apparatus) lag behind and are excluded from the main nuclei in the daughter cells. These fragments form their own membranes and appear as Feulgen-positive bodies in the cytoplasm of the daughter...
cells. The daughter cells mature and are exfoliated. The exfoliated cell micronucleus test has been shown to be a sensitive technique for quantifying the level of chromosomal breakage in tissues of individuals exposed to carcinogenic agents (40, 41).

Fig. 1A is a histogram showing MN frequencies in exfoliated cells collected by centrifugation of urine samples from 37 male patients with \textit{S. haematobium} infection. For comparison, Fig. 1C shows urothelial MN frequencies of 32 villagers, matched for age, sex, and socioeconomic status but free of infection. Infection status is confirmed by analyzing urine from each individual for eggs and hematuria on at least two sampling occasions. A wide range of MN frequencies is present in the infected group (range, 0–2.67%); however, the frequencies are significantly elevated above those observed in the control group (Table 2, \( P < 0.0001 \)). Fig. 1B shows the MN frequencies of each infected patient in a second urine sample taken 2 months after treatment with praziquantel. A significant decrease in MN frequencies is observed in response to the treatment (Fig. 1B, \( P < 0.0001 \)). Posttreatment MN values did not differ significantly from those observed in the control population (Table 2, \( P = 0.35 \)).

These data show that schistosomiasis patients have elevated levels of genetic change in their bladders and suggest that this change requires the presence of the infection. When the parasite is killed, MN frequencies return to levels observed in control populations. In order to obtain more information concerning the source of the genetic damage in these patients we evaluated the same specimens for inflammatory cells. Elevated epithelial proliferation will also increase genetic damage, further studies are required to show that the relationship is causal or to determine the mechanism by which these cells would be inducing the damage. A major problem in these studies is in distinguishing between effects due to altered cell proliferation in response to injury and effects resulting from the action of inflammatory cells. Elevated epithelial proliferation will also increase genetic damage (for reviews see Refs. 4, 6, and 40).

Furthermore, the inflammatory cells will themselves play a role in mediating proliferative changes in the infected bladder via the generation of eicosanoids and other factors. We are currently extending this research to better define interactions among chromosomal breakage, proliferation, and oxidative damage by using a battery of biomarkers for these events (for reviews see Refs. 3, 7, 40, and 42).

\textbf{Is Altered Sensitivity to Oxidative Stress a Driving Force for Accumulation of Genetic Change?}

Reactive oxygen species are continuously produced during normal cell metabolism as well as generated in response to an array of xenobiotics. A complex defense system has evolved to deal with these species. This system includes low molecular weight antioxidants and detoxifying enzymes (superoxide dismutase, catalase, and peroxidases) in addition to a repair process that prevents fixation of DNA damage resulting from radical attack. When the radical load increases beyond the capacity of these defenses (such as in the bladder of schistosomiasis patients) or the system becomes defective, a situation known as \textit{"oxidative stress"} (43) or a \textit{"prooxidant state"} (44) is created and genetic damage occurs. One of the interests of this laboratory is the identification of host factors that increase sensitivity to oxidative damage and, more specifically, the products of activated inflammatory cells.

Recent studies suggest that loci on chromosome 11 may be involved in the control of oxidative DNA damage in cells. Parshad et al. (45) reported an abnormally high frequency of chromatid breaks and gaps when human tumors are X-irradiated during the G2 phase of the cell cycle. This effect occurs in tumors of different tissue origin and/or histopathology. Insertion of normal chromosome 11 into these tumor cell lines results in a reduction of radiation-induced damage to the level observed in normal cells. These workers attribute this effect to a restoration of a defective DNA repair process and suggest that this

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
Subjects & No. of patients & \% of cells with MN (mean) & Av. egg count & \% of individuals with indicated level of inflammation/infection \\
\hline
Schistosomiasis patients & & & & \\
Before treatment & 37 & 0.82$^{ef}$ & 28.5$^{f}$ & 85.3 \hline
After treatment & 37 & 0.19$^{e}$ & 0.4$^{f}$ & 29.4 \hline
Controls & 32 & 0.12$^{e}$ & 0$^{f}$ & 16.1 \hline
\end{tabular}
\caption{MN frequencies of bladder cells in schistosomiasis patients$^{a}$}
\end{table}

$^{a}$ Original study described by Anwar and Rosin (39).
$^{b}$ Eosinophils (EOS) > 3, PMNs > 5, and bacteria > 3 per high-power field (×400 magnification).
$^{ef}$ Indicates comparison significantly different at \( P < 0.0001 \).
defect is a critical early event in tumorigenesis (46). It is possible that the same mechanism is affecting the capacity of bladder cells to deal with DNA damage by ROS. Chromosome 11 alteration is a common occurrence in tumors of the urinary bladder (47).

In order to test this hypothesis we assayed two cell cultures for sensitivity to oxidative stress: A1698, a bladder carcinoma culture (termed parent), and A1698 + del(11), a cell line created from A1698 by microcell-mediated transfer of a normal chromosome 11 (termed hybrid). The cultures were exposed for 1 h to X/XO (a treatment which produces a mixture of superoxide and hydrogen peroxide) or coincubated with TPA-activated human neutrophils. Micronucleus frequencies were significantly lower in the hybrid cultures containing the chromosome insert (Table 3).

We have yet to determine the mechanism underlying this protection. However, the altered sensitivity does not appear to be due to a difference in the level of single-strand DNA breakage and its rejoining. In a recent study, we assayed parent and hybrid cells for single-strand DNA breakage after X/XO treatment using the single-cell gel electrophoresis assay (alkaline comet assay). This assay analyzes individual cells within a population and provides data concerning the heterogeneity of DNA breakage and repair. There was no significant difference between parent and hybrid cultures in either the initial amount of single-strand DNA breakage at treatment (P > 0.1) or after 20 min of repair (P > 0.1) (48).

It is not yet known whether the phenomenon described above will be restricted to the cell culture being used or be more universal in nature. However, it is possible that alterations such as this will play a major role in driving the progression of a tissue from a normal to a neoplastic state. Clones of cells with an inherited alteration in the capacity to repair oxidative DNA damage would show an elevation in genetic instability and an increased probability of acquiring the multiple alterations to specific oncogenes or suppressor genes required for tumor development (40). Schistosomiasis patients would appear to be an appropriate model system in which to test such an hypothesis.

**Conclusion**

In this paper we have summarized some of the first evidence supporting an association between chronic inflammation in humans and an elevated level of genetic damage in the infamed site. Further work is necessary to prove that this damage is actually due to the inflammation and, if so, to define the mechanism by which the inflammation is acting to produce the damage. We have stressed the possibility that ROS are involved. However, we have indicated alternatives that need to be considered. The inflammatory cells could also be increasing genetic damage through nitrosamine formation, by bioactivation of carcinogens, and/or through enhanced stimulation of cell proliferation.

In summary, we have described a human model system that is very amenable to studies of the role of inflammation in cancer. Further expansion of these studies should involve the use of the biomarkers currently available for oxidative damage, genetic change, and cell proliferation to test these hypotheses.

**Acknowledgments**

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**References**


Table 3 MN induction in response to oxidative stress in bladder carcinoma cell lines with and without a chromosome 11 insert.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>% of MN in untreated cultures</th>
<th>MN induction in response to TPA-activated PMNs*</th>
<th>X/XO (1)</th>
<th>X/XO (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1698</td>
<td>3.3 ± 0.4</td>
<td>5.5 ± 0.7</td>
<td>8.0 ± 1.1</td>
<td>9.6 ± 0.1</td>
</tr>
<tr>
<td>A1698 + del(11)</td>
<td>4.0 ± 0.3</td>
<td>0.5 ± 0.6</td>
<td>0.4 ± 0.4</td>
<td>2.6 ± 0.7</td>
</tr>
</tbody>
</table>

* Original study described by Ward et al. (48). Cultures were kindly provided by Dr. O. Pereira-Smith (Baylor College of Medicine, Houston, TX).

**MN frequencies in untreated cultures have been subtracted to provide induced values:**

- For treated cells: MN frequencies in treated cultures - MN frequencies in untreated cultures.
- For untreated controls: MN frequencies in untreated controls - MN frequencies in untreated cultures.

Data represent results of 3 replicate experiments for each treatment.


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