Smoking-induced immune deviation contributes to progression of bladder and other cancers

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We propose here that cigarette smoke (CS), in addition to its established genotoxic effects, elicits chronic albeit sub-clinical immune suppression, which is a major contributor to cancer progression. This hypothesis, presented here primarily in the context of bladder cancers (BCs), is applicable to other cancers, including those without a confirmed link to smoking.

Introduction

Smoking is strongly associated with BC, also referred to as transitional cell carcinoma or urothelial carcinoma. Indeed, cigarette smoking is the single biggest risk factor for BC with an estimated 40–60% causally related. Using data from >450,000 participants in the NIH-AARP Diet & Health Study (1995–2007), Freedman et al. concluded, “former smokers were twice as likely to develop BC as those who never smoked, and current smokers were four times more likely...smoking cessation was associated with reduced BC risk.” The current hypothesis for this epidemiological association is that arylamines from CS enter the bloodstream, and thence proceed to the kidneys where they are concentrated in the urine which is subsequently seques-tered in the bladder until micturition. This prolonged exposure of the urothelium to the arylamines causes mutagenesis (formation of DNA adducts) leading to transformation. This explanation, which posits transformation by a direct effect of the components of smoke on the target cells, is supported by ample evidence. It explains the primary event in the genesis of BC, i.e., the malignant transformation of the urothelium. In terms of the direct genotoxic effect of carcinogens in CS on the target tissue epithelium, it is also consistent with the mechanism of carcinogenesis in lung cancer (see): lungs of smokers harbor thousands of mutations, as described recently in several studies utilizing high throughput DNA sequencing (and subsequent studies).

Interestingly, exposure of mice to CS reveals a complex pattern of tumorigene-sis, perhaps partly because mice and rats do not inhale CS, but actually avoid it. Broadly speaking, “it is difficult to reproduce the carcinogenicity of CS in animal models.” There are significant differences between the effects of sidestream CS (SCS) and mainstream CS (MCS), and between mice exposed to CS neonatally vs. adult mice. Specifically, with respect to BC, the results of testing of tumorigenic-ity of CS as a complex mixture in rodents are inconsistent. Two recent studies, among many others, make that point clearly. Ohnishi et al. who exposed C57BL/6 mice to MCS and SCS for several months, failed to observe neoplastic or even pre-neoplastic bladder lesions, even as they did observe a transient increase in urothelial proliferation by Ki67 labeling index at 3 mo but not at any later time point. The proliferation was attributed to regenerative proliferation secondary to urothelial cytotoxicity. Kato et al. used an initiation-promotion model instead. “After initiation of carcinogenesis with N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) followed by 22 weeks of exposure to CS, there were no significant increases in bladder tumors compared with the BBN-initiated clean
air controls. There was, however, a non-significant increase in bladder tumors in the CS-exposed group, suggesting that longer exposure to CS may enhance bladder carcinogenesis in this model. These inconsistent results in CS-elicited bladder carcinogenesis in mice are in sharp contrast to consistent bladder carcinogenesis in transgenic mouse models where SV40 T antigen expression is driven by the urothelium-specific uroplakin II promoter.

**Hypothesis**

Difficulties in reproducing the effects of CS in animal models, and the many reasons for inconsistencies, have been discussed elsewhere, and in the primary papers documenting the inconsistent results. Here, we contribute to that discussion by suggesting that in addition to exerting its undisputed genotoxic effects, smoking acts by mediating immune suppression, which promotes progression of the transformed urothelium into a full-grown cancer. Some previous investigators have occasionally referred to immune suppression as a consequence of exposure to CS (discussed later); however, the explicit suggestion that smoking-mediated immune suppression is an essential contributor to progression of BC, is novel. In this reasoning, small animal models such as mice and rats are inherently limited by their life spans to provide the long-term chronic immune-suppressed milieu in which transformed cells grow into malignant tumors and progress to full-blown BCs (Figure 1). Our hypothesis thus augments the mechanisms through which smoking may cause and exacerbate BC.

The role of the immune system in counteracting tumorigenesis/tumor progression is well established. As an example, patients whose immune systems are suppressed because they have received a transplant of some organ (and take immunosuppressant medicines to avoid rejection of the transplant) have a 5 to 6 fold higher incidence of BC (and of other malignancies) than individuals with normal immune systems. Studies from experimental work with mice have largely substantiated the role of immune surveillance, although evidence to the contrary also exists.

There is a considerable albeit, fragmented literature documenting the effects of CS on some components of the immune system. A considerable amount of work has focused on CS-associated inflammation. A smaller number of studies have described how CS inhibits innate immunity. Concisely, Mehta et al. review the evidence that CS impairs phagocytic functions of macrophages, alters the ratio of helper to suppressor cells, lowers the natural killer (NK) activity, and reduces the production of interferon γ by lymphocytes. In a study focused on human monocyte-derived dendritic cells (DCs), such DCs differentiated in the presence of nicotine showed suppressed secretion of IL-12 and TNFα by lipopolysaccharide (LPS)-stimulation as compared to that seen in DCs grown without nicotine. DCs cultured in presence of nicotine also “displayed a diminished capacity to induce allogeneic T cell proliferation with a reduced production of IFNγ, and maintained/enhanced LPS-mediated expression of co-inhibitory molecules.” In a study on the effects of CS extracts on T cell function in vitro, Hernandez et al. observed, “activated T cells cultured in the presence of CS extract displayed a dose-dependent decrease in cell proliferation, which associated with the induction of cellular apoptosis. T cell apoptosis by CS extract was independent of caspases and mediated through reactive oxygen and nitrogen species endogenously contained within CS extract.”

Only rarely have studies shown a functional and mechanistic connection between CS and immunological impairment and a disease outcome. In one such notable study, Feng et al. exposed mice to CS and infected them with *Mycobacterium tuberculosis* or influenza A. They observed that CS “inhibited the lung T-cell production of IFNγ, compared with controls, during stimulation in vitro with anti-CD3, after vaccination with a

![Figure 1. Current (in black, upper arm) and proposed (in red, lower arm) hypotheses for explaining the mechanisms by which smoking mediates bladder carcinogenesis.](image-url)
construct expressing an immunogenic mycobacterial protein, and during infection with *M. tuberculosis* and influenza A virus *in vivo*. Reduced IFNγ production was mediated through the decreased phosphorylation of transcription factors that positively regulate IFNγ expression. CS exposure increased the bacterial burden in mice infected with *M. tuberculosis* and increased weight loss and mortality in mice infected with influenza virus.” This recent study was the first demonstration of CS-mediated inhibition of T cell function *in vivo* leading to increased morbidity and mortality in excellent mouse models of animal disease.

The aforementioned study by Feng et al. provides a particularly relevant vantage point to frame the discussion of our hypothesis, since it demonstrates immune-suppressive effects of CS on disease progression in a disease where T cells play an important role. In contrast to the acute infection with a bacterium or a virus where disease progression is swift, genesis and progression of BC (as in other cancers) is a long-term process that involves one or more primary events followed by considerable evolution of disease. It is our premise that the immune system applies brakes to this long-term process of cancer evolution at multiple points, such that in the short life span of the mouse, the immune system prevails, and no consistent CS-induced bladder carcinogenesis is seen, even though CS-induced primary genotoxic events do occur. In contrast, in the human situation, the relatively long human life span allows the evolutionary mechanisms of oncogenesis to play out the battle with the immune system, and eventually prevail.

A small number of small epidemiological studies also point to the association of CS and immune suppression. In a cross-sectional study of 75 healthy women, who participated in the Data Bank and Bio Repository program at Roswell Park Cancer Institute, higher peripheral blood levels of CD4(+) CD25(+) FOXP3(+) regulatory T (T-reg) cells were significantly associated with smoking, among other parameters.15 Such T-reg cells play a major role in down-regulating immune response; hence these observations are consistent with the thesis that smoking suppresses immune response. In a study of 27 patients under the age of 40 treated for invasive vulvar cancer at the Women’s Cancer Center, University of Minnesota, smoking and a history of an immunosuppressive medical illness were the most common parameters in this patient population.18 In a study of 441 patients consecutively diagnosed with Herpes Zoster, smoking was observed to be an independent predictor of post-herpetic neuralgia.19

The evidence linking the numbers and types of tumor infiltrating lymphocytes (TILs) to clinical outcomes is the clearest in the case of colon25 and ovarian cancers26 and melanomas.27 However, evidence is now emerging for BC. In a multivariate analysis of 514 BC patients and a follow-up of over >9 years,28 TILs were a highly significant indicator of a favorable prognosis (p = 0.007). More recently, Sharma et al.29 analyzed the presence of intratumoral CD8⁺ T cells... in BC samples. Immunohistochemical staining for intratumoral CD8⁺ T cells in tissue samples from 69 patients with BC showed that patients with advanced urothelial carcinoma (UC) (pT2, pT3, or pT4) and higher numbers of CD8⁺ TILs within the tumor (8 or more) had better disease-free survival (p < 0.001) and overall survival (p = 0.018) than did patients with similar-staged UC and fewer intratumoral CD8⁺ TILs.” (39).

Altogether, our hypothesis is based on the compelling evidence of the role of the immune response in modulating the course of disease in BC, and the considerable anecdotal, yet largely non-mechanistic studies of the effects of CS exposure on immune responses. It may be logically argued that if smoking leads to systemic immune suppression (and since immune response seems to play a vital role in controlling cancers in general), smoking should be associated with almost all cancers. However, smoking seems to be a risk factor specifically for cancers of the lung, head and neck, urinary tract (including bladder), and pancreas. In this regard, it is our thinking that smoking acts at two distinct levels (see Figure 1), one at the level of direct effects of the components of smoke in causing malignant transformation of the exposed cells, (i.e., at the initiation of malignancy), and two, at the level of immune suppression leading to progression of malignancy. The effects of smoking at these two levels are synergistic, which is why smoking is an obvious risk factor only for those cancers where components of the smoke directly interact with the target cells, i.e., those of the lung, head and neck, urinary tract (including bladder), and pancreas. We suggest that smoking shall turn out be a weaker and less obvious risk factor for other cancers as well. Indeed, the Surgeon General’s report30 on the Health Consequences of Smoking, opines that “evidence is sufficient to infer a causal relationship” between smoking and hepatocellular carcinoma, as well as colorectal adenomas and colorectal cancer. It also says “evidence is suggestive but not sufficient to infer a causal relationship” between tobacco smoke and breast cancer.

**Testing the Hypothesis**

We suggest three methods to interrogate the hypothesis. A longitudinal study of detailed immune status of early smokers
stratified by intensity of CS exposure, including flow cytometric analyses of well-known biomarkers, as well as assays of T cell function (allo-reactivity or T cell response to influenza as examples), can provide a strong test of the idea. Secondly, conducting a detailed retrospective analysis of a sufficiently large cohort of BC patients with respect to smoking status, TIL content, and overall survival shall be instructive. Correlation between TIL content and survival has already been sought and obtained to a degree; analysis of similar data with the superimposition of instructive. Correlation between TIL content, and overall survival shall be.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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