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The TNF-α 238A polymorphism is associated with susceptibility to persistent bone marrow dysplasia following chronic exposure to benzene

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Abstract

Chronic exposure to benzene can result in transient hematotoxicity (benzene poisoning, BP) or persistent bone marrow pathology including dysplasia and/or acute myeloid leukemia. We recently described a persistent bone marrow dysplasia with unique dysplastic and inflammatory features developing in individuals previously exposed to benzene (BID) [Irons RD, Lv L, Gross SA, Ye X, Bao L, Wang XQ, et al. Chronic exposure to benzene results in a unique form of dysplasia. Leuk Res 2005;29:1371–80]. In this study we investigated the association of single nucleotide polymorphisms (SNP) (−863 (C → A), −857 (C → T), −308 (G → A), −238 (G → A)) in the promoter region of the cytokine, tumor necrosis factor-alpha (TNF-α) on the development of BP, persistent BID and *de novo* myelodysplastic syndrome (MDS) in 394 individuals. Only the −238 (G → A) polymorphism was significantly associated with the development of BID (odds ratio (OR) = 7.4; 95% C.I. 1.23–44.7) and was specific for BID and not *de novo* MDS or BP. These findings are consistent with a role for inflammation in the development of BID and suggest that cell-specific alterations in TNF-α expression may promote clonal selection in the evolution of neoplastic hematopoietic disease.

Keywords: Benzene poisoning; TNF-α promoter gene polymorphisms; Leukemogenesis; Clonal selection; Genetic variation; Inflammation; Epidemiology; Toxins/drugs/xenobiotics

1. Introduction

Benzene is a widely known hematotoxic agent. However, the severity of hematotoxicity associated with chronic exposure to benzene varies widely. Individuals undergoing comparable exposure may demonstrate no significant hematologic abnormalities while others exhibit signs of benzene poisoning (BP) or even bone marrow failure. Some individ-

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uals with BP will undergo complete recovery after cessation of benzene exposure while others will go on to develop bone marrow dysplasia that persists for years after exposure has ended and/or develop acute myeloid leukemia (AML). We recently described a unique form of persistent bone marrow dysplasia developing in patients previously exposed to benzene (BID), which is characterized by severe inflammatory changes, including eosinophilic progenitor dysplasia and hematophagocytosis as well as dyserythropoiesis, dysgranulopoiesis and stromal degeneration [1]. In some individuals the dysplastic changes observed in BID are consistent with a defined subtype of myelodysplastic syndrome (MDS), predominantly refractory cytopenia with multiliineage dysplasia (RCMD). The pathology in BID also is frequently accompanied by lymphocyte activation, and prominent clonal proliferation of regulatory T lymphocyte subsets in the bone marrow of affected individuals. The underlying mechanisms that explain these inflammatory changes in the bone marrow microenvironment remain unknown. However, these findings appear to predate the development of clonal structural cytogenetic abnormalities frequently observed in MDS, and present as a unique diagnostic feature that distinguishes BID from the majority of cases of *de novo* MDS [1].

The cytokine, tumor necrosis factor-alpha (TNF-α), plays a pivotal role in inflammation, immunity, hematopoiesis and apoptosis [2,3]. Altered production of TNF-α has been implicated in the development and severity of several diseases including rheumatoid arthritis, psoriasis, congenital and acquired aplastic anemia [4,5], MDS [2,3], viral and bacterial infections and cancer [6]. Also, TNF-α recently has been implicated as playing an important role in mediating the regulatory activity and survival of CD34+ hematopoietic progenitor cells in HLA-mismatched bone marrow transplantation [7,8]. TNF-α production in human cells is influenced by genetic polymorphisms, several of which have been implicated in the development of disease [9–11]. However, neither the mechanisms whereby these polymorphisms influence TNF-α gene expression and transcription, nor their role in the pathogenesis of disease or hematopoietic stem cell biology is completely understood. The TNF-α gene is located within the highly polymorphic major histocompatibility complex (MHC) class III region on chromosome 6p21.3. It contains at least nine single nucleotide polymorphisms (SNP), including four located within the promoter region that have been extensively studied (occurring at positions relative to the transcription start site) −308 (G → A), −238 (G → A), −863 (C → A), −857 (C → T) [10]. The −308A haplotype is associated with increased production of TNF-α, is linked to erosive joint disease in rheumatoid arthritis and an increase in the severity of certain infections, such as malaria and leishmaniasis [11]. The −238A haplotype is not linked with increased TNF-α production but is associated with derepression of TNF-α expression in vitro in some, but not all cell lines studied [12]. The −238A haplotype also has been implicated in the severity of infections (*e.g.* hepatitis B, tuberculosis, malarial anemia) [13] and psoriasis [14]. The −863A allele is variously associated with increased TNF-α production as well as decreased serum TNF-α concentrations in healthy males [15] and also has been implicated in erosive joint disease in rheumatoid arthritis [16]. The −857T allele alternatively confers an increased risk of gastric ulcer and a decreased risk of lymphoma associated with *Helicobacter pylori* infection [17,18].

The association between inflammation and cancer development is well-established, and evidence has emerged to suggest that bone marrow failure in MDS may involve activation of immune cells that target antigens in the hematopoietic microenvironment accompanied by an increase in pro-inflammatory cytokines, including TNF-α [19–22]. Recent studies in our laboratory also demonstrated that the benzene metabolite, hydroquinone, sensitizes human bone marrow progenitor cells to TNF-α-induced apoptosis via a mechanism that involves inhibition of nuclear factor-κB (NF-κB) [23]. These observations, taken together with the prominent inflammatory changes described in BID, led us to investigate the potential role of TNF-α in the pathogenesis of benzene-induced hematotoxicity. We investigated the frequency of TNF-α genetic polymorphisms in individuals with current and previous chronic exposure to benzene and correlated them with evidence of hematotoxicity occurring during benzene exposure (BP) as well as the development of dysplasia in individuals previously but not currently exposed to benzene (BID).

2. Materials and methods

2.1. Subjects

Three hundred and ninety-four cases and controls, ≥18 years of age, were enrolled from two sources: (1) in-patients recruited from Shanghai hospitals, and (2) benzene-exposed workers recruited from factories involving the rubber, petrochemical, asbestos manufacturing and painting industries. Informed consent was obtained according to the Declaration of Helsinki, 2004 and the NIH Common Rule (45CFR46), and together with the protocol, were approved by the Combined Multiple Institutional Review Board of the University of Colorado at Denver and Health Sciences Center and the Internal Review Board at Fudan University in Shanghai, China.

Three different case groups were defined: (a) patients diagnosed with MDS ($N=95$), (b) workers diagnosed with bone marrow dysplasia persisting after previous chronic exposure to benzene (BID) ($N=23$) [1], and (c) workers diagnosed with benzene poisoning (BP) ($N=46$). Control groups included: (a) healthy workplace-based subjects ($N=141$) recruited from the same factories as BP and BID cases, and (b) hospital-based patients diagnosed with non-hematopoietic diseases ($N=89$). Information on age, gender, smoking and alcohol use was obtained through personal interviews when feasible, and subsequently accounted for