Neutrophil Antigen (FcγRIIIB) SH Gene Frequencies in Six Racial Groups

To the Editor:

Human leukocyte surface receptors specific for the Fc portion of IgG play a critical role in immune function by coupling the humoral and cellular immune systems. The Fcγ receptor III (FcRIII) (CD 16), one of three Fc receptor classes, is encoded by two highly homologous genes on chromosome 1. FcRIIIA encodes the transmembrane receptor expressed on macrophages and natural killer cells and is not known to be polymorphic. FcRIIIB encodes the glycosylphosphatidylinositol (GPI)-linked receptor expressed on neutrophils and has three isoforms: NA1, NA2, and the recently described SH.2 SH-positive individuals possess an additional copy of the FcRIIIB gene, which may occur in tandem with NA2-FcRIIIB.3 The nucleotide sequence of the NA1 and NA2 genes both predict a protein containing 233 amino acid residues; however, the two alleles differ by five base substitutions (nts 141, 147, 227, 277, and 349) within exon 3, which predict amino acid changes at positions 36, 65, 82, and 106 of the translated peptide. SH differs from NA2 by only one base substitution (nt 266, C→A); this substitution predicts an amino acid change (Ala→Asp) at position 60.2

The NA antigens, which are difficult to identify by serologic tests (ie, granulocyte immunofluorescence or agglutination), are clinically important because they are the most frequent targets of neutrophil antibodies in neonatal alloimmune neutropenia (NAN), transfusion-related acute lung injury, and chronic benign autoimmune neutropenia of infancy. An SH-positive frequency of 5% has been reported for Caucasians; however, its prevalence in other racial groups is unknown.2,3 Because alloimmunization to SH has been reported to cause NAN, its distribution in other racial groups is important.2,5 Therefore, individuals (n = 799) of African American, Asian Indian, Caucasian, Hispanic, Korean, and Native American descent were SH genotyped by allele-specific polymerase chain reaction (AS-PCR; Fig 1) essentially as described by Bux et al, and gene frequencies were established (Table 1).2

The highest SH gene frequency (22.5%) was observed in African Americans, whereas SH was not observed in 101 Koreans. NA1 and NA2 gene frequencies for African Americans, Asian Indians, Caucasians, Hispanics, Native Americans, and Asian populations have been reported by our laboratory and others.6,7 In general, NA2 is more common in African, Asian Indian, and Caucasian populations (gene frequencies of 70% to 63%, respectively) and becomes less common as one examines populations endogenous to the Americas and Asia (gene frequencies of 55% to 30%, respectively). In this study, the overall distribution of SH paralleled the NA2 frequency, being more common in Western populations than in Eastern populations. All SH-positive individuals were also NA1/NA2 genotyped; no SH-positive, NA1 homozygotes were observed. This is consistent with the proposed tandem genomic localization of NA2 and SH.3 The high SH frequency in African Americans suggests that this group may be at a higher risk for alloimmunization to this antigen.

Table 1. SH Genotyping of Six Racial Groups

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>SH-positive</th>
<th>SH-Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African American</td>
<td>204</td>
<td>46</td>
<td>22.5</td>
</tr>
<tr>
<td>Asian Indian</td>
<td>88</td>
<td>14</td>
<td>15.9</td>
</tr>
<tr>
<td>Caucasian</td>
<td>222</td>
<td>10</td>
<td>4.5</td>
</tr>
<tr>
<td>Hispanic</td>
<td>90</td>
<td>7</td>
<td>7.8</td>
</tr>
<tr>
<td>Korean</td>
<td>101</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Native American</td>
<td>94</td>
<td>1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Fig 1. SH typing by ASPCR. Twenty-five μL of each reaction was electrophoretically analyzed on a 2% ethidium bromide stained agarose gel. Lane 1, size standard; lane 2, negative control; lane 3, SH-positive sample; and lane 4, SH-negative sample.

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Mitomycin C–Induced DNA Damage in Fanconi Anemia: Cross-Linking or Redox-Mediated Effects?

To the Editor:

The article by Carreau et al1 reports on the in vivo effects of mitomycin C (MMC) in mice carrying the Fanconi anemia (FA) group C mutation (Fac−/−). Among the mechanistic scenarios underlying FA pathogenesis, the authors refer to a phenotypic feature of FA cells related to oxygen hypersensitivity. Unfortunately, the use of citations on this subject appears to be quite inappropriate. First, the authors attributed a “secondary” role for oxygen sensitivity in FA cells2 which, however, may have been made oxygen-resistant after the immortalization procedure. In fact, the loss of O2 sensitivity in transformed cells has been recognized as a general phenomenon, not confined to FA cell lines.3 A general statement was then made4 about the published results of studies which “have demonstrated overproduction of reactive oxygen species (ROS) and increased susceptibility to oxygen, as well as an increase in ROS-induced DNA lesions, particularly 8-hydroxy-2′-deoxyguanosine (8OHdG).” Unfortunately, the three references reported4–6 (cited as 37–39 in the report) neither dealt with FA nor with MMC-induced DNA damage. The above statement about excess ROS production and 8OHdG formation in FA was true, but rather should refer to the reports by Takeuchi and Morimoto7 and Degan et al8 It is worthwhile to consider the subject of oxidative stress in FA based on both in vitro and ex vivo evidence, as reviewed by us recently.9

A role for oxidative stress in FA has been documented for two decades, with reports providing evidence for an improvement of either chromosomal instability or cell growth after exposure of either primary lymphocyte cultures or fibroblasts from FA patients to: (1) catalase or superoxide dismutase, (2) low-molecular-weight antioxidants, or (3) lymphocyte cultures or fibroblasts from FA patients to: (1) catalase or superoxide dismutase, (2) low-molecular-weight antioxidants, or (3) culturing cells in 5% O2,15 and a major role was suggested for free iron in inducing G2 arrest in FA cells.16 The report by Takeuchi and Morimoto7 provided evidence for excess oxidative DNA damage (8OHdG) in FAA cells challenged with H2O2 that was related, at least in part, to catalase deficiency. A recent report by Ruppitsch et al17 provided elegant evidence for the loss of both MMC and diepoxybutane (DEB) sensitivity of FAA cells transfected with CDNA causing overexpression of thioroxygenase, a nonenzymatic antioxidant protein.18 Hence, both exogenous and endogenous antioxidants can decrease the phenotypic defect of FA cells, both including O2 and MMC sensitivity. In turn, the action mechanisms of MMC can either be ascribed to DNA cross-linking or to redox cycling, as reported in early studies of MMC.19,20 That MMC sensitivity in FA cells may be attributed to redox mechanisms rather than to DNA cross-linking has been shown by four independent reports.11,12,21,22 focused on as many different endpoints (chromosomal instability, cytotoxicity, apoptosis, and mutagenesis). Together, the results of these studies showed that: (1) MMC-induced toxicity was confined to normoxic conditions which, unlike hypoxia, were associated to enhanced redox-cycling mechanisms, not to DNA cross-linking,21,22 and (2) MMC toxicity was both removed by antioxidants and by low-molecular-weight antioxidants.11,13

The observation of redox abnormalities in FA is not confined to in vitro conditions. A series of ex vivo studies provided evidence for abnormal O2 metabolism in FA patients and in their parents. Freshly drawn white blood cells from both FA homozygotes and heterozygotes produced excess ROS as detected by luminol-dependent chemiluminescence (LDCL),23,24 and displayed excess 8OHdG levels that were significantly correlated with LDCL as well as with chromosomal instability.4 Thus, both ex vivo and in vitro evidence pointed to a direct link between ROS formation, oxidative DNA damage, and chromosomal breakages in FA.

Based on the available evidence, one might suggest that the authors could carry out a new series of experiments by exposing Fac−/− mice to different oxygen levels, with or without MMC administration. As additional endpoints worth being evaluated in Fac−/− mice, one might suggest to include the evaluation of oxidative DNA damage as well as ROS-detoxifying activities. This study could provide a formidable insight both into the FAC defect and the in vivo action mechanisms of MMC.

In conclusion, the current view attributing the FA-associated defect(s) to the phenotypic sensitivity to MMC and DEB related to cross-linking mechanisms may be viewed as a fading dogma relying on the definition of FA as a DNA repair disorder. While no conclusive evidence has thus far related FA gene products to any function in DNA repair, a thriving body of evidence has associated MMC (and DEB) sensitivity to an impairment of redox balance in FA cells, both in vitro and in vivo. This evidence should no longer be disregarded in the forthcoming studies of FA.

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