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Normal Somatic Hypermutation of Ig Genes in the Absence of 8-Hydroxyguanine-DNA Glycosylase

David B. Winter,1* Quy H. Phung,* Xianmin Zeng,* Erling Seeberg,† Deborah E. Barnes,‡ Tomas Lindahl,§ and Patricia J. Gearhart2*

The hypermutation cascade in Ig V genes can be initiated by deamination of cytosine to uracil by activation-induced cytosine deaminase and its removal by uracil-DNA glycosylase. To determine whether damage to guanine also contributes to hypermutation, we examined the glycosylase that removes oxidized guanine from DNA, 8-hydroxyguanine-DNA glycosylase (OGG1). OGG1 has been reported to be overexpressed in human B cells from germinal centers, where mutation occurs, and could be involved in initiating Ab diversity by removing modified guanines. In this study, mice deficient in Ogg1 were immunized, and V genes from the H and κ L chain loci were sequenced. Both the frequency of mutation and the spectra of nucleotide substitutions were similar in ogg1−/− and Ogg1+/+ clones. More importantly, there was no significant increase in G:C to T:A transversions in the ogg1−/− clones, which would be expected if 8-hydroxyguanine remained in the DNA. Furthermore, Ogg1 was not up-regulated in murine B cells from germinal centers. These findings show that hypermutation is unaffected in the absence of Ogg1 activity and indicate that 8-hydroxyguanine lesions most likely do not cause V gene mutations. The Journal of Immunology, 2003, 170: 5558–5562.

During hypermutation of Ig genes, nucleotide substitutions are introduced at a high frequency into a 2-kb region surrounding rearranged H and L chain V genes. Although it is not understood what targets the hypermutation mechanism to this region, the process is believed to start when DNA bases are modified and undergo error-prone repair. Several types of DNA lesions could initiate this process. First, single- and double-strand DNA breaks have been detected by ligation-mediated PCR techniques in the vicinity of V genes (1–3). It had been proposed that the activation-induced cytosine deaminase (AID) protein might deaminate cytosine(s) in an mRNA encoding an endonuclease, thereby enabling an active form of the enzyme to be produced (4, 5). This interpretation has been questioned by recent findings that AID-deficient B cells do not have reduced levels of DNA double-strand breaks (6–8).

Second, cytosine in DNA may be deamincated to uracil. This is then removed by uracil-DNA glycosylase to produce an abasic site that would be cleaved by an abasic endonuclease. The resulting single-nucleotide gap could be filled in by a low-fidelity DNA polymerase during repair, or error-prone synthesis could occur opposite the abasic site during replication. Recently, it has been reported that AID mutates DNA when it is expressed in bacteria (9).

Furthermore, uracil appears to be an initiating lesion for hypermutation in chicken cells (10) and mice (11), because inhibition of uracil-DNA glycosylase changes the pattern of mutation. The data indicate that uracil-DNA glycosylase is a key enzyme for producing mutations at G:C base pairs via generation of an abasic site. However, the molecular mechanism(s) for generating mutations at A:T base pairs remains unclear.

Third, guanine in DNA undergoes frequent oxidation to 8-hydroxyguanine (8-oxoG). This base lesion is removed by 8-oxoG-DNA glycosylase (OGG1) (12–18), and the resulting abasic site is usually repaired in an error-free manner by DNA polymerase β and DNA ligase III. However, 8-oxoG residues could be involved in generating Ab diversity if guanines were oxidized when they were opposite the uracils that had been generated by AID. Because it may be difficult to determine the number of lesions in the V gene loci of hypermutating B cells, which are a minor population in lymphoid tissues, the level of OGG1 could be assessed in these cells. Intriguingly, human OGG1 is overexpressed in B cells from germinal centers in tonsils (19), suggesting that 8-oxoG occurs frequently in these rapidly dividing cells. As shown in the scheme presented in Fig. 1A, OGG1 could conceivably be involved in hypermutation by removing modified guanines and leaving an abasic site. A recent model (20), in which a DNA glycosylase might act on unmodified guanine opposite uracil, would have a similar effect, but such an activity is only hypothetical. Mutations could, in either case, then be introduced during base excision repair by a low-fidelity DNA polymerase that fills in the one nucleotide gap to produce mutations opposite uracil, or by filling in a longer gap that is generated by exonuclease activity or strand displacement to misinsert mutations opposite other neighboring bases. Mutations could also be introduced during replication by an inaccurate DNA polymerase that can bypass the abasic site with any nucleotide. In this model, the absence of OGG1 might be expected to cause a decrease in the frequency of mutation. Furthermore, as shown in Fig. 1B, 8-oxoG could persist in DNA and base pair with A during chromosomal replication by a high-fidelity DNA polymerase (21). Thus, if 8-oxo-G lesions were being used for hypermutation, there
would be an increase in G:C to T:A transversion mutations in Ogg1-deficient mice.

To determine whether Ogg1 is involved in hypermutation, we first measured its expression in B cells from murine germinal centers. Mutations were then identified in V region clones from H and \( \kappa \) L chain loci in mice that were deficient in the glycosylase (22), and were compared with the frequency and spectra of mutations from wild-type clones.

### Materials and Methods

### Ogg1 expression

Expression of murine Ogg1 was analyzed in two independent experiments using BALB/cJ (Ogg1+/+) mice. Splenic lymphocytes were prepared 11 days after i.p. immunization with phenyloxazolone coupled to chicken serum albumin (23). The cells were stained with PE-labeled Ab to B220 (BD PharMingen, San Diego, CA) and fluorescein-labeled peanut agglutinin (PNA; E-Y Laboratories, San Mateo, CA). The cells were stained with PE-labeled Ab to B220 (BD PharMingen, San Diego, CA) and fluorescein-labeled peanut agglutinin (PNA; E-Y Laboratories, San Mateo, CA). The cells were stained with PE-labeled Ab to B220 (BD PharMingen, San Diego, CA) and fluorescein-labeled peanut agglutinin (PNA; E-Y Laboratories, San Mateo, CA).

#### Results

Ogg1 is not up-regulated in murine germinal center B cells

It has been previously reported that OGG1 is expressed at high levels in germinal center B cells from human tonsil (19). To examine its expression in germinal center B cells from mice, spleen cells from immunized *Ogg1*+/+ mice were isolated, and *Ogg1* expression was measured by RT-PCR in PNA+ cells that were undergoing mutation as well as in PNA− cells that were not undergoing mutation. The ratio of RT-PCR products corresponding to the Ogg1 transcript vs 18S rRNA was compared for each cell type (Fig. 2). The average values of two experiments were 1.03 for PNA+ cells and 1.09 for PNA− cells, indicating that Ogg1 is...
equally expressed in both cell types. The level of β-actin compared with 18S rRNA was included as a control, and was 1.00 and 1.02, respectively, for the samples (data not shown).

Frequency of hypermutation is similar in V genes from ogg1<sup>−/−</sup> and ogg1<sup>+/+</sup> mice

Hypermutation was measured in V genes at both the κ and H chain loci. All mutations in clones with different VDJ joins were scored, and only unique mutations in clones with the same joins were counted. For V<sub>j</sub> genes, mutation was measured in the rearranged V<sub>j</sub>Ox1 gene in splenic B cells from immunized mice. For ogg1<sup>−/−</sup> mice, 60% of the clones had mutations (24 of 40), and for ogg1<sup>+/+</sup> mice, 61% of the clones had mutations (17 of 28) (Fig. 3A). The overall frequency was similar between the two groups: 0.5% mutations per bp for ogg1<sup>−/−</sup> clones and 0.8% for ogg1<sup>+/+</sup> clones (p = 0.10). The mutations were similarly distributed in the V<sub>j</sub>exon for both genotypes (not shown). For example, both groups of clones had an accumulation of codon changes in complementarity-determining region 1, which are associated with increased affinity for the immunizing Ag, phenylazoxalone (30).

For V<sub>H</sub> genes, mutation was measured in the 3′ intron downstream of J<sub>H</sub>4 gene segments that are rearranged to J558 V<sub>H</sub> gene segments in Peyer’s patch B cells from immunized mice. For ogg1<sup>−/−</sup> mice, 64% of the clones had mutations (49 of 76), and for ogg1<sup>+/+</sup> mice, 51% of the clones had mutations (45 of 88) (Fig. 3B). The overall frequency was the same between the two groups: 1.0% mutations per bp for ogg1<sup>−/−</sup> clones and 0.9% mutations per bp for ogg1<sup>+/+</sup> clones (p = 0.48). Some 98% of the intron mutations in both groups were base substitutions. In summary, Ogg1 deficiency did not substantially decrease the frequency of hypermutation and did not affect the production of high affinity Abs.

![FIGURE 3. Mutation frequencies in V<sub>j</sub> genes and J<sub>H</sub> introns from Ogg1-deficient and proficient mice. The total number of clones analyzed is shown in the center of each circle. The pie segments represent the proportion of clones that contained the specified number of mutations indicated. A, Mutations in V<sub>j</sub>Ox1 genes from spleen cells of immunized mice. B, Mutations in the intron downstream of rearranged J<sub>H</sub>4 genes from Peyer’s patch cells.](http://www.jimmunol.org/)

**Table I. Base changes in Ogg1-deficient clones**

<table>
<thead>
<tr>
<th>Substitution&lt;sup&gt;a&lt;/sup&gt;</th>
<th>V&lt;sub&gt;j&lt;/sub&gt;Ox1 mut (%)</th>
<th>J&lt;sub&gt;H&lt;/sub&gt;4 intron mut (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A to: G</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>T</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>T to: C</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>A</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G to: A</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>T</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>C to: T</td>
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<td>10</td>
</tr>
<tr>
<td>A</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>G</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data show the percentage of each type of mutation in V<sub>j</sub>Ox1 coding and rearranged J<sub>H</sub>4 intron sequences. Values were corrected to represent a sequence with equal amounts of the four nucleotides. All mutations are shown from the coding strand.

ogg1<sup>−/−</sup> clones have similar types of substitutions as Ogg1<sup>+/+</sup> clones

The spectra of base changes between the two groups of clones were compared, and the data are summarized in Table I. In V<sub>j</sub>Ox1 genes, the high incidence of A to T and C to A mutations in both groups is due to selection for codons in complementarity-determining region 1, which are associated with increased affinity for the immunizing Ag, phenylazoxalone (30). For V<sub>H</sub> genes, the high incidence of A to T and C to A mutations in both groups is due to selection for codons in complementarity-determining region 1, which are associated with increased affinity for the immunizing Ag, phenylazoxalone (30).

Discussion

Although all four nucleotides in V genes undergo mutation, substitutions of G and C seem to occur as the default mechanism. For example, lower species such as horned shark (31) and frogs (32) have a predominance of G:C mutations; mice deficient in mismatch repair proteins Msh2 (33–36) and Msh6 (37) have a large proportion of G:C mutations; and cell lines that mutate in vitro accumulate mostly G:C mutations (38–41). This has led to the proposal that mutations of G and C occur first, and then another pathway subsequently introduces mutations of A and T (36). G:C mutations could be generated by two distinct mechanisms. First, the mutations could occur during synthesis by an inaccurate DNA polymerase that preferentially inserts mismatches opposite G and C vs A and T. However, there is no strong evidence to date for a DNA polymerase that specifically miscycles G and C. DNA polymerases μ (42), λ (42), κ (43), ξ (44), and τ (P. J. Gearhart, manuscript in preparation) do not appear to alter the mutational spectra because mice lacking these proteins or with reduced levels have a normal pattern of V gene substitutions. DNA polymerase η has been proposed to be an A:T mutator, because humans without the polymerase have fewer mutations of A and T (45, 46).

Second, G:C mutations could occur because the bases are modified more frequently during hypermutation. There is compelling...
evidence for lesions at C residues in DNA (9, 10), and mice deficient for uracil-DNA glycosylase have an altered mutational spectrum with a preponderance of C to T transitions (11). This suggests that uracil initiates mutation in the V gene, and the base excision repair pathway that usually removes it in an error-free manner is subverted to a mutagenic role. To test whether specific lesions at G residues also cause hypermutation, we examined mice deficient for Ogg1, the major DNA glycosylase that removes damaged guanines. The lesion is strongly mutagenic if it persists in DNA, because it can mispair with A and cause G:C to T:A transversions. The rationale for examining Ogg1 in hypermutation came from a report that the enzyme is overexpressed in human germinal center cells (19), and the speculation that it may be involved in the bias toward G:C mutations in Msh2-deficient mice (47). Ogg1 may also generate strand breaks in the switch regions of constant genes due to its lyase activity which incises DNA. Mice deficient in Ogg1 accumulated a 3-fold increase of 8-oxoG in liver and an increase in G:C to T:A transversions in a reporter gene (22, 48, 49). However, they do not develop spontaneous malignancies, which may be due to a backup system to repair 8-oxoG involving the Csb protein (50). Nonetheless, Ogg1 may be important, because a parallel situation exists with the presence of several uracil-DNA glycosylases in mice, and yet only one alters hypermutation (11, 51).

In this study, we show that Ogg1 is not overexpressed in murine B cells from germinal centers. The discrepancy between human and murine expression of OGG1 in germinal centers may be due to the contrasting nature of the lymphoid organs being studied. Human tonsil germinal center cells are constitutively stimulated, undergo a high rate of apoptosis, and may accumulate large amounts of oxidative damage that could elevate the level of OGG1 expression (52). Murine splenic germinal center cells are transiently stimulated and may not acquire substantial oxidative damage over a short time. Furthermore, Ogg1-deficient mice had a similar frequency of mutation as Ogg1-proficient mice in V genes from the \( \kappa \) and L chain loci. The spectra of nucleotide changes were also similar between the two groups, with around 50% of the mutations occurring at A:T base pairs and 50% at G:C base pairs. More importantly, there was not a significant increase in G:C to T:A transversions in the \( \text{oggl}^{-/} \) alleles. Taken together, these findings show that hypermutation is unaffected in the absence of Ogg1 activity and indicate that 8-oxoG lesions most likely do not cause V gene mutations.

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