XRCC1 suppresses somatic hypermutation and promotes alternative nonhomologous end joining in IgH genes

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Activation-induced deaminase (AID) deaminates cytosine to uracil in immunoglobulin genes. Uracils in DNA can be recognized by uracil DNA glycosylase and abasic endonuclease to produce single-strand breaks. The breaks are repaired either faithfully by DNA base excision repair (BER) or mutagenically to produce somatic hypermutation (SHM) and class switch recombination (CSR). To unravel the interplay between repair and mutagenesis, we decreased the level of x-ray cross-complementing 1 (XRCC1), a scaffold protein involved in BER. Mice heterozygous for XRCC1 showed a significant increase in the frequencies of SHM in IgH variable regions in Peyer’s patch cells, and of double-strand breaks in the switch regions during CSR. Although the frequency of CSR was normal in Xrcc1+/− splenic B cells, the length of microhomology at the switch junctions decreased, suggesting that XRCC1 also participates in alternative nonhomologous end joining. Furthermore, Xrcc1+/− B cells had reduced IgH/c-myc translocations during CSR, supporting a role for XRCC1 in microhomology-mediated joining. Our results imply that AID-induced single-strand breaks in IgH variable and switch regions become substrates simultaneously for BER and mutagenesis pathways.
also had an altered spectrum of substitutions, showing that UNG participates in mutagenesis (Rada et al., 2002; Saribasak et al., 2006; Rajagopal et al., 2009). Thus, UNG is required for both repairing mutations and generating them, which may explain why the mutation frequency was not dramatically increased in UNG-deficient mice. In addition, UNG-deficient mice had strikingly decreased CSR, implying that uracil excision precedes recombination (Rada et al., 2002). In mice with a haploinsufficiency of APE1, the frequency of uracil excision precedes recombination (Guikema et al., 2007), indicating that APE1 also participates in both canonical BER and CSR. Therefore, the conundrum is why UNG and APE1 do not funnel all of the single-strand breaks into the high-fidelity BER pathway but leave some of them to become substrates for the mutagenesis pathway.

Another interpretation could be that UNG functions mainly in the mutagenesis pathway, and another uracil glycosylase, such as SMUG1, directs uracil repair into the faithful BER pathway (Di Noia et al., 2006). This is unlikely, however, because UNG is the most active uracil glycosylase in B cells, and the competition between repair and mutagenesis would mostly be for the single-strand breaks produced by APE1 incision of the abasic sites generated by UNG. In this regard, it should be noted that there will be breaks in the V region to allow entry for either DNA polymerase β to repair the break or DNA polymerase η and other low-fidelity polymerases to introduce nucleotide substitutions. DNA polymerases require a 3’ hydroxyl end to initiate synthesis, which could be provided by nicks from APE1 cleavage or from mismatch repair. Of course, single-strand breaks in the S regions are more abundant and easily detected as a result of the high frequency of AID hotspots and RNA pausing.

Another BER protein, DNA polymerase β, which replaces the excised nucleotide, was examined for its effect on SHM and CSR. In polymerase β-deficient B cells, the mutation frequency was increased in V and S regions, and there were more double-strand breaks in S regions and slightly increased CSR (Poltoratsky et al., 2007; Wu and Stavnezer, 2007). Therefore, polymerase β appears to function solely within the BER pathway to repair AID-induced strand breaks, and in its absence, SHM and CSR are elevated. The synthesized ends are then sealed by DNA ligase 3 to provide a corrected product. The role of this ligase in SHM and CSR can only be predicted because DNA ligase 3–deficient mice are not viable (Puebla-Osorio et al., 2006).

In this study, we examine the involvement of a scaffold protein in the BER pathway, x-ray cross-complementing 1 (XRCC1), in repairing AID-induced lesions. Although it has no enzymatic activity, XRCC1 interacts with DNA polymerase β and DNA ligase 3 to stabilize the proteins (Caldecott, 2003; Parsons et al., 2008). This suggests that a deficiency in XRCC1 would impair their function during BER, and increase the frequency of SHM and CSR. XRCC1 also interacts with PARP1 (Audebert et al., 2004), which may bind to the DNA breaks that are induced during CSR. Mice deficient in PARP1 had a normal frequency of CSR, but the Sμ–Sγ3 joins had less microhomology than joins from wild-type mice (Robert et al., 2009). Thus, PARP1, and possibly XRCC1, may function during CSR, through alternative nonhomologous end joining (A-NHEJ), which is characterized by microhomology at the junctions, rather than through classical NHEJ (C-NHEJ), which produces mostly blunt joins. In addition, A-NHEJ has been shown to facilitate chromosomal translocations (Yan et al., 2007; Boboila et al., 2010a; Simsek and Jasin, 2010), implying that XRCC1 could affect the frequency of translocations. XRCC1 may, therefore, be a versatile player in both BER and A-NHEJ and have an effect on SHM, CSR, and translocations in B cells. Because XRCC1 is required for embryonic development (Tebbels et al., 1999), we used heterozygous mice to study its role in the frequencies of mutation in the V region, double-strand breaks in Sμ, CSR to various isotypes, microhomology at Sμ–Sγ3 joins, and translocations between Igh and c-myc loci.

**Figure 1.** *Xrcc1*+/− cells have reduced levels of protein and are more sensitive to MMS damage. (A) Protein levels of XRCC1. Protein from lysates from resting B cells was measured by Western blot and is shown relative to β-actin. Error bars represent the SD of values from three independent experiments with one to two mice per genotype per experiment. Significance was determined by the Student t test. (B) Protein levels for DNA polymerase β and (C) DNA ligase 3 in *Xrcc1*+/− and *Xrcc1*−/− B cells. Western blot analysis was performed on whole cell lysates from day-3 cells activated with LPS and IL-4 and are shown relative to β-actin levels. Error bars represent the SD of values from four independent experiments with two mice per genotype per experiment. (D) DNA repair after MMS damage. B cells were activated ex vivo, treated with MMS on day 1, and viability was measured by staining with propidium iodide on day 2. Error bars represent the SD of values from two independent experiments using two mice per genotype for one experiment and one mouse per genotype for another experiment.
RESULTS AND DISCUSSION

**Xrcc1**+/− B cells have reduced BER

To ensure that XRCC1 is down-regulated in heterozygous mice, we measured RNA and protein levels in resting splenic B cells. Transcript levels were detected by quantitative PCR of cDNA, which was amplified with primers located downstream of the deleted exon 8 (Tebbs et al., 1999). There was a 38% reduction of XRCC1 transcripts in **Xrcc1**+/− cells compared with **Xrcc1**+/+ cells (unpublished data). Protein levels were measured by Western blotting of whole cell extracts with antibody to XRCC1, and the results confirmed a 40% reduction in the heterozygous cells (Fig. 1 A; P = 1.4 × 10−7). Reduced XRCC1 protein could affect the stability of DNA polymerase β and DNA ligase 3. We determined the levels of these enzymes by western analyses and found that the amounts of DNA polymerase β (Fig. 1 B) and DNA ligase 3 (Fig. 1 C) were similar in both **Xrcc1**+/+ and **Xrcc1**+/− B cells, indicating that the residual XRCC1 protein was sufficient to maintain stability of these two critical proteins. To determine if **Xrcc1** haploinsufficiency affected BER function, splenic B cells were activated with LPS plus IL-4 and incubated with various concentrations of methyl methanesulfonate (MMS), which leads to base loss and DNA strand breakage. As shown in Fig. 1 D, **Xrcc1**+/− cells were more sensitive to MMS compared with **Xrcc1**+/+ cells, indicating that the physiological role of XRCC1 to repair breaks is impaired in these cells. Collectively, the reduced XRCC1 protein levels and lower repair activity suggest that the heterozygous mice are a good model to determine if high-fidelity BER competes with the mutagenesis pathway for access to DNA single-strand breaks.

**Higher SHM frequency in V<sub>μ</sub> regions**

SHM was detected in a 492-nucleotide region containing the intron downstream of rearranged J<sub>μ4</sub> genes from germinal center-derived Peyer’s patch B cells. The frequency of mutation was determined in 4-mo- and 9-mo-old littermate mice and is shown in Fig. 2 A. The frequency in mutated sequences from 4-mo-old mice was significantly higher (Fig. 2 B; P = 0.007) in **Xrcc1**+/− clones (15.4 × 10−3 mutations/base) than in **Xrcc1**+/+ clones (6.6 × 10−3). The frequency in 9-mo-old mice also was higher in **Xrcc1**+/− clones (28.5 × 10−3 mutations/base) compared with **Xrcc1**+/+ clones (17.9 × 10−3). The overall increase in mutations in older mice is in agreement with the study reporting that mutations accumulate with time in Peyer’s patch B cells (González-Fernández et al., 1994). These results are striking because the haploinsufficient cells retained at least 50% of the protein. The types of mutations were similar between the two genotypes (Fig. 2 C), indicating that the increased mutations were generated by both the UNG and MSH2 pathways affecting G:C and A:T substitutions, respectively (Rada et al., 2004). Thus, the increased frequency of mutation was likely a result of inefficient repair in the heterozygous cells, because XRCC1 failed to adequately recruit DNA polymerase β and DNA ligase 3 to complete BER (Parsons et al., 2008). We propose that XRCC1 plays a role in promoting the accurate repair of single-strand breaks in V regions, and when the level of XRCC1 is reduced, more breaks are channeled into the mutagenesis pathway.

**Increased double-strand breaks in S<sub>μ</sub>**

In the S<sub>μ</sub> region, breaks are frequent as a result of multiple targeting sites for AID activity and prolonged RNA pausing in R-loops (Rajagopal et al., 2009; Wang et al., 2009). UNG and APE1 then produce single-strand breaks, which can be repaired by BER. Because XRCC1 is a major contributor to the repair of DNA single-strand breaks (Caldecott, 2003), it should be present in the S regions during CSR. We therefore measured the amount of protein at the S<sub>μ</sub> region by chromatin immunoprecipitation (ChIP) analysis in ex vivo stimulated cells. Significantly higher levels of protein were detected bound to S<sub>μ</sub> from **Xrcc1**+/+ and **Xrcc1**+/− mice compared with **Aid**+/− mice (P = 0.009 and 0.038, respectively), and there was no increase in **Xrcc1** bound to the C<sub>μ</sub> gene (Fig. 3 A). Thus, the heterozygous cells had a greater abundance of XRCC1 protein relative to AID-deficient cells, indicating that the protein is recruited to DNA breaks in S<sub>μ</sub>. Single-strand
had significantly more breaks than Xrcc1+/+ cells (P = 0.002), and Aid−/− cells had very few breaks (Fig. 3 C). These data indicate that XRCC1 plays a role in repairing AID-induced breaks in the Sμ region.

**Similar frequency of CSR but shorter microhomology at switch junctions**

The increased number of DNA breaks predicted that the frequency of CSR would be elevated. Before testing for CSR, we determined that Xrcc1+/− B cells underwent similar amounts of DNA synthesis as Xrcc1+/+ spleen cells after stimulation with LPS and IL-4, as seen by incorporation of breaks in close proximity on both strands can then be processed into double-strand breaks for recombination to downstream S regions. To measure the amount of double-strand breaks, a linker was ligated to DNA from cells stimulated for 2 d ex vivo, and the reactions were amplified by ligation-mediated PCR (LM-PCR) and separated by gel electrophoresis (Kong and Maizels, 2001; Guikema et al., 2007). Products containing Sμ DNA were identified by hybridization to Sμ. Representative blots are shown. (C) Quantification of breaks from the LM-PCR assay was performed using the highest dilution of DNA and was calculated from 10 independent PCR reactions each from two Xrcc1+/+ and two Aid−/− mice and 30 independent reactions from six Xrcc1+/+ mice. Horizontal bars show the mean intensity of hybridization. Statistical analysis was performed using a two-tailed Student’s t test.

**Figure 4.** Xrcc1+/− cells have shorter overlaps in switch junctions. (A) DNA synthesis in Xrcc1+/+ and Xrcc1+/− splenic B cells. Cells were cultured with LPS and IL-4 in the presence of EdU. Error bars represent the SD of values from two independent experiments with one mouse per genotype per experiment. Wild-type (WT) levels (dotted line) were 37% for IgG1, 17% for IgG2a, 32% for IgG2b, and 3% for IgG3. Error bars signify the SD of values from three independent experiments with two mice per genotype per experiment. (B) Microhomology in Sμ−Sμ3 switch joins from cells stimulated with LPS. Lengths of overlaps are depicted on the x-axis, and mean overlap for number (n) of sequences from two independent experiments with two mice per genotype per experiment is shown in the inset. Statistical difference was determined by the Mann-Whitney test.
ethyl-2-deoxyuridine (EdU), an analogue of thymidine (Fig. 4 A). Isotype switching was determined 4 d after addition of LPS plus various cytokines to induce switching from IgM to IgG1, IgG2a, IgG2b, or IgG3. There was no increase in the level of CSR to all isotypes (Fig. 4 B), even under suboptimal conditions of stimulation using less LPS and cytokines (Wu and Stavnezer, 2007; not depicted). Thus, despite the greater incidence of double-strand breaks, XRCC1 haploinsufficiency did not affect the overall frequency of CSR. Similar results were seen in DNA polymerase β–deficient cells, where increased double-strand breaks did not generate a corresponding increase in CSR (Wu and Stavnezer, 2007). In contrast, cells with decreased breaks as a result of reduced APE1 cleavage or altered switch mechanisms still showed substantial recombination (Guikema et al., 2007; Zarrin et al., 2007). This indicates that only a few breaks can lead to recombination, and more breaks may not increase the frequency of CSR. Therefore, the frequency of switching does not accurately reflect the number of double-strand breaks.

Double-strand breaks in CSR are joined by either C-NHEJ, which is characterized by S-S joins with blunt or short overlapping nucleotides, or A-NHEJ, which generates joins with longer microhomologies. Experiments from several laboratories have demonstrated that in the absence of proteins that promote C-NHEJ, such as Ku, XRCC4, and DNA ligase 4, CSR occurs by A-NHEJ (Boboila et al., 2010b; Pan-Hammerström et al., 2005; Souls–Spraul et al., 2007; Yan et al., 2007). However, limited information is available about the proteins that are involved in A-NHEJ during CSR. PARP1 (Robert et al., 2009), MRN (Dinkelmann et al., 2009), Ku70 (Boboila et al., 2010b), and CtIP (Lee-Theilen et al., 2011) have recently been reported to play roles. Because XRCC1 has been shown to participate in A-NHEJ after DNA damage (Audebert et al., 2004; Wang et al., 2005), we reasoned that a reduction in XRCC1 would drive recombination toward the C-NHEJ route and produce S-S joins with more blunt joins and short overlaps. We sequenced Sµ-S3 junctions from spleen cells stimulated for 4 d with LPS (Fig. 4 C) because the length of S3 is shorter than other S regions, making it easier to identify homology after recombination. Microhomology was measured by the number of identical bases shared between Sµ and S3 at the break site (Fig. S1). There was a significant decrease in the mean length of microhomology in Xrc1+/− cells (1.7 bp) compared with Xrc1+/+ cells (2.5 bp; P = 0.043), suggesting that XRCC1 is involved in A-NHEJ during CSR, likely through its interactions with DNA ligase 3.

**XRCC1 facilitates Igh/c-myc translocations**

AID–dependent chromosomal translocations between the oncogene c-myc and the Sµ region (Ramiro et al., 2004) are catalyzed by A-NHEJ (Boboila et al., 2010a). The data in the previous section shows that XRCC1 favored joining using longer microhomologies, which suggested that the protein has a role in generating translocations. To assess the frequency of translocations, we cultured splenic B cells from Xrc1+/+ and Xrc1+/− mice with LPS and IL-4 for 3 d. Translocations were measured by long-range PCR with both chromosome 12 and 15 primers and identified by Southern hybridization (Fig. 5, A and B). In amplifications from 2.7 × 107 cells per genotype, there were 18 translocations in Xrc1+/+ cells and 9 translocations in Xrc1+/− cells. Therefore, the frequency in Xrc1+/+ cells was 6.5 translocations per 107 cells, in accord with a previously published study (Boboila et al., 2010a; Fig. 5 C). In Xrc1+/− cells, there was a significant reduction in the frequency (3.4 per 107 cells; P = 0.038), suggesting that XRCC1 plays a role in the microhomology based pathway to promote chromosome translocations. Although a previous study (Robert et al., 2009) found no requirement for PARP1, a protein in A-NHEJ, in translocations during CSR, their conditions for stimulation in wild-type cells produced translocation frequencies of only 1.5 per 107 cells.
which would make it difficult to detect less frequent events. Thus, even though the frequency of recombination between S-S regions in \( \text{Xrc1}^{+/+} \) was normal, we have documented a decrease in recombination between \( S_h \) and \( c-myC \), perhaps because the latter is a rare event which reveals a quantitative difference.

**BER is active in the Ig loci**

BER resolves small base modifications, abasic sites, and single-strand breaks, all of which are abundant in V and S regions after AID is unleashed. The high frequency of mutations and seeming dearth of repair has been postulated to be a result of either a lack of faithful BER in the Ig loci or the very high level of damage induced by AID that could overwhelm BER. To what extent does BER compete with mutagenesis for DNA strand breaks? Our results show that it is relatively easy to change the outcome by removing \(~40\%\) of a BER scaffold protein, \( \text{XRCC1} \), suggesting that alterations to the DNA synthesis and ligation steps of BER regulate the balance between accurate repair and mutagenesis. Our results confirm previous results showing increased mutations and double-strand breaks in B cells deficient for DNA polymerase \( \beta \) (Polhotrasky et al., 2007; Wu and Stavnezer, 2007), and they extend the SHM analysis for V regions undergoing mutation in vivo. The increase in SHM in BER-compromised cells implies that many AID-induced lesions are repaired faithfully by BER. The U-G mismatch is also recognized by proteins in the mismatch repair pathway, and a similar conclusion was reached for a balance between mismatch repair and mutagenesis after AID is unleashed. The high frequency of mutations in BER-compromised cells implies the result of either a lack of faithful BER in the Ig loci or the very high level of damage induced by AID that could overwhelm BER. The U-G mismatch is also recognized by proteins in the mismatch repair pathway, and a similar conclusion was reached for a balance between accurate repair and mutagenesis. Our results confirm previous results showing increased mutations and double-strand breaks in B cells deficient for DNA polymerase \( \beta \) (Polhotrasky et al., 2007; Wu and Stavnezer, 2007), and they extend the SHM analysis for V regions undergoing mutation in vivo. The increase in SHM in BER-compromised cells implies that many AID-induced lesions are repaired faithfully by BER. The U-G mismatch is also recognized by proteins in the mismatch repair pathway, and a similar conclusion was reached for a balance between accurate repair and mutagenesis. Our results confirm previous results showing increased mutations and double-strand breaks in B cells deficient for DNA polymerase \( \beta \) (Polhotrasky et al., 2007; Wu and Stavnezer, 2007), and they extend the SHM analysis for V regions undergoing mutation in vivo. The increase in SHM in BER-compromised cells implies that many AID-induced lesions are repaired faithfully by BER. The U-G mismatch is also recognized by proteins in the mismatch repair pathway, and a similar conclusion was reached for a balance between accurate repair and mutagenesis.

**MATERIALS AND METHODS**

**Mice**. \( \text{Xrc1}^{+/+} \) mice on a C57BL/6 background were rederived from cryopreserved embryos as previously described (McNeill et al., 2011). Breeding pairs were set up as crosses between heterozygous and wild-type mice. Genotypes were confirmed by PCR of tail DNA using the primers listed in this section. To detect the neo cassette in exon 8 of the \( \text{Xrc1} \) genomic locus, NEO-F01 (5'-GCTTGGCAGATATCATGGTG-3') and TAR-D (5'-ATTAGGGTTGGTCCCATCGAG-3') generated a 450-bp band in heterozygous mice and no band in wild-type mice. To detect both targeting and recombination events, the remaining cell suspension was washed and eluted, and the cross-links were reversed by heat-denaturation at 37°C for 15 min. The reaction was quenched by adding glycine to a final concentration of 0.125 M at room temperature for 5 min. The cells were washed twice with 5 ml of cold phosphate-buffered saline supplemented with protease inhibitor cocktail tablet (Roche), lysed in 400 ml SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8), and incubated on ice for 10 min. The reaction was then mixed with 600 ml ChIP dilution buffer (0.01% SDS, 1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8, 16.7 mM NaCl, 1 mM PMSE, and protease inhibitor cocktail). Chromatin was sonicated to a mean length of \(~300\) bp. Supernatant was saved and diluted fourfold in ChIP dilution buffer after centrifugation at 10,000 \( \times \) 30 min. One tenth of the diluted cell suspension was saved as input. To get rid of nonspecific IgG binding proteins, the remaining cell suspension was incubated with 36 ml Dynabeads Protein G (Invitrogen) and 5 mg of normal mouse IgG antibody (12–371; Millipore) at 4°C for 3 h. The suspension was then transferred into fresh 1.5-ml tubes and incubated with 36 ml Dynabeads Protein G and 5 mg rabbit anti-XRCC1 antibody (ab9147; Abcam) at 4°C for 16 h. DNA was washed and eluted, and the cross-links were reversed by heating the Dynabeads at 65°C for 5 h followed by proteinase K treatment at 45°C for 2 h. DNA was recovered by phenol:chloroform:isoamyl alcohol 25:24:1 (Sigma-Aldrich) extraction and ethanol precipitation. \( S_h \) and \( C_h \) sequences were quantified by quantitative PCR, relative to input values, \( S_h \) primers: forward, 5'-ACACTTACATCTCTTGTATCAATCTAAGTGGT-3', and reverse, 5'-GGATTTAAGCAGTTCGACTTCC-3'; \( C_h \) primers: forward, 5'-TCTGACGAGGGCAAGAGAACAGATTCCTA-3', and reverse, 5'-GACACAGATTTTACAGGACCGG-3'.

**Double-strand break assay**. Genomic DNA from 10⁶ cells was isolated from cells stimulated with LPS plus IL-4 for 2 d, as previously described.
(Guikema et al., 2007). DNA was digested in agarose plugs with proteinase K, washed, and equilibrated in 50 µl T4 ligase buffer. The agarose was melted, and 20 µl DNA was removed and mixed in a 60-µl reaction with a preannealed linker L/LL2 (Kong and Maizels, 2001) and T4 ligase for 16 h at 16°C. After ligation, samples were dialyzed fivefold in water and heated to 70°C for 10 min. To compare DNA recovery, threefold dilutions (spanning from 3- to 243-fold) were made and used as a template to amplify Gapdh as previously described (Maul et al., 2011). After standardization to Gapdh, double-strand breaks were amplified by nested PCR. The first round used forward primer LMPCR1 (5'-GAATTTACGAGTTAATTCTGGCTCTTCTTAAGCAG-3') and reverse primer LL4 (Kong and Maizels, 2001), and the second round used forward primer LMPCR2 (5'-GAATCTATTCGCTCCTTAAATTGCTTCC-3') and reverse primer LL2 (Kong and Maizels, 2001). PCR products were separated by electrophoresis through a 1.2% agarose gel, blotted onto Hybond N+ membrane (GE Healthcare), and hybridized with a 32P-ATP labeled Sα probe (5'-CTTACTGATTCTAATAATTGCTCC-3'). The intensity of hybridization was quantified by exposure to a phosphorimager and analyzed using image quant software (GE Healthcare).

Igh/c-myc translocations. DNA was collected from B cells stimulated with LPS and IL-4 for 3 d and resuspended at a ratio equivalent of 105 cells per µl. Nested PCR analysis of Igh/c-myc translocations on both chromosomes 12 and 15 was performed as previously described (Ramiro et al., 2004), and Aid was amplified as a positive control as previously described (Robert et al., 2009). 10 µl PCR products were separated by gel electrophoresis and stained with ethidium bromide. The gel was then blotted onto a Hybond N+ membrane (GE Healthcare) and analyzed sequentially with Igh and c-myc-specific probes (Ramiro et al., 2004).

Online supplemental material. Fig. S1 lists the Sα-S3 joins with nucleotide overlays for microhomology analyses. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20111135/DC1.

We thank R. Wersto, C. Morris, J. Scheers, C. Nguyen, and T. Wolf for flow cytometry analyses, and Raman Sen and Sebastian Fugmann for valuable comments.

This research was supported entirely by the Intramural Research Program of the National Institutes of Health, National Institute on Aging.

REFERENCES


Rada, C., G.T. Williams, H. Nissen, D.E. Barnes, T. Lindahl, and M.S. Neuberger. 2002. Immunoglobulin isotype switching is inhibited and...


