Molecular Immunology 53 (2013) 214–217

Contents lists available at SciVerse ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Short communication

Analysis of Ig gene hypermutation in *Ung*$^{-/-}$*Polh*$^{-/-}$ mice suggests that UNG and A:T mutagenesis pathway target different U:G lesions

Shuyin Li[a,b], Yaofeng Zhao[a], Ji-Yang Wang[b,*]

[a] State Key Laboratory of AgroBiotechnology, College of Biological Sciences, China Agricultural University, Beijing 100094, China
[b] Laboratory for Immune Diversity, Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Yokohama, Yokohama 230-0045, Japan

ARTICLE INFO

Article history:
Received 14 July 2012
Accepted 7 August 2012
Available online 4 September 2012

Keywords:
Immunoglobulin gene hypermutation
Activation-induced cytidine deaminase
Uracil DNA glycosylase
DNA polymerase η
Cell cycle

ABSTRACT

The activation-induced cytidine deaminase (AID) initiates Ig gene hypermutation by converting cytosine to uracil (U) and generating a U:G lesion. Genetic and biochemical studies suggest that the AID-triggered U:G lesions are processed by three mutagenic pathways to induce mutations at both C:G and A:T pairs. First, direct replication of the U:G lesion leads to C to T and G to A transitions. Second, U can be excised by the uracil DNA glycosylase (UNG) and the replication/processing of the resulting abasic site leads to transversions and transitions at C:G pairs. Third, the U:G lesion is recognized by an atypical mismatch repair (MMR) pathway which generates mutations at A:T pairs in a DNA polymerase η (POLH)-dependent manner. To further explore whether these three mutagenic pathways function competitively or independently, we have analyzed Ig gene hypermutation in mice deficient in both UNG and POLH. Compared with WT mice, UNG deficiency caused elevated frequency of C:G mutations, suggesting that UNG-mediated U excision led to error-free as well as error-prone repair. In contrast, UNG deficiency did not affect the frequency and patterns of A:T mutations, suggesting that the MMR did not target U:G lesions normally recognized and processed by UNG. In addition, POLH deficiency did not affect the frequency and patterns of C:G mutations and UNG POLH double deficiency showed an additive effect of single deficiency. Based on these observations and previous results, along with the recent finding that UNG excises AID-triggered U predominantly during G1 phase of the cell cycle, it appears that UNG and MMR targets U:G lesions generated during G1 and S phases of the cell cycle, respectively.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

During an immune response against protein antigen, B cells are activated and undergo rapid expansion in the germinal centers (GC) of the periphery lymphoid tissues. GC B cells undergo Ig gene somatic hypermutation (SHM), which introduces random point mutations into Ig genes and is essential for the generation of high-affinity antibodies. SHM is initiated by the activation-induced cytidine deaminase (AID) (Muramatsu et al., 2000), which is thought to catalyze the deamination of cytosine to uracil and generate a U:G lesion on DNA (Chaudhuri et al., 2003).

Based on the genetic and biochemical data, Neuberger et al. have provided an excellent model explaining how various mutations are generated (Di Noia and Neuberger, 2007; Maul and Gearhart, 2010; Rada et al., 1998, 2004; Reynaud et al., 2009). Accordingly, mutations are induced during replication and repair of the AID-triggered U:G lesion by three major pathways. First, direct replication of U:G can lead to C to T and G to A transitions (Ts) as U is structurally similar to thymine (T) and normally pairs with adenine (A). Second, U can be excised by the uracil DNA glycosylase (UNG) and the replication of the resulting abasic site is thought to generate transversions (Tv) as well as Ts at C:G pairs. Third, the U:G lesion is recognized by an atypical mismatch repair (MMR) pathway, resulting in the generation of A:T mutations in a DNA polymerase η (POLH)-dependent manner (Bardwell et al., 2004; Delbos et al., 2005, 2007; Faili et al., 2004; Frey et al., 1998; Langerak et al., 2007; Martomo et al., 2004, 2005; Rada et al., 1998; Zeng et al., 2001).

It remains less clear as to whether these three mutagenic pathways function competitively or independently. To further understand the relationship of these three pathways, we have established mice deficient in both UNG and POLH and compared their mutation frequency and patterns of Ig genes with those in WT and singly deficient mice. We found that absence of UNG increased the frequency and changed the patterns of C:G mutations but had no affect on A:T mutations. Conversely, absence of POLH did not

Abbreviations: AID, activation-induced cytidine deaminase; GC, germinal center; MMR, mismatch repair; POLH, DNA polymerase η; SHM, somatic hypermutation; Ts, transition; Tv, transversion; UNG, uracil DNA glycosylase.

* Corresponding author at: Laboratory for Immune Diversity, RIKEN Research Center for Allergy and Immunology, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan. Tel.: +81 45 503 7041; fax: +81 45 503 7040.
E-mail address: oh@rcai.riken.jp (J.-Y. Wang).

0161-5890/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.molimm.2012.08.009

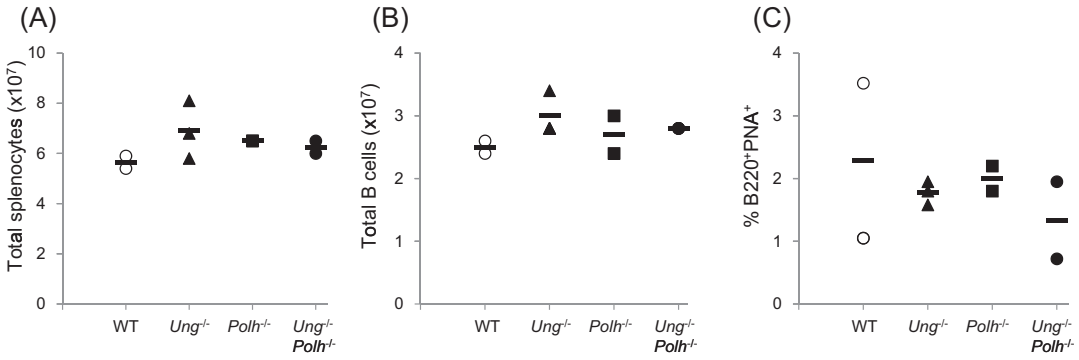

Fig. 1. Normal numbers of splenocytes, B cells and the frequency of GC B cells in WT, *Ung*$^{-/-}$, *Polh*$^{-/-}$ and *Ung*$^{-/-}$*Polh*$^{-/-}$ mice at ages of 11–13-wk. (A) Total numbers of splenocytes. Single cell suspension of spleen from each mouse was treated with ACK lysis buffer (0.15 M NH_4Cl, 1 mM $KHCO_3$ and 0.1 mM Na_2EDTA, pH 7.4) to eliminate red blood cells and then counted with a hemocytometer in the presence of trypan blue. The total numbers of live cells in the spleen of each mouse are shown. (B) Total numbers of B cells in the spleen of each mouse after purification with an IMAG B Lymphocyte Enrichment Set. (C) Frequency of the B220$^+$PNA$^+$ GC B cells in each mouse before sorting. A bar indicates the average.

affect C:G mutations and UNG POLH double deficiency showed an additive effect of single deficiency. These observations suggested that UNG-mediated C:G and POLH-mediated A:T mutagenesis did not interfere with each other. Along with the findings that UNG excises AID-triggered U predominantly during G1 phase (Sharbeen et al., 2012) and that rapid DNA synthesis is important for induction of A:T mutations (Kano et al., 2011), it appears that UNG and A:T mutagenesis targets U:G lesions generated during G1 and S phase of the cell cycle, respectively.

2. Materials and methods

2.1. Mice

Ung$^{-/-}$ (Nilsen et al., 2003) and *Polh*$^{-/-}$ mice (Ohkumo et al., 2006) were kindly provided by Dr. Deborah Barnes and Dr. Fumio Hanaoka, respectively. *Ung*$^{-/-}$ mice had a mixed genetic background of 129 and C57BL/6 and were backcrossed to C57BL/6 for three generations before crossing with *Polh*$^{-/-}$ mice. *Polh*$^{-/-}$ mice had been backcrossed with C57BL/6 for 12 generations (Kano et al., 2012). *Ung*$^{+/-}$*Polh*$^{+/-}$ were crossed to obtain WT, *Ung*$^{-/-}$, *Polh*$^{-/-}$ and *Ung*$^{-/-}$*Polh*$^{-/-}$ mice. The mice were maintained under specific pathogen-free conditions and all experiments were approved by the Animal Facility Committee of RIKEN Yokohama Institute (Permission number 20-025).

2.2. Immunization and analysis of Ig gene hypermutation

Two WT, 3 *Ung*$^{-/-}$, 2 *Polh*$^{-/-}$ and 2 *Ung*$^{-/-}$*Polh*$^{-/-}$ mice at ages of 11–13-wk-old were immunized *i.p.* with 100 μg of 4-hydroxy-3-nitrophenyl-acetyl coupled to chicken γ globulin (NP-CGG) in Imject Alum Adjuvant (Thermo Fisher Scientific). Two weeks later, Spleen B cells were purified using negative sorting with the IMag B Lymphocyte Enrichment Set (BD Biosciences) and then stained with APC-B220 (BD Biosciences) and FITC-PNA (Vector Laboratories). B220$^+$PNA$^+$ GC B cells were then sorted using an Aria cell sorter and >10^4 GC B cells were collected. Genomic DNA was extracted and the J_H4 intronic region was amplified with forward primer J558Fr3 (5′-CAGCCTGACATCTGAGGACTCTGC-3′) and reverse primer JHCHint (5′-CTCCACCAGACCTCTCTAGACAGC-3′) as described (Kano et al., 2012). The PCR products were cloned into the pCR2.1 vector for sequencing. Only unique sequences were analyzed in each mouse.

3. Results and discussion

3.1. Ig gene hypermutation in WT, *Ung*$^{-/-}$, *Polh*$^{-/-}$ and *Ung*$^{-/-}$*Polh*$^{-/-}$ mice

Ung$^{-/-}$*Polh*$^{-/-}$ mice developed normally with no obvious abnormalities by appearance and had normal numbers of total splenocytes (Fig. 1A and B) and B cells (Fig. 1B). In addition, the frequency of the B220$^+$PNA$^+$ GC B cells was not significantly different from that in WT and singly deficient mice (Fig. 1C), suggesting that B cell activation and expansion in vivo in response to antigen stimulation were grossly normal in these mice. We sorted GC B cells from 2 WT, 3 *Ung*$^{-/-}$, 2 *Polh*$^{-/-}$ and 2 *Ung*$^{-/-}$*Polh*$^{-/-}$ mice and analyzed mutations in the intronic region 3′ of J_H4. We chose J_H4 intronic region since mutations in this region do not affect antibody affinity and therefore represent unbiased mutations. The results are summarized in Table 1. The overall mutation frequency in WT mice was 0.761×10^{-2}/bp. This value was slightly lower compared with that in our previous studies (Masuda et al., 2009; Kano et al., 2012), which could be due to the use of a different alum conjugate in the present study. Nevertheless, the same NP-CGG precipitated with alum was used for immunization of all genotypes and should not affect the comparison of Ig gene hypermutation among these

Table 1
Mutation frequency in the J_H4 intronic region of WT, *Ung*$^{-/-}$, *Polh*$^{-/-}$ and *Ung*$^{-/-}$ *Polh*$^{-/-}$ mice.

	WT (2 mice)	*Ung*$^{-/-}$ (3 mice)	*Polh*$^{-/-}$ (2 mice)	*Ung*$^{-/-}$ *Polh*$^{-/-}$ (2 mice)
Number of sequences	270	343	263	375
Mutated sequences (%)	202 (74.8%)	260 (75.8%)	171 (65.0%)	264 (70.4%)
Total length of mutated sequences	102,818	132,340	87,039	134,376
Total number of mutations	782	1190	402	1004
Overall mutation frequency ($\times 10^{-2}$/bp)	0.761	0.899	0.462	0.747
Mutation frequency at C:G ($\times 10^{-2}$/bp)	0.373	0.528[a]	0.401	0.662[a]
Mutation frequency at A:T ($\times 10^{-2}$/bp)	0.388	0.371	0.061[b]	0.085[b]
% mutation at C:G vs. A:T	49.0:51.0	58.7:41.3	86.8:13.2	88.6:11.4

[a] $p < 0.05$ compared with mutation frequency at C:G of WT mice (unpaired *t*-test).
[b] $p < 0.01$ compared with mutation frequency at A:T of WT mice (unpaired *t*-test).

Download English Version:

https://daneshyari.com/en/article/2831083

Download Persian Version:

https://daneshyari.com/article/2831083

Daneshyari.com