Somatic hypermutation is initiated by the activation-induced deaminase (AID) protein, which is expressed in activated B lymphocytes. AID functions by deaminating cytosine to uracil in DNA (Maul et al., 2011), and the U:G mismatch produces a mutational storm to generate extreme diversity in the immunoglobulin (Ig) loci. Proteins are drawn in from base excision and mismatch repair pathways (Rada et al., 2004), as well as low-fidelity DNA polymerases (Saribasak et al., 2012), to produce nucleotide substitutions and single-strand breaks. Peaks of mutation are found over V regions on the heavy (H) and light chain loci, and over switch (S) regions preceding constant (C) genes on the H chain locus (Maul and Gearhart, 2010). Mutations occur downstream of promoters, which implicates transcription in the process (Lebecque and Gearhart, 1990; Peters and Storb, 1996; Xue et al., 2006). However, the mechanism of how transcription focuses AID to these two regions is unclear.

For S regions, recent findings have revealed that the DNA sequence is important for recruiting AID. These 2–8 kb regions of intronic DNA are composed of repeats of 3–4 G clusters, which form stable RNA-DNA hybrids (R-loops) when transcribed (Huang et al., 2007), and WGC (W = A or T) motifs, which bind AID (Kohli et al., 2009; Wang et al., 2010). RNA polymerase II (pol II) accumulates as it transcribes the repetitive region (Rajagopal et al., 2009; Wang et al., 2009), leading to recruitment of AID via interaction with Spt5 (Pavri et al., 2010) and the RNA exosome (Basu et al., 2011). AID then deaminates C on both nontranscribed and transcribed strands.

Variable (V) genes of immunoglobulins undergo somatic hypermutation by activation-induced deaminase (AID) to generate amino acid substitutions that encode antibodies with increased affinity for antigen. Hypermutation is restricted to germinal center B cells and cannot be recapitulated in ex vivo–activated splenic cells, even though the latter express high levels of AID. This suggests that there is a specific feature of antigen activation in germinal centers that recruits AID to V genes which is absent in mitogen-activated cultured cells. Using two Igh knock-in mouse models, we found that RNA polymerase II accumulates in V regions in B cells after both types of stimulation for an extended distance of 1.2 kb from the TATA box. The paused polymerases generate abundant single-strand DNA targets for AID. However, there is a distinct accumulation of the initiating form of polymerase, along with the transcription cofactor Spt5 and AID, in the V region from germinal center cells, which is totally absent in cultured cells. These data support a model where mutations are prevalent in germinal center cells, but not in ex vivo cells, because the initiating form of polymerase is retained, which affects Spt5 and AID recruitment.
Figure 1. AID expression and somatic hypermutation in germinal center– and ex vivo–stimulated B cells. (A) AID expression. mRNA levels were measured relative to 18S rRNA levels in B1-8hi mice 7 d after immunization (4 independent experiments with 1 mouse per experiment) or 3 d after LPS and IL-4 stimulation (4 independent experiments with 1 mouse per experiment). Error bars represent SD. *, P < 0.0002 (unpaired two-tailed Student’s t test) compared with germinal center. (B) Region sequenced for mutations. (C) Mutation analysis in germinal center cells. Mutation frequencies were measured in the V region of B1-8hi Ung–/–, 8D10-GL, and B1-8hi Aid−/− mice (3–5 independent experiments with one mouse per experiment). *, P-values compared with the B1-8hi Aid−/− control. (D) Mutation analysis in ex vivo cells. Mutation frequencies were measured in B1-8hi Ung–/– and B1-8hi Aid−/− mice (3–4 independent experiments with 1 mouse per experiment).

Amassed Spt5 in V genes | Maul et al.

Robust somatic hypermutation in germinal center cells but not in ex vivo–activated cells

To study mutation in V regions on the Igh locus, we used two independent knock-in mice that contained a rearranged V–diversity (D)–joining (J) gene on both alleles: the V186.2 gene from the J558 V186.2 family rearranged to D and JH2 segments, and cloned into the JH4 intron (B1-8hi mice; Shih et al., 2002); and the VGK7 gene from the VGAM3.8 VH family rearranged to D and JH2 segments, and cloned into the JH4 intron (8D10-GL mice, this work). For germinal center cells, mice were immunized with phycoerythrin, an antigen which has broad specificity for many V genes—including V186.2 (Pape et al., 2011)—and GL7+ splenic B cells were isolated on day 7. For ex vivo activation, naive spleen cells from B1-8hi mice were stimulated with LPS and IL-4 for 2–5 d in culture. We first determined the level of expression of AID in cells under both conditions of activation. AID mRNA was measured by qPCR.

RESULTS

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pol II accumulates for an extended distance in V regions from both germinal center and ex vivo cells

We then analyzed RNA pol II activity by nuclear run-on experiments—which measure polymerase activity in specific regions—in B1-8hi cells activated ex vivo for 2 d. Transcription in isolated nuclei was initiated in vitro, and transcripts were hybridized to 500-bp DNA probes spanning a distance of 1.5 kb from the TATA box to the intron enhancer (Ej; Fig. 2 A, green probes V1–5), and a control probe in Cμ1. Data were corrected for dTTP content between the different probes because relative to 18S ribosomal RNA; there was fivefold more AID expressed after ex vivo activation compared with germinal center activation (Fig. 1 A). Thus, the lack of mutation in cultured cells is not due to deficient AID expression.

We then measured mutation frequencies in V regions by sequencing ~600 bp around the VDJ exon (Fig. 1 B) in cells from both types of activation. For the B1-8 gene, we used B1-8hi Ung–/– cells, which are compromised for base excision repair due to the lack of uracil DNA glycosylase (UNG), to increase the mutation frequency. In germinal center cells 7 d after immunization, both B1-8hi Ung–/– and 8D10-GL mice had significant levels of mutation compared with B1-8hi Aid−/− control mice (Fig. 1 C). In ex vivo cells 5 d after LPS and IL-4 activation, B1-8hi Ung–/– cells had no significant increase in mutation compared with B1-8hi Aid−/− cells (Fig. 1 D), which confirms previous reports (Manser, 1987; Reina-SanMartin et al., 2003). Thus, hypermutation of V genes is robust in germinal center cells but debilitated in cells stimulated in culture. Other attempts to establish ex vivo systems to study somatic hypermutation have been elusive as well (McHeyzer-Williams et al., 1991; Decker et al., 1995; Källberg et al., 1996; Williams et al., 1991; Decker et al., 1995; Källberg et al., 1996; Nojima et al., 2011; Matthews et al., 2014).
radiolabeled UTP was incorporated. Compared with the C probe, polymerase loading was significantly higher over the V2, V3, and V4 probes located in the VDJ exon and JH intron in day 2 cells relative to day 0 (Fig. 2 B). To examine if the increase is a common feature of highly transcribed genes, the γ-actin gene was also analyzed with probes similarly located downstream of the TATA box (Fig. 2 A, green probes G1-5). In contrast to the V region, there was no increase in the γ-actin gene on day 2 and no difference in levels between the different probes (Fig. 2 B). For a negative control, a probe located in the promoter region of the nontranscribed CD3 gene showed no transcription. Thus, pol II uniquely accumulated over V regions in ex vivo–activated B cells.

To determine whether pol II also accumulated in V regions from germinal center cells, we performed chromatin immunoprecipitation (ChIP) assays. For B1-8hi and 8D10-GL mice, V and I primers were located 0.6 and 1.2 kb downstream of the TATA box, and E and C primers were placed 1.6 and 6.4 kb downstream in the Eκ and Cκ regions, respectively (Fig. 2 A, red primers). For the γ-actin locus, primer a was located in the TATA box, and primers b and c were placed 0.6 and 1.6 kb downstream of the TATA box. For a negative control, a primer was located over the TATA box of the nontranscribed β-globin gene. As shown in Fig. 2 C, total pol II levels from germinal center and ex vivo cells were significantly higher over the V and I primers compared with primers for E, C, γ-actin, and β-globin from both B1-8hi and 8D10-GL mice. Pol II levels differed slightly at probes and primers when comparing run-on and ChIP data, which is likely due to the different techniques. For run-on, signals were received directly at the probe location. For ChIP, signals were obtained from large shear sizes of DNA, which could occur ~500 bp on either side of the primer location. In summary, both germlinal center and ex vivo activation produced significant accumulation of pol II for an extended distance of 1.2 kb from the TATA box.

**Pol II abundance correlates with single-strand DNA and hypermutation in germinal center cells**

To see if the increased pol II density exposed more single-strand DNA, which is the substrate for AID deamination, we measured its frequency in germinal center cells. To eliminate AID-dependent background deaminations, spleens from B1-8hi Aid−/− mice were used 7 d after immunization with phycoerythrin. Nuclei were isolated and treated with sodium bisulfite, which deaminates single-stranded cytosine to uracil, similar to AID. The products were sequenced for C:G to T:A transitions to distinguish deamination on the nontranscribed (C to T) and transcribed (G to A) strands in three 700-bp segments of DNA (Fig. 3 A). As seen in Fig. 3 B (top half), activated cells had substantially increased single-strand DNA compared with naive cells (dotted lines). Because pol II could accumulate on the transcribed strand, single-strand cytosines might be more frequent on the nontranscribed strand. However, the frequency was similar on both DNA strands, which indicates that both strands are accessible during transcription for deamination by bisulfite, and presumably AID. It has been suggested that the RNA exosome removes nascent RNA from the transcribed strand in S regions (Basu et al., 2011), and this may occur in V regions as well (Milstein et al., 1998; Rada et al., 2004; Ronai et al., 2007; Parsa et al., 2012). To compare frequencies, the deamination events were grouped into V, I, and E segments and corrected for the nucleotide composition of C:G bases. The single-strand DNA frequency

![Figure 2. Transcription analyses of the V region and γ-actin loci from activated B cells.](image)
To confirm that somatic hypermutation does indeed mirror RNA pol II accumulation and single-strand DNA density, we collected extensive data from sequencing the B1–8th region from germinal center B cells from Peyer’s patches. As shown in Fig. 3 B (bottom half) and Fig. 3 D, the frequency of somatic hypermutation was significantly higher in the V and I segments compared with E. Although the E segment was not associated with many mutations or RNA pol II accumulation, the single-strand DNA detected there may be due to activity of the intronic promoter (Lennon and Perry, 1985). Collectively, elevated pol II levels correlate with increased single-strand DNA and mutations in the VDJ and JH intron regions in germinal center cells. However, the lack of mutations after ex vivo activation indicates that pol II density and single-strand DNA alone are not sufficient to generate mutations.

Initiating Pol II, Spt5, and AID are present in V regions from germinal center cells but absent in ex vivo cells

To identify what is missing in cultured cells, we first characterized the type of RNA pol II accumulation. Initiating polymerases are marked by having the C-terminal domain phosphorylated at Ser5 (Ser5P; Buratowski, 2009). Using an antibody for Ser5P, ChIP analysis showed that this form of pol II was abundant at the V and I primers in both B1–8th and 8D10–GL germinal center B cells but not in ex vivo–activated cells (Fig. 4, A and B). Furthermore, Spt5, which is a member of the DSIF complex associated with the initiating form of pol II, was associated with the V and I primers in germinal center cells but was missing in ex vivo cells (Fig. 4 C). Concomitantly, AID was abundant at the V and I locations in germinal center cells but was nonexistent in ex vivo cells (Fig. 4 D). For controls, the transcribed γ-actin gene had significant amounts of the Ser5P form of pol II and Spt5 at primer a over the promoter in both germinal center and ex vivo cells, whereas the promoter of the nontranscribed β-globin gene was negative for these two proteins. AID was not present in the off-target γ-actin and β-globin genes. Thus, there is good correlation between the Ser5P form of RNA pol II, Spt5, and AID proteins bound to V and I sequences after in vivo immunization. These proteins are totally absent in ex vivo–stimulated cells.

Initiating Pol II, Spt5, and AID are present in Sμ regions from both germinal center and ex vivo cells

Proteins bound to the Sμ region have been well-characterized in ex vivo–stimulated cells, but little is known about their participation in germinal center cells. Because the Sμ region undergoes somatic hypermutation after both types of activation, we determined if recruitment of transcription and AID proteins was identical by ChIP. As shown in Fig. 5 A, there is an intronic promoter located downstream of the Eμ enhancer that is constitutively activated and initiates transcription through the Sμ region. We used E, S, and C primers located before the promoter, in the 5’ Sμ region, and in Cμ, respectively, to measure proteins in germinal center– and ex vivo–activated cells from B1–8th Ung−/− mice. In Fig. 5 B, pol II was significantly higher in the V and I segments compared with the E segment (Fig. 3 C). A similar analysis of single-strand DNA from ex vivo–activated cells also revealed a higher frequency in V and I regions compared with E (unpublished data).
was significantly accumulated at the S primer, and it was the initiating form characterized by Ser5P. Furthermore, Spt5 and AID were significantly associated with the stalled polymerases in both germinal center and ex vivo cells. These results confirm previous reports of ex vivo transcription, which is defined by stalled polymerases (Rajagopal et al., 2009; Wang et al., 2009), Ser5P (Wang et al., 2009), Spt5 (Pavri et al., 2010), and AID (Pavri et al., 2010) and extend the findings to germinal center transcription. Because these proteins are downregulated in Eμ, the results suggest that they are brought in during de novo transcription from the intronic promoter and become amassed during the stalled migration of pol II through R-loop structures. All these markers of transcription and mutation are made manifest by inducing somatic hypermutation was significantly accumulated at the S primer, and it was the initiating form characterized by Ser5P. Furthermore, Spt5 and AID were significantly associated with the stalled polymerases in both germinal center and ex vivo cells. These results confirm previous reports of ex vivo transcription, which is defined by stalled polymerases (Rajagopal et al., 2009; Wang et al., 2009), Ser5P (Wang et al., 2009), Spt5 (Pavri et al., 2010), and AID (Pavri et al., 2010) and extend the findings to germinal center transcription. Because these proteins are downregulated in Eμ, the results suggest that they are brought in during de novo transcription from the intronic promoter and become amassed during the stalled migration of pol II through R-loop structures. All these markers of transcription and mutation are made manifest by inducing somatic hypermutation.

**Figure 4.** ChIP analyses of proteins bound to V region DNA from activated B cells. (A) Location of ChIP primer sets (red) in Igh (V, I, E, and C) and γ-actin (a, b, and c) are shown above the loci. Gray oval in the Igh locus represents the area of somatic hypermutation. (B–D) Ser5P (B), Spt5 (C), and AID (D) ChIPs from day 7 germinal center (red) and day 2 ex vivo (blue) activation. Error bars represent the SD of values from 9 independent experiments for B1-8h, and 6 experiments for 8D10-GL, with 1 mouse per experiment. For the Igh genes, P < 0.05 compared with values for C (unpaired two-tailed Student’s t test). For the γ-actin gene, results were averaged between B1-8h and 8D10-GL cells. *, P < 0.05 compared with c.

**Figure 5.** ChIP analysis and mutation frequency in the Sμ region from activated B cells. (A) Location of ChIP primer sets for E, S, and C (red) are shown above the loci. Double-sided arrow represents the region sequenced for mutations. Green arrow, intronic promoter. Gray oval represents the area of somatic hypermutation. (B) ChIP from day 7 germinal center (red) and day 2 ex vivo (blue) activation. Error bars represent the SD of values from 9 independent experiments for B1-8h with 1 mouse per experiment. *, P < 0.05 compared with values for C (unpaired two-tailed t test). (C and D) Hypermutation analysis in germinal center and ex vivo activated cells. Germinal center mutation frequencies (red) were measured in B1-8h Ung−/−, 8D10-GL, and B1-8h Aid−/− (3-4 independent experiments with 1 mouse per experiment). *, P < 10−4 compared with the B1-8h Aid−/− control. Ex vivo mutation frequencies (blue) were measured in B1-8h Ung−/− and B1-8h Aid−/− (3-4 independent experiments with 1 mouse per experiment). *, P < 10−4 compared with B1-8h Aid−/−.
in the Sµ region from germinal center cells (Fig. 5 C) and ex vivo cells (Fig. 5 D). Therefore, the Sµ region is accessible for mutation under both conditions of cell activation, perhaps because of the constitutively active intronic promoter and unique DNA sequence.

Deletion of the 3′ regulatory region (3′RR) reduces Pol II and ablates Spt5 and AID in Jµ 4 regions

The 3′RR on the heavy chain locus is implicated in controlling transcription, somatic hypermutation, and class switch recombination in mature B cells (Vincent-Fabert et al., 2010; Rouaud et al., 2013). To determine if it affects the recruitment of Spt5 and AID to the V region in germinal center cells, we examined mice which have a 30-kb deletion of the 3′RR (Vincent-Fabert et al., 2010). Mice were immunized with phycoerythrin for 7 d, and GL7+ B cells were isolated from spleens for ChIP assays. Because the mice contain a heterogeneous repertoire of VDJ rearrangements, we focused on the Jµ 4 intron, with the caveat that only 25% of the cells may contain rearrangements to Jµ 4. To see if transcription could be measured in these heterogeneous cells, RNA pol II ChIPs were performed using Jµ 4 and E primers (Fig. 6 A). The results in Fig. 6 B showed that in WT cells, pol II was reduced fourfold compared with similar regions in the knock-in B1-8 mice (Fig. 2 C), confirming that WT mice had fewer rearrangements to Jµ 4. In 3′RR cells, pol II molecules were drastically reduced 80% in both Jµ 4 and E regions, compared with WT cells. Pol II binding in the mutant mice was unaffected in primers located at the TATA box in the γ-actin gene, and the β-globin gene was a negative control. In accord with the ChIPs from knock-in mice, Spt5 was present in Jµ 4 from WT cells, albeit fourfold lower than from B1-8th cells, but not in the E region, suggesting that the initiating form of pol II is retained over Jµ 4. However, Spt5 was absent over Jµ 4 in 3′RR cells. As controls, Spt5 was bound to primers located in the promoter of the γ-actin gene from both types of mice. In confirmation of a previous study (Rouaud et al., 2013), the AID protein was also missing in the Jµ 4 region from 3′RR mice. Therefore, in the absence of the 3′RR, transcription was markedly reduced, and both Spt5 and AID proteins were not recruited to the Jµ 4 region.

DISCUSSION

V genes have long been enigmatic in efforts to understand how they are substrates for AID activity during transcription. In this first report of AID targeting in germinal center B cells, we examined features of transcription that are unique to V genes, which provide insight into their ability to sustain high levels of somatic hypermutation. Two distinct events occur after transcription.

First, RNA polymerases were paused for a long distance downstream of the TATA box. Using both nuclear run-on and ChIP techniques, pausing was detected over 1.2 kb encompassing the VDJ exon and Jµ 4 intron in B cells activated either ex vivo or in vivo. In contrast, there was no pausing in the transcribed γ-actin or CD19 genes (unpublished data) from these cells. Prolonged pausing in the V region could be induced during co-transcriptional splicing of the leader and V exons. For example, the transcriptional machinery may be sluggish because of multiple splicing reactions at the leader and rearranged V exons on the heavy and light chain loci, and slow-moving transcription bubbles would increase pol II density. It is notable that CTNNBL1 and PTBP2 factors associated with the spliceosome also bind to AID (Conticello et al., 2008; Nowak et al., 2011). Pol II accumulation could also be due to cis DNA sequences in the Jµ 4 introns, which are shared by all V gene rearrangements. Intron sequences have been suggested to form secondary structures during transcription (Golding et al., 1987; Wright et al., 2008), which may impact histone assembly and polymerase migration. Both these predictions can be tested by modifying DNA sequences of rearranged genes in cell lines to see how they affect pol II accumulation. Furthermore, when pol II progression was blocked in DT40 and Ramos cell lines, mutations were increased (Kodgire et al., 2013; Wang et al., 2014). In summary, pausing for more than 1 kb after the promoter is an intrinsic feature of rearranged V genes and can occur regardless of the type of cell stimulation. Paused polymerases then establish the boundary of mutation by exposing single-strand DNA as the substrate for AID in cells from either germinal centers or ex vivo culture. However, although pol II density and single-strand DNA are necessary for somatic hypermutation, they are not sufficient because
the V and I segments from ex vivo activation have virtually no mutations.

Second, although RNA pol II density profiles are similar between germinal center and ex vivo cells, the initiating form of pol II characterized by Ser5 phosphorylation in the C-terminal domain is retained only in germinal center cells. Typically, the conversion to transcriptional elongation occurs within 100 bp after the start of transcription, followed by a rapid loss of Ser5P (Brookes et al., 2012), which we detected in the control γ-actin gene. Thus, it is notable that the Ser5P form of pol II was elevated in the V and I primers located 0.6 and 1.2 kb, respectively, downstream from the TATA box preceding the VDJ gene. It is not known what causes the delay from initiation to elongation, but the process catalyzes the recruitment of Spt5 and AID to V genes. Our ChIP data conclusively show that Spt5 and AID are present at the V and I primers in germinal center cells but are totally absent in ex vivo cells. Thus, there is a strong correlation between Ser5P, Spt5, AID, and mutations, as summarized in Fig. 7. The data also indicate that pol II pausing occurs independently of Spt5 recruitment in ex vivo cells and may precede it in germinal center cells. We then examined whether the 3′ RR enhancers affect Spt5 and AID accumulation using mice with a 30-kb deletion of the region. Pol II density in germinal center cells was decreased 80% in the J14 and Ep regions, and Spt5 and AID were nonexistent, explaining the lack of somatic hypermutation in these mice (Rouaud et al., 2013). However, a definitive role for the 3′ RR is elusive because transcription was greatly reduced in the knockout mice. Perhaps sub-enhancers—such as h3a, h1l-2, h3b, and h4—within this region may be differentially used by WT cells during activation in vivo compared with ex vivo.

The other targets of somatic hypermutation are the S regions on the IgH locus. These regions are a sponge for AID due to the draconian R-loop structures which produce a pile-up of RNA pol II molecules. Wang et al. (2009) have reported that the Ser5P form is also enriched in S regions located 3 kb from transcription start sites in introns. This suggests that retention of the initiating form for long distances in the V and S regions is quite different from the promoter-proximal pausing described in other genes (Kwak and Lis, 2013). Paused initiating polymerases can then more efficiently recruit Spt5, AID, and other factors involved in hypermutation (Willmann et al., 2012; Aida et al., 2013; Singh et al., 2013).

Genome-wide studies of ex vivo–stimulated cells have shown that AID localization mirrors RNA pol II and Spt5 density (Pavri et al., 2010; Yamane et al., 2011). Because RNA pol II and Spt5 are present at most promoters, as many as 6,000 genes could be potential targets of AID (Yamane et al., 2011). We demonstrate here that transcribed V genes are not a target for Spt5 or AID in ex vivo cells and suggest that the number of potential targets may be very different in germinal center B cells. Furthermore, not all genes are mutated equally (Liu et al., 2008; Yamane et al., 2011), which indicates that additional factors regulate AID activity. For example, the mutated c-myc gene has a specific RNA pol II pause site in close proximity to the area of mutations (Krumm et al., 1992), and the murine Bkl6 gene has a significant number of nucleotide repeats near the site of mutations (Liu et al., 2008). Therefore, the sequences of these genes may cause transcriptional perturbation, followed by subsequent pol II accumulation and AID activity. In contrast, we show that the highly transcribed γ-actin gene has high levels of the Ser5P form of pol II and Spt5 protein at the promoter region, but no increased accumulation of pol II in the gene body and no detectable AID localization. Thus, a model evolves where AID may be present at open promoters, but the ability to access downstream DNA is regulated by the combined effects of RNA pol II density, DNA sequence, and AID cofactors.

What causes RNA pol II to retain its Ser5P initiating form for a long distance over the V gene and downstream intrinsic DNA is not known. Conversion to the elongating form in germinal center cells could be blocked by differential usage of transcription factors from either the enhancers or promoter after activation. It has been proposed that the same enhancer may have different factors bound at various times (Pinaud et al., 2011), and the same gene may be controlled by different enhancers during various activation states (Kieffer-Kwon et al., 2013). Another question is what role Spt5 is playing during V gene transcription. The protein is generally associated with the DSIF complex which coordinates pol II promoter release (Kwak and Lis, 2013), or it could potentially be taking a larger role in regulating V region transcription similar to recent results for the A20 gene (Diamant et al., 2012).

In summary, transcription sets up the V locus as a single-strand substrate by augmenting paused RNA polymerases, perhaps due to the DNA sequence of the region. Proteins associated with hypermutation, such as Spt5 and AID, are recruited to the initiating form of pol II specifically in cells...
activated in germinal centers. Differential activation programs in B cells induced in germinal centers versus ex vivo may produce an altered landscape of transcription factors that favors initiating polyornases and the cascade of mutation proteins.

MATERIALS AND METHODS

**Mice.** B1-8<sup>th</sup>-knock in mice (Shuh et al., 2002) were obtained from Rafael Casellas (National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health). B1-8<sup>th</sup>-10<sup>]−/−</sup> and B1-8<sup>th</sup>-Ad<sup>−/−</sup> mice were generated at the National Institute on Aging. 8D10-GL mice were made at Brandeis and Harvard Universities. 8D10-GL expresses the unmutated version of the mutated heavy chain (VGAM3.8 family VK7 germline gene rearranged to D and JH<sub>2</sub>) from 8D10, a hybridoma derived from the National Institutes of Health (NIH), National Institute on Aging. 8D10-GL mice were backcrossed onto the 129S6 background and were bred to EIIa-Cre <sup>(129S6) females (Taconic), and the F1 progeny were screened by Southern blot analysis.**

**Materials and Methods.** Gene rearrangement to D and JH<sub>2</sub>) from 8D10, a hybridoma derived from the National Institutes of Health. 8D10-GL mice were generated at the National Institute on Aging. 8D10-GL mice were backcrossed onto the 129S6 background and were bred to EIIa-Cre <sup>(129S6) females (Taconic), and the F1 progeny were screened by Southern blot analysis.**

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