Minireview

Secret sharers in the immune system: a novel RNA editing activity links switch recombination and somatic hypermutation

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Published: 13 October 2000

Genome ***Biology*** 2000, **1(4)**:reviews1025.1–1025.3

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2000/1/4/reviews/1025

Abstract

A new mechanism for regulation in the immune system has been identified: a cytidine deaminase is critical for both class switch recombination and somatic hypermutation, revealing an unanticipated link between these two processes.

Genetic tampering in the immune system

We have become accustomed to the notion that the immune system tampers with the genome. First, rearrangement of V,D, and J gene segments creates the antigen receptor variable regions. V(D)J recombination depends on the site-specific recombination proteins, RAG1 and RAG2, and occurs early in development of both B cells and T cells (reviewed in [1,2]). Subsequent encounter with antigen stimulates two additional and distinct processes that modify the genomes of B cells, but not T cells: class switch recombination and somatic hypermutation (see Figure 1). Switch recombination joins an expressed heavy chain variable (VDJ) region to a new downstream constant (C) region, changing the antigen clearance properties of an antibody without altering its specificity for antigen (reviewed in [3,4]). Switch recombination is a region-specific recombination process that deletes many kilobases of DNA and produces heterogeneous recombination junctions. Circles containing the deleted DNA can be found in B cells that have recently completed switch recombination, suggesting that regions targeted for recombination can synapse to form a recombination intermediate, which is then cleaved and religated. In contrast, somatic hypermutation is a targeted mutation process resulting in single nucleotide substitutions. These single base changes occur in an expressed heavy or light chain variable region, providing the genetic raw material for subsequent selection of clones producing high-affinity antibodies (reviewed in [5]). The currently prevailing model for hypermutation envisions an initial DNA break that is repaired by an error-prone polymerase, but no specific nuclease or polymerase has as yet been conclusively linked to somatic hypermutation.

Recent results, appearing in the September 1 issue of *Cell* [6,7], now show that the immune system's tampering is not limited to DNA, but that RNA editing is an additional tool in its bag of tricks. These exciting results have many implications. First, they identify a new mechanism by which the immune system alters nucleic acid sequence. Secondly, they add a new activity to a short (but clearly growing) list of examples of RNA editing enzymes in mammalian cells. Thirdly, they establish the cause of a human genetic disease, hyper-IgM syndrome (HIGM2). And finally, they point to a developmental and/or mechanistic link between switch recombination and somatic hypermutation.

A cytidine deaminase essential for both switch recombination and somatic hypermutation

The laboratories of Honjo [6] and Durandy [7] have identified a lymphocyte-specific cytidine deaminase which is required for B cells to carry out both class switch recombination and somatic hypermutation. The new activity is called 'activation-induced deaminase (AID)' because it is induced in B cells that have been activated to respond to antigen. The *AID* gene was identified as a candidate for function in class switch recombination by subtractive hybridization of mRNAs from a murine B cell lymphoma that enriched for transcripts displaying increased expression upon induction

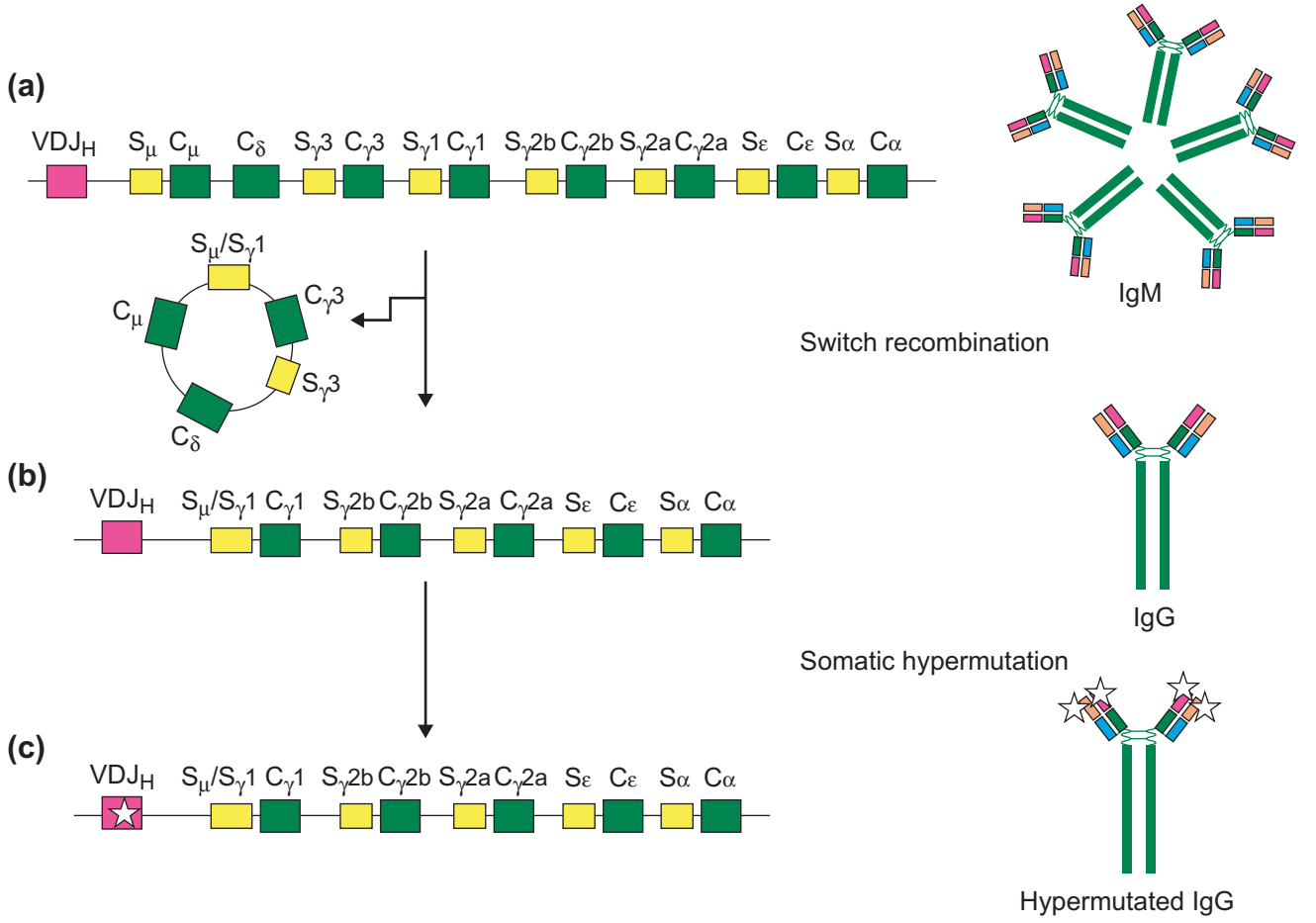

Figure 1
Switch recombination and somatic hypermutation at the immunoglobulin heavy chain locus. **(a)** The murine heavy chain locus (left) has undergone VDJ recombination and encodes a μ heavy chain. The resulting IgM antibodies (right) are pentamers of a dimer containing two heavy and two light chains. **(b)** Class switch recombination joins a new constant region to the expressed variable (VDJ) region, resulting in synthesis of antibody of a new class. Shown is switch recombination from Cμ to Cγ1, to produce a dimeric IgG1 antibody (right). **(c)** Somatic hypermutation modifies the variable region sequences of both heavy chains (left) and light chains. Following affinity selection, hypermutated antibodies (right) have increased affinity for antigen. Stars denote mutations in the DNA (left) and protein (right). Somatic hypermutation is shown following switch recombination, but neither process is prerequisite for the other (see text for further details). VDJ, heavy chain variable region; S, switch region; C, constant region.

of switch recombination [8]. Consistent with a specific function for AID in the immune system, further analysis showed that *AID* gene expression is induced where and when B cells are activated *in vivo*. The likely function of the AID protein was deduced by sequence comparison, and recombinant protein was shown to deaminate cytidine *in vitro*. The initial description of AID was of considerable interest, as only half a dozen mammalian RNA editing activities have been described. APOBEC-1, the first mammalian RNA editing activity to be described, changes a C to U in apolipoprotein B100 mRNA to create a premature stop codon, resulting in a truncated polypeptide of altered function [9]. There are also adenosine deaminase activities that alter specific mRNAs and tRNAs (reviewed in [10]). Phylogenetic analysis showed that AID is most closely related to the APOBEC enzyme family.

Impairment of AID has a profound effect on the immune response in mice and - more importantly - people. Using a genome-wide search of polymorphic microsatellite markers, the human HIGM2 susceptibility locus was first mapped to chromosome 12p13 [7]. As the murine *Aid* gene had been mapped to this interval [11], AID was tested as a candidate in a screen that identified 10 independent AID mutations in 12 different HIGM2 families [7]. Both people and mice lacking functional AID activity are characterized by the absence of switch recombination and somatic hypermutation, and an unusual lymphoid hyperplasia. In mammals, the lymphoid

tissues (spleen, lymph nodes and tonsils) provide specialized microenvironments in which B-cell activation occurs. Within these tissues, regions called germinal centers form within a few days after immunization and harbor cells carrying out switch recombination and somatic hypermutation (reviewed in [12]). In the absence of functional AID, germinal centers are not only evident but greatly enlarged, so proliferation signals appear to continue despite the block in successive developmental steps.

What is the target of the AID editing activity?

What specific target(s) of the AID editing activity would explain the profound effect of AID deficiency on both switch recombination and somatic hypermutation? It is at least a formal possibility that the target of AID is not a specific mRNA or tRNA, but rather the pools of nucleotide precursors. Two enzymes involved in purine metabolism, adenosine deaminase (ADA) and purine nucleotide phosphorylase (PNP), are essential for lymphocyte development at the stage of V(D)J recombination, and impairment of either activity in humans produces severe immundeficiency. But the fact that AID resembles the APOBEC family of site-specific mRNA editing enzymes would argue against this possibility. Another possibility is that AID regulates a critical developmental step; in this case, the target for modification could be an mRNA that encodes a key developmental protein. A third possibility, favored by Honjo and collaborators [8], is that the absence of both switch recombination and somatic hypermutation in the *Aid*$^{-/-}$ mice is due to the inability of the mice to produce a protein essential to the nitty gritty DNA mechanics of both processes.

Consistent with the notion that AID targets a developmental regulatory pathway, switch recombination and somatic hypermutation are temporally and spatially linked - these processes happen in quick succession upon B-cell activation, and occur in restricted lymphoid microenvironments. Moreover, neither process appears to be a prerequisite for the other: B cells can be identified in which somatic hypermutation but not switch recombination has occurred, and vice versa. It may seem unlikely that switch recombination and somatic hypermutation share important mechanistic components, because the genomic modifications these processes generate are superficially quite different. Nonetheless, close examination of the products of switch recombination and somatic hypermutation reveals certain similarities. Switch recombination is a process of regulated DNA deletion, and a limited amount of DNA deletion appears to accompany hypermutation [13,14]. Conversely, somatic hypermutation creates predominately single-nucleotide substitutions, and a limited amount of unfaithful DNA synthesis is evident in the proximity of switch junctions [15]. Moreover, at least one gene appears to participate in both processes. In *Msh2*$^{-/-}$ mice, which lack a key repair factor, both somatic hypermutation and switch recombination are diminished; switch junctions are not heterogeneous but clustered at hotspots; and the spectrum of hypermutation is also altered to increase the fraction of mutations that are at hotspots [16,17].

Questions abound. Is the target (or targets) of AID editing activity mRNA, tRNA, or even nucleotide pools? Does AID deficiency affect DNA recombination and/or repair directly, or indirectly by arresting B- or T-cell development? And, now that this new molecular trick is out of the bag, will other genes prove to be common to both switch recombination and somatic hypermutation?

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