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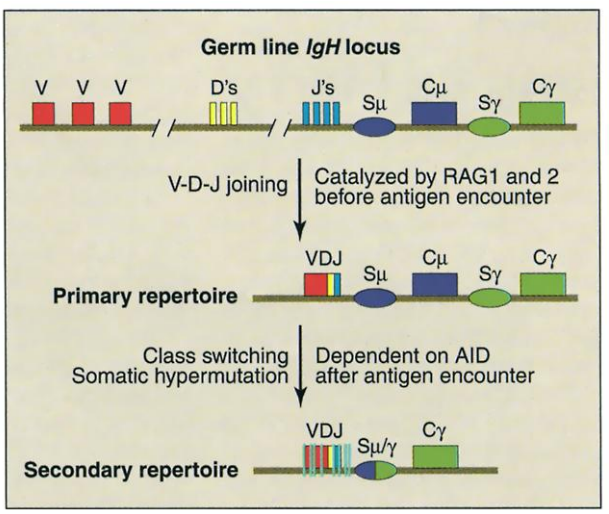
PERSPECTIVES: IMMUNOLOGY

RNA Editing AIDs Antibody Diversification?

Michael S. Neuberger and James Scott

The B lymphocytes of the immune system use multiple genetic mechanisms—gene rearrangement, somatic hypermutation, and gene conversion—to drive the generation of antibody diversity. Now, Honjo and colleagues (*1*) and Durandy and co-workers (*2*) provide evidence in a recent issue of *Cell* for an unexpected contribution to antibody gene diversification from RNA editing. The authors suggest that RNA editing is crucial for production of the secondary antibody repertoire in mature B cells.

Antigen-specific antibodies are formed in two stages (see the figure). In the first stage, a primary repertoire of immunoglobulin M (IgM) antibodies is produced in immature B lymphocytes present in the fetal liver or bone marrow. The diversity of antigen-binding sites on the antibodies expressed by these B cells is generated by gene rearrangement (see the figure). After antigen enters the body, a secondary repertoire is generated. Those B cells expressing an IgM molecule that recognizes the antigen are induced to proliferate and form germinal centers within secondary lymphoid organs. Here, two further assaults occur on the genetic integrity of antibody gene loci. The variable (V) regions (which encode the antigen-binding part of the IgM) are further diversified—this time by somatic hypermutation—allowing the generation of antibodies with improved affinity for antigen. The IgM constant region (responsible for effector activity of the antibody) is replaced by the constant region of another class of immunoglobulin (IgG, IgA, or IgE), a phenomenon termed class switching.



Generation of antibody repertoires. B lymphocytes developing in fetal liver or adult bone marrow use RAG1 and RAG2 proteins to rearrange their immunoglobulin V (variable), D (diversity), and J (joining) gene segments, producing a functionally integrated VDJ segment that is linked to the μ constant region (Cμ). This yields a primary antibody repertoire composed of IgM antibodies. Subsequent encounter with antigen causes those B cells expressing cognate IgM antibodies to proliferate, forming germinal centers in secondary lymphoid organs. Here, their rearranged immunoglobulin genes undergo class (isotype) switching and hypermutation, allowing the production of high-affinity IgG antibodies (the secondary repertoire). Class switching occurs by region-specific recombination between the switch (S) regions located upstream of Cμ and Cγ. Hypermutation introduces multiple single-nucleotide substitutions into a region of ~2 kilobases encompassing the rearranged VDJ. Deficiency in activation-induced deaminase (AID) abolishes the switching and hypermutation of the secondary repertoire.

The new work (*1*, *2*) reports that a deficiency in a single gene product, activation-induced deaminase (AID), is sufficient to obliterate generation of the secondary antibody repertoire in both human and mouse B cells. Both somatic hypermutation and class switching fail to take place, although lymphoid germinal centers are produced and are, indeed, even larger than normal.

Activation-induced deaminase was originally identified in Honjo's laboratory in a subtractive hybridization screen for genes that were activated upon the induction of class switching in a B lymphoma cell line (*3*). Examining the profiles of expressed genes revealed expression of the *AID* gene to be largely restricted to activated B cells. The sequence of the AID protein also provided a clue to its function: AID has homology to cytidine deaminases and, in particular, is closely related to Apobec-1. This protein is a catalytic component of the complex that edits apolipoprotein B messenger RNA (mRNA) and catalyzes the deamination of $C^{6666} \rightarrow U$, thereby generating a stop codon and caus-

M. S. Neuberger is at the Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK. J. Scott is in the Department of Medicine, Imperial College School of Medicine, London W12 0NN, UK.

ing production of a truncated apoB polypeptide by cells of the small intestine. It is likely that in vivo AID is an RNA-editing enzyme, although the possibility that it could act on DNA cannot be totally excluded.

How can AID be an essential component in both somatic hypermutation and antibody class switching, two processes that are quite distinct? In somatic hypermutation, multiple single-nucleotide substitutions are introduced into the rearranged V regions of antibody heavy and light chains. Class switching occurs by deletional recombination between sequences that flank the 5′-side of the constant regions and only takes place at the heavy-chain locus. Moreover, although both processes occur within the germinal center B cell population, each can take place independently of the other. Nevertheless, both hypermutation and class switching are linked to transcription, and the observation that the two processes are similarly affected by deficiency in the mismatch recognition protein Msh2 has led to the proposal that their mechanisms have aspects in common (*4*).

Thus, the fact that both hypermutation and switching are abolished by AID deficiency could reflect a requirement for AID-directed mRNA editing, allowing the production of polypeptides necessary for the two processes. Less conventional, but possibly more exciting, is the idea that AID-directed editing of immunoglobulin transcripts occurs while these transcripts are still attached to their genomic template, thereby providing an important signal in the switching and hypermutation processes. Thus, editing-induced mismatches could trigger the double-strand breaks necessary for switch recombination, and the error-prone repair for hypermutation. In such a scenario, the question would then arise as to how AID can be specific for its immunoglobulin RNA substrate. The answer might be provided by other proteins that associate with AID, assuming that, like Apobec-1, AID works as part of a multiprotein editing complex (*5, 6*). Although these speculations serve to highlight our lack of knowledge of the way in which AID deficiency abolishes production of the secondary antibody repertoire, it is evident that the identification of AID will have a major impact on future research into the mechanism of antibody diversification.

The close homology between AID and Apobec-1 suggests that they share a common evolutionary ancestor; indeed, the two genes are linked on human chromosome 12 (*7*). The dependence of somatic hypermutation on AID in both human and mouse suggests that an AID homolog is likely to be present in those lower vertebrates that use hypermutation to generate their antigen receptor repertoires. In that case, AID is likely to predate Apobec-1 and may well have provided a precursor from which Apobec-1 evolved.

Finally, it is striking that the inactivation of a single enzyme destroys generation of the secondary (but not primary) antibody repertoire in B cells, with little apparent effect on other cell lineages. AID may therefore prove to be an attractive drug target in the therapeutic modulation of antibody-dependent autoimmune diseases.

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NOTA BENE: BIOMEDICINE

Sleep, Eat, and Be Merry

Collapsing from muscle weakness upon hearing a funny joke is just one of the improbable symptoms afflicting the 1 in 2000 people who suffer from narcolepsy. Characteristics of this life-long disorder include not just the loss of muscle tone triggered by emotions (cataplexy), but also profound daytime sleepiness and disturbed rapid eye movement (REM) sleep—debilitating symptoms that are far from a laughing matter. Last year, exciting work in narcoleptic mice (*1*) and dogs (*2*) identified defective versions of hypocretin and its receptor—known regulators of food intake—as the cause of murine and canine narcolepsy, respectively. Now, Mignot's group (*3*) and Thannickal *et al.* (*4*) propose that decreased hypocretin production due to loss of hypocretin-secreting neurons in the hypothalamus could be the principal cause of human narcolepsy.

Since it was first described 120 years ago, human narcolepsy has been a puzzle. Unlike the canine disease, which is clearly inherited, most human cases are sporadic, hinting that a defective gene may not be the culprit. At least 85% of narcoleptic patients carry a major histocompatibility complex class II antigen HLA DQB1*0602 on their immune cells, implying that narcolepsy may be an autoimmune disease.

Earlier this year, the Mignot group reported undetectable levels of hypocretin in cerebrospinal fluid (CSF) from seven of nine narcoleptic patients, implicating a defect in hypocretin metabolism. In the new work (*3*), they looked for disease mutations in the genes encoding the two hypocretin receptors and the preprohypocretin precursor protein (from which hypocretin is derived) in 74 narcoleptic patients. They found a mutation in only one patient with an unusually severe form of narcolepsy. The single point mutation introduced an arginine into the signal peptide of preprohypocretin resulting in its aberrant processing in the endoplasmic reticulum and a drastic decrease in hypocretin secretion (no hypocretin was detected in this patient's CSF).

Then they searched for hypocretin-secreting cells in the lateral and posterior hypothalamus of postmortem narcoleptic and control brain tissue. They found no hypocretin mRNA expressed in the hypothalamus of narcoleptic brains, in contrast to the high levels expressed in control tissue. There was abundant expression of melanin concentrating hormone (MCH) mRNA in both narcoleptic and control hypothalamic tissue demonstrating that loss of hypocretin-secreting cells in narcoleptic tissue was selective. With immunohistochemistry, Thannickal *et al.* (*4*) confirmed that 85 to 95% of hypocretin-secreting neurons in postmortem narcoleptic hypothalamic tissue had disappeared, whereas the number of MCH neurons was normal.

Destruction of hypocretin-secreting cells could account for the majority of sporadic human narcolepsy cases. Given its late onset and possible autoimmune etiology, Mignot's group looked for evidence of immune cell activation in narcoleptic and normal brain tissue with the DQB1*0602 haplotype. They found no evidence of acute inflammation in the brain sections that they analyzed, in contrast to Thannickal *et al.* who discovered activated astrocytes in narcoleptic but not control hypothalamic tissue. The results leave open the possibility of autoimmune attack (perhaps triggered by an environmental toxin) resulting in the selective destruction of the 15,000 to 20,000 hypocretin-secreting cells in the hypothalamus. Loss of these cells could easily account for the symptoms of narcolepsy because their axons connect with monoaminergic neuronal clusters controlling wakefulness and muscle tone, and with cholinergic neurons controlling REM sleep.

The new work should accelerate clinical trials to test whether treatment with hypocretin or hypocretin receptor agonists will alleviate the distressing symptoms of narcolepsy.

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—ORLA SMITH