a result of increases in the densities of the atmosphere and neutral clouds due to a volcanic eruption, appears to be operating at Io. If accepted and verified, these observations provide an important piece of the puzzle in the continuing quest to untangle the web of activity that manifests itself on one of the most compelling objects in the solar system.

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IMMUNOLOGY

Reuse of B Lymphocytes in Germinal Centers

Yong-Jun Liu

The remarkably diverse immunoglobulins (Igs), which can exist either as secreted antibodies or as membrane-bound antigen receptors on B lymphocytes, can recognize a broad universe of foreign antigens. This is primarily because the gene coding for the Ig antigen-binding site is constructed by a combinatorial joining of several distinct germline gene segments—V, D, and J for the Ig heavy chain, and V and J for the light chain (*1*). V(D)J recombination is initiated by the proteins encoded by recombination-activating genes 1 and 2 (RAG1 and RAG2), which recognize the recombination signal sequences adjacent to each of the V, D, and J coding segments. These proteins generate double-stranded DNA breaks and hairpin structures. After deletion and addition of nucleotides at the coding ends, a DNA binding protein complex that contains Ku70, Ku80 subunits, RAG1, RAG2, and DNA ligase executes V(D)J joining (*2*). The expression of RAG1 and RAG2 is restricted to T and B cells. During early B cell development in fetal liver and bone marrow, RAG1 and RAG2 are induced by an unknown signal or signals at the pro- to pre–B cell stage and initiate Ig gene rearrangement. They are then down-regulated once developing B cells express surface IgM, to prevent expression of two or more antigen receptors (*3*).

It has been thought that B lymphocytes have only one chance to undergo a productive Ig rearrangement during their lifetime. But in fact RAG1 and RAG2 can be induced again in sIgM-positive immature B cells within bone marrow as a consequence of antigen receptor triggering (*4*). This leads to a secondary Ig gene rearrangement, allowing autoreactive B cells (otherwise destined to die) to replace their "bad" autoreactive antigen receptors with new antigen receptors and to become mature normal peripheral B cells.

Now a second exception is described in two reports on pages 298 and 301 of this issue (*5*, *6*). Secondary Ig rearrangement also occurs in germinal centers (GCs) during T cell–dependent antibody responses. In the GC, B cells further increase the diversity and improve the affinity of their antigen receptors by somatic hypermutation in rearranged IgV region genes (see the figure). The somatic mutants undergo affinity selection by antigens presented by follicular dendritic cells (FDCs). High-affinity Ig variants pick up antigen from FDCs, present the antigen to T cells in the GC, and differentiate into memory B cells and plasma cells. B cells with low-affinity Ig receptors fail to acquire sufficient antigen and die by apoptosis (*7*). The first hint that mature B cells may undergo secondary Ig gene rearrangement in GCs comes from the observation that GC B cells express RAG (*8*–*10*). The ability of mature B cells to reexpress RAG was further supported by the demonstration that lipopolysaccharide (LPS) and interleukin-4 (IL-4) are able to induce RAG expression in mature naïve B cells (*9*). Now, using a locus-specific, ligation-mediated polymerase chain reaction assay, Han *et al.* and Papavasiliou *et al.* detected J_κ breaks and new VJ_κ junctions in GC B cell populations, but not in naïve B cell populations from immunized mouse spleen. Signals such as LPS and IL-4 that are capable of inducing RAG expression in naïve B cells induce J_κ breaks and VJ_κ joints in these cells in culture (*5*, *6*).

Does secondary V(D)J recombination in activated mature B cells result in the replacement of "old" antigen receptors with "new" ones? Using an antibody that recognizes the idiotype produced by a unique VDJ region (the antigen-binding site), Nussenzweig and colleagues (*5*) showed that secondary Ig rearrangement within these mature B cells correlates with the disappearance of a preexisting idiotype on the cell surface. However, this does not occur in LPS- and IL-4–stimulated B cells whose Ku80 gene involved in V(D)J joining is deleted. These experiments suggest

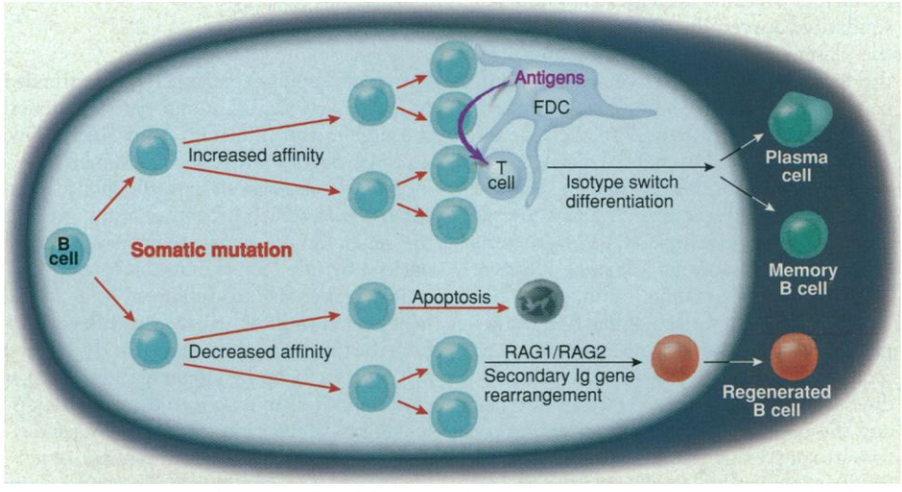

ILLUSTRATION: K. SUTLIFF

Action in the germinal center. The GC reaction is initiated by oligoclonal expansion of antigen-specific B cells. Somatic hypermutation occurs in rearranged Ig genes during expansion. Somatic mutation can increase the affinity of antigen receptors on some B cells. These B cells pick up antigen from FDCs, present it to GC T cells, and differentiate into memory B cells and plasma cells. Somatic mutation can also decrease the affinity of antigen receptors. These low-affinity B cells undergo either apoptosis or secondary Ig gene rearrangement by RAG1 and 2 to become new B cells.

The author is in the Department of Immunobiology, DNAX, Palo Alto, CA 97304-1104, USA. E-mail: liu@dnax.org

that the secondary Ig gene rearrangement in GC B cells results in the replacement of a preexisting IgV region.

Because the main function of GCs is to allow affinity maturation of the antibody response, it is hard to imagine how random V(D)J recombination can maintain or improve the antigen receptor specificity of GC B cells. Then what is the function of secondary Ig gene rearrangement in GCs? A clue comes from the observation of Kelsoe and colleagues (6) that RAG expression and secondary V(D)J recombination are enriched in $B220^{low}$ relative to $B220^{high}$ GC B cell populations. $B220^{high}$ GC B cells apparently express high-affinity antigen receptors, whereas $B220^{low}$ GC B cells express lower affinity antigen receptors. On the basis of this evidence, they propose that the absence of antigen binding by low-affinity GC B cells triggers RAG expression and secondary V(D)J recombination. In this way, secondary Ig gene rearrangement could rescue GC B cells from apoptosis by allowing them to express new antigen receptors, whereas GC B cells with high-affinity antigen receptors would not be affected. One problem with this hypothesis is that because the antigens held on FDCs are believed to be the primary survival signal for GC B cells (7), it is difficult to explain how random V(D)J recombination can generate high-affinity antigen receptors, allowing GC B cells to bind antigens on FDCs.

In conclusion, the new work clearly demonstrates that mature B cells undergo secondary Ig gene rearrangement in GCs during T cell–dependent immune responses. But we still do not know to what extent secondary Ig gene rearrangement actually contributes to the establishment of the peripheral B cell repertoire. The regulation of RAG expression and V(D)J recombination during primary B lymphopoiesis is largely unknown. Because the molecular controls of the primary and the secondary V(D)J recombination may be similar, the ability to induce V(D)J recombination in GCs and in cell culture may provide insights into the signaling cascade regulating primary V(D)J recombination.

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NEUROSCIENCE

Good Pain, Bad Pain

Michael J. Iadarola and Robert M. Caudle

Pain is never a good thing, right? With this view, investigators in the field of pain research have concentrated on mapping pain pathways and pharmacologically or surgically eliminating pain altogether. But pain also has an important protective function in the preservation of the organism. The problems arise when this "good" pain turns into the "bad" pain that occurs in pathophysiological conditions such as nerve injury, causing debilitating disease. Two reports on pages 275 and 279 of this issue provide significant advances in understanding and counteracting this process (*1*, *2*). Mantyh and colleagues use a ligand-toxin conjugate to specifically kill a population of pain-sensing neurons in the spinal cord, an approach conceptually similar to the use of immunotoxins to kill cancer cells (*3*). Malmberg *et al.* examine nociceptive processes in mice lacking the neuron-specific γ isoform of protein kinase C (PKC). In both cases, only pathological (bad) pain is reduced, leaving good pain functioning.

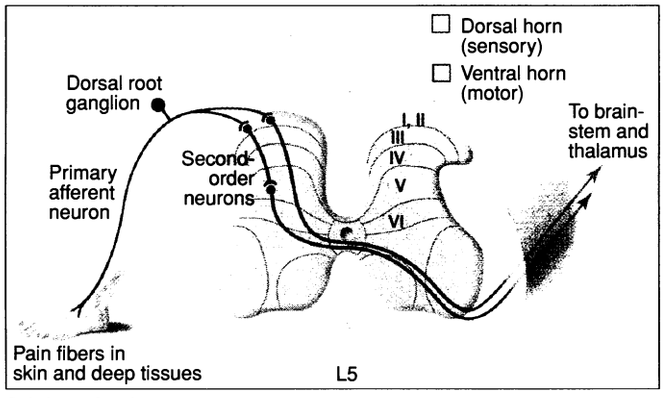

ILLUSTRATION: K. SUTLIFF

Origins of pain.

A striking feature of postinjury and neuropathic pain is that somatosensory signals get mixed up. A normally pleasant light brush on the skin can produce excruciating pain in patients with neuropathic conditions. This painful response to a normally nonpainful stimulus is called allodynia. Furthermore, in these patients the sensations from a mildly painful stimulus are greatly exaggerated (hyperalgesia). Anyone who has been sunburned and steps into a warm shower has an inkling of what this is like.

This hypersensitivity usually serves a protective function after injuries—we don't use a broken arm, and our pain-sensing neural circuits protest vigorously if we try—but usually resolves as the injury heals. However in neuropathic pain disorders, allodynia, hyperalgesia, and spontaneous pain are constant features of life, and this bad pain is often very difficult to treat effectively.

What is less appreciable through personal experience is that persistent pain triggers intense alterations in neuronal gene expression at the very first synaptic processing station for pain: the spinal cord dorsal horn (see the figure). Persistent pain induces changes in neural plasticity at the most fundamental molecular level, altering genes involved in transcriptional control (*c-fos*, *c-jun*, *NGF-IA*) and genes that encode neuropeptides (dynorphin, enkephalin, substance P, NPY) and their receptors (*4*). While a simple pinprick does not induce these changes, 5 or 10 minutes of persistent pain does, and these increases parallel the time course of the peripheral inflammation or pathophysiology. Thus, a transcriptional network is activated in these pain-processing neurons and, if the stimulus is of a sufficient degree and duration, a network of target genes even further downstream is also activated. There are still unknowns about the signal-transduction processes and second-messenger cascades activated by pain in the cytoplasm of these

The authors are in the Pain and Neurosensory Mechanisms Branch, National Institute of Dental Research, NIH, Bethesda, MD 20892-4410, USA. E-mail: iadarola@yoda.nidr.nih.gov