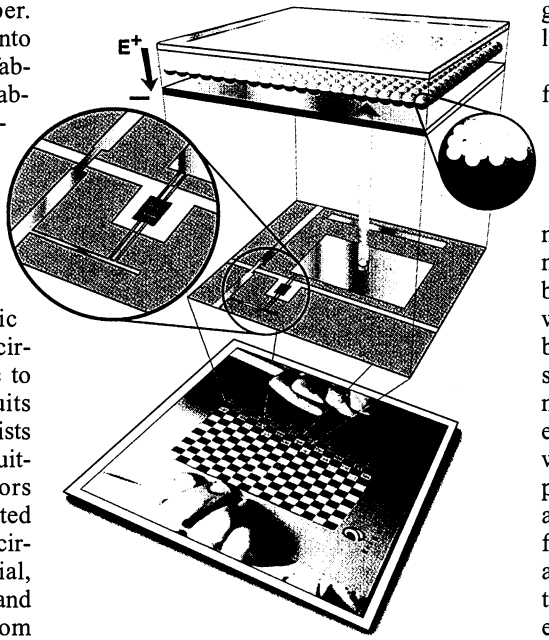
seem to be useful for electronic paper. They have recently been incorporated into small laboratory demonstrator circuits fabricated on plastic (*5*, *6*) with low-cost fabrication methods, such as ink-jet printing and a high-resolution form of rubber stamping known as microcontact printing (*7*). A photochemical patterning process has also been developed for devices that use certain types of photosensitive plastics (*8*).

Our recent work combines organic semiconductors with rubber-stamped circuit elements on thin sheets of plastic to produce high-quality, large-area circuits for displays (*9*). A typical system consists of a square array of several hundred suitably interconnected organic transistors with micrometer feature sizes distributed over areas of 6 inches by 6 inches. The circuits incorporate five layers of material, patterned in registry with one another and processed entirely outside a clean-room environment. The compatibility of the stamping method with high-speed, continuous reel-to-reel printing approaches, the large area coverage, and the good performance of the transistors are all important features of these flexible circuits.

The figure shows a photograph of the Bell Labs electronic paper display and an artist's impression of the different components of this system. It uses rubber-stamped plastic circuits and microencapsulated electrophoretic inks. The entire device is less than 1 mm thick and weighs about 20% as much as a liquid crystal display of similar size. The exploded view (not to scale) illustrates the layout of a unit cell. Each pixel is associated with an organic transistor that acts as a voltage-regulated switch to control the color of the ink.



The nuts and bolts of electronic paper. The exploded view shows the elements in a unit cell (not to scale). Arrays of rubber-stamped plastic transistors (inset on the left; blue, organic semiconductor; gold, source/drain electrodes; gray, gate electrode) control the color of a layer of microencapsulated electronic ink (inset on the right).

The ability of the stamping method to form micrometer-sized features on plastic substrates is critically important for this circuit. It enables the transistors to achieve the necessary switching speed, even with semiconductors that have modest electrical performance. Furthermore, it allows the same circuit design to be extended to high-resolution displays with large numbers of pixels. This scalability and the gradual emergence of other suitable materials and processing techniques point to a bright future for flexible electronic systems. These technologies will enable not only electronic paper displays and other applications that we can anticipate today (such as low-cost identification tags) but also completely new and unexpected devices that will change the way that we think about consumer electronics.

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PERSPECTIVES: IMMUNOLOGY

B Cell Receptor Rehabilitation—Pausing to Reflect

Leslie B. King and John G. Monroe

One of the most crucial steps during B cell development in the bone marrow is the expression of the B cell receptor for antigen (BCR). The BCR, a cell surface form of antibody, is responsible for initiating the B cell response to pathogens and other antigens. Unique antigen-specific antibodies are expressed by individual B cells after the developmentally regulated rearrangement of gene segments within the heavy and light chain loci of antibody genes. The random nature of the rearrangement, coupled with the complexity contributed by heavy and light chain pairing, creates an incredibly diverse antibody repertoire. Although such diversity allows for the immune recognition of a vast array of foreign pathogens, it concomitantly yields B cells that are potentially self-reactive. To avoid autoimmune responses elicited by activation of these cells, the immune system has two ways in which it induces tolerance to self antigens: physical elimination of self-reactive cells through apoptosis (clonal deletion) or impairment of their activity (clonal anergy). More recently, receptor editing, during which there is a secondary rearrangement of the antibody light chain locus, has been proposed as another way to eliminate autoreactive B cells (*1*–*3*). On page 1541 of this issue, Casellas *et al.* (*4*) now report that receptor editing is likely to be a major force in shaping the B cell antibody repertoire (see the figure). They show that receptor editing takes place during a 2-hour delay in B cell development and that at least 25% of pre-B cells end up expressing edited versions of their originally self-reactive antibodies.

Receptor editing provides a simple way for autoreactive B cells that have received a verdict of either death (deletion) or life imprisonment (anergy) following encounter with antigen to commute their sentence by producing a rehabilitated nonautoreactive BCR. Such a scenario is supported by a number of studies in transgenic mice. In these studies, transgenic mice were engineered so that pre-rearranged antibody heavy and light chain antigen-combining regions encoding autoreactive BCRs were "knocked in" (inserted by homologous recombination) to their respective endogenous loci, preserving the potential for secondary rearrangements (*5*, *6*). In these transgenic animals, autoreactive BCRs were edited to a much greater extent than their more innocuous counterparts. However, the involvement of

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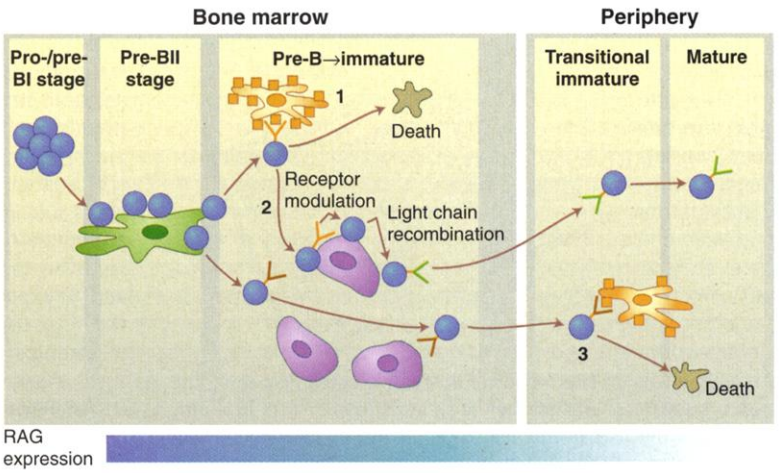
receptor editing in eliminating autoreactive B cells in nontransgenic mice, which have a more variable antigenic repertoire, remains somewhat controversial. Initial attempts to assess the frequency of normal B cells that have undergone receptor editing depended on the determination of the proportion of mature B cells expressing λ light chains that had also rearranged their κ locus (*7*).

Casellas and colleagues now take these studies a step further by engineering double "knock-in" mice. In these animals, one antibody κ light chain locus carries a pre-rearranged antigen-combining region capable of undergoing secondary rearrangements and the other locus contains a polymorphism that facilitates detection of B cells that have undergone receptor editing. In these studies, the normal B cell repertoire is more closely approximated than in previous studies that used pre-rearranged heavy and/or light chains from autoreactive BCRs. The reason for this is that the pre-rearranged antibody light chain is innocuous and is allowed to pair with endogenous antibody heavy chains. Furthermore, B cells that have undergone secondary rearrangements can be identified immediately by the characteristics of the antibody that they express. Using several different pre-rearranged innocuous and potentially autoreactive light chain "knock-ins," the authors determined that about 25% of developing B cells had undergone secondary light chain rearrangements. This suggests that receptor editing can dramatically influence the antibody repertoire of B cells.



Eliminating unwanted B cells. Pre-B cells (blue circles) develop in the bone marrow and after further maturation emigrate into the periphery as transitional immature B cells. Along the way, their expression of RAG1 and RAG2—proteins necessary for antibody gene recombination—gradually decreases. This process is interrupted by encounter with antigen (orange squares). Antigen receptor signals generated by this encounter trigger either (**1**) cell death (deletion) or (**2**) a delay in the differentiation of pre-B cells into immature B cells. The consequences of a developmental delay are modulation of the antigen-reactive receptor and the prolonging of antibody light chain recombination. If cells that are able to generate a new BCR through receptor editing (orange and green antibody) fail to recognize antigen, they then proceed through the remaining stages of B cell development. The decision to die or to pause during development may be determined by localization to a bone marrow niche (pink cells) that protects the pre-B/immature B cell from BCR-induced apoptosis. This protection allows the continuation of light chain recombination despite the induction of proapoptotic pathways triggered by BCR engagement with antigen. The absence of these protective cells in the periphery predicts that antigen recognition by transitional immature B cells leads only to cell death (**3**). Thus, the outcome of antigen recognition by developing B cells depends both on slowing maturation at a stage where RAG expression is sufficient to allow continued recombination and on the microenvironmental niche where antigen recognition occurs.

Although together these studies suggest that antigen is important for promoting receptor editing, it has been difficult to ascertain whether receptor editing is induced by encounter with autoantigen or instead results from random recombination events followed by extensive selection. To further address this issue, Casellas and co-workers undertook a side-by-side comparison of developing B cells that continued to express the pre-rearranged antibody light chain (and therefore were not undergoing receptor editing) with those in which receptor editing was taking place. They found that cells undergoing receptor editing spent longer in the pre-BII stage of development than those that had not undergone receptor editing. These data argue that editing is not the result of random rearrangements at the very early pro-B cell stage followed by antigen-driven selection later in development. Together with the "knock-in" experiments, these findings imply instead that antigenic encounter promotes receptor editing at a relatively late developmental stage in the bone marrow.

In most current models of receptor editing, antigen-reactive immature B cells are envisioned to re-express *RAG*—the recombination activating genes that are necessary for antibody gene rearrangement. This results in the reinitiation of light chain recombination after interaction with self antigens (*8–10*). Paradoxically, immature B cells are extremely susceptible to BCR-induced apoptosis initiated by antigen binding (*11, 12*). This begs the question: How can immature B cells initiate receptor editing while they are simultaneously receiving an execution signal? Two models have been proposed to resolve this conundrum. The first model suggests that the BCR-induced response of immature B cells is developmentally regulated. Newly emerging immature B cells are specifically induced to undergo receptor editing after BCR engagement with antigen, whereas later stage immature B cells undergo apoptosis (*9*). In a second model, all immature B cells are sensitive to BCR-induced apoptosis, but survival signals provided by specialized bone marrow cells provide them with a temporary stay of execution, giving them a second chance to produce a nonautoreactive BCR (*13*). Common to both models is the premise that the editing process is inducible.

In contrast to these inductive models for secondary light chain rearrangement, the Casellas data suggest that encounter with antigen immediately after the initial deposition of BCR on the cell surface leads to developmental arrest in the pre-BII compartment where RAG levels remain sufficient to promote continued light chain rearrangement. This model represents a new outlook because interaction with self antigens would maintain (rather than induce) competency to undergo continued light chain rearrangement. Although this concept is not new, analysis of developmental arrest in an earlier study was complicated by the use of a pre-rearranged antibody heavy chain capable of driving premature B cell development (*5*). The operational difficulty in distinguishing immature B cells that have modulated their BCR after antigenic encounter from pre-BII cells that do not yet express BCR makes the distinction appear artificial, but the implications of each model are quite different. In the inductive model, reinitiation of light chain rearrangement in immature B cells after antigenic encounter implies that BCR signaling is crucial for rendering cells competent to undergo secondary light chain rearrangements. In the Casellas model, BCR signaling induces developmental arrest at a rearrangement-

competent developmental stage, but has no direct effect on RAG expression or antibody gene rearrangement.

Most studies designed to assess the significance of receptor editing are predicated on elimination of autoreactivity as a major impetus; nonetheless, one can easily envision other situations in which continued light chain rearrangement would be beneficial. If receptor editing is not directly induced by BCR signaling but instead leads to developmental arrest in the rearrangement-competent pre-BII stage, then any situation that results in arrest at this stage may enable continued antibody light chain rearrangement. Because antibody gene rearrangement is an error-prone, inefficient process that often generates antibody products that are out-of-frame or incapable of forming heavy chain–light chain pairs, a large number of candidate B cells fail to express a functional BCR. The inability to express a functional BCR would block B cell development at the pre-BII stage, potentially allowing these cells to generate another antibody light chain before undergoing "death by neglect."

Finally, if the goal of receptor editing is to promote the generation of nonautoreactive BCRs, how does a cell know when this has been successfully accomplished? One possibility is that light chain rearrangement continues in pre-BII cells until a signal provided by cell surface expression of a functional BCR that displays little or no reactivity to self antigens promotes maturation into bona fide immature B cells, with a consequent down-regulation of *RAG* (*14, 15*) and termination of further receptor editing. If a pre-BII cell is unsuccessful in generating such a BCR, it may very well continue to undergo receptor editing until it draws its last breath. The recent identification of a protective niche in the bone marrow where the BCR-induced apoptotic response of immature B cells is blocked (*13*) suggests that the local microenvironment in which an autoreactive immature B cell first encounters antigen may also play an important part in determining its fate. An immature B cell outside the protective niche would undergo rapid apoptosis, whereas one inside the niche would have time to generate a nonautoreactive BCR by receptor editing. It is yet to be determined whether immature B cells that have functionally interacted with self antigens are drawn to the protective niche, or whether only those cells that are lucky enough to be in close proximity to the protective niche can be rescued. In either case, it is tempting to speculate that signals produced by a functional, nonautoreactive BCR serve as the impetus for the rehabilitated cell to leave its nurturing microenvironment and to make its own way in the world.

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PERSPECTIVES: EVOLUTION

A Horn for an Eye

Paul H. Harvey and Charles J. Godfray

Despite his encyclopedic knowledge of natural history, Charles Darwin was puzzled by dung beetles. The males of many dung beetle species have elaborate horns, and Darwin's first thought was that these horns had evolved by sexual selection to make males more efficient in competing with other males for mates. But what confused Darwin was that the size and location of the horns varied—in some cases they were on the front of the head, in others on the thorax. Emlen's study of *Onthophagus* dung beetles (*1*) on page 1534 of this issue provides an elegant solution to Darwin's dilemma. Emlen discovered that possessing a pair of extravagant horns involves a cost—a reduction in the size of nearby organs, such as the wings, antennae, or eyes. The need for well-developed eyes versus well-developed antennae, or wings differs depending on the life history of the beetle species. Thus, the position of the horns is determined by the organ that a beetle species needs the least.

There are two types of sexual selection: The first is fighting (and other direct interactions) between males, and the second is the effect of female choice. Frequently, males fight for mating access to females and so have developed associated weaponry—the horns of beetles, the antlers of deer—to improve their chances. Alternatively, females may choose their mate according to an evolved preference—the peacock's iridescent tail is the classic case.

Darwin essentially held our modern view of how competition among males leads to the evolution of structures such as horns and antlers. However, he failed to solve the problem of how female choice could give rise to structures such as the peacock's tail, calling them ornaments and invoking innate aesthetic female preferences as the driving force. The horns of *Onthophagus* male beetles are extraordinarily variable in their size, shape, and location on the beetle's body (see the figure). Owing to these observations and the fact that Darwin could find no evidence that dung beetle horns were used in combat, he concluded that "they have been acquired as ornaments." This conclusion "is that which best agrees with the fact of their having been so immensely, yet not fixedly developed, as shewn by their extreme variability in the same species, and by their extreme diversity in closely allied

Horn of plenty. The Australian dung beetle *Onthophagus neostenocerus* (**left**) and the Central American dung beetle *Onthophagus crinitis panamensis* (**right**). The position of horns on the beetle's body influences the size of nearby organs such as the wings or eyes.

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