ciable. The figure provides visual evidence. Supplies of H_2S are often so large that reactive metals cannot consume it all. The excess is available to react with any organic molecules that might be present. Carotenoid pigments, which are rich in double bonds, would be particularly reactive. And here is the difficulty. The range of reduction products found in sediments is huge. One can look at it and say "it's a mess, it must be abiotic." But that does not eliminate the possibility of varying degrees of microbial alteration.

Hebting *et al.* work closely with natural-products organic chemists. This expertise has helped them to separate the components of the "mess." Using a formidable array of spectroscopic techniques, they have elucidated molecular structures. Wherever it has been possible to pin down the stereochemistry at chiral centers, the results show that the hydrogenations were not stereoselective and, therefore, not microbially catalyzed.

Must the conventional wisdom be revised, at least for hydrogenations? Probably. The proven microbial hydrogenations are quite specific, whereas the pathway now demonstrated is general. And practically every aerobic-anaerobic boundary includes a sulfide-rich zone. In fact, the present group was one of those (*8*, *9*) who showed earlier that formation of sulfide bridges—a sort of natural vulcanization—is an important process by which reactive organic molecules are immobilized by attachment to insoluble macromolecules in sediments.

A second phase of the present work is therefore important. In laboratory experiments, Hebting *et al.* (*1*) have demonstrated hydrogenation of double bonds in carotenoids and steroidal ketones incubated at 50° to 90°C in saturated solutions of H_2S. Yields are low, but, as the authors say, the results, and observation of the expected intermediates in some natural sediments, support a hypothetical, two-step process: addition of H_2S to a polyene to yield an allylic thiol, followed by reduction of the thiol. If a double bond is conjugated with a carbonyl group rather than another olefinic bond, initial sulfurization is apparently not required, and a different mechanism applies.

Without doubt, this report erects a new, and highly significant, milepost on the pathway of carbon in nature.

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IMMUNOLOGY

Discriminating Microbe from Self Suffers a Double Toll

The autoimmune disease lupus is caused by immune cells that secrete antibodies to the patient's own antigens. A mouse version of the disease reveals two genes that lead to this disregulation.

Christopher C. Goodnow

Sensing microbial infection and countering it with a vigorous immune reaction is a double-edged challenge facing all multicellular organisms. Mobilizing a swift immune response to viruses and bacteria is often a critical survival advantage. However, many microbial components are chemically similar to constituents of our own cells, and failure to tell the two apart leads to devastating autoimmune reactions against self. The fine line that the immune system treads between antimicrobial immunity and autoimmunity is highlighted in reports by Pisitkun *et al.* on page 1669 of this issue (*1*) and by Kumar *et al*. on page 1665 (*2*). The groups have identified alterations in two mouse genes that illuminate the pathogenesis of a particularly sinister group of autoimmune diseases affecting 46 out of 100,000 people: systemic lupus erythematosus, Sjogren's syndrome, scleroderma, and polymyositis, where antibodies are secreted against DNA, RNA, and the associated proteins of our own cells.

Why chromatin self components—nucleic acids and associated proteins in the cell nucleus—so frequently recur as targets of systemic autoimmunity has been a long-standing mystery. The findings by Pisitkun *et al*. cap off an impressive body of evidence for "the Toll hypothesis" (*3*–*7*) that these autoimmune diseases stem from imperfect discrimination of microbe from self by an ancient receptor system for detecting microbial DNA and RNA. In plants and animals, a diverse set of leucine-rich repeat receptors sense unique chemical constituents (antigens) of microbes and trigger innate immune reactions to counter these infections (*8*–*10*). The Toll-like receptor (TLR) subfamily of transmembrane proteins is especially crucial in invertebrates and vertebrates.

TLR7 and TLR9 highlight the fine line distinguishing microbial and self-constituents, because they sense nucleic acids that are common to all living things. TLR9 best triggers immune reactions in the presence of bacterial DNA sequences with multiple CpG nucleotides. On a molar basis, mammalian DNA is a much less potent stimulator of TLR9 because it contains fewer such motifs, and these are mostly "masked" by methylation (*10*). TLR7 also shows preference for single-stranded viral genome RNA that is rich in G/U nucleotides and lacks mammalian-specific methylation and other modifications (*11*). A second level of specificity for the nucleic acid–sensing Toll-like receptors is their residence within intracellular endosomal vesicles, and dependence upon endosomal acidification to trigger immune responses (*10*, *12*). Many viruses and bacteria use receptor-mediated trafficking to acidified endosomes as a route to invade cells. In the small volume of an endosome, the single-stranded RNA genome contained in a single virion can release micromolar concentrations of TLR7 ligands. In contrast, self nucleic acids have limited access to this compartment because they are rapidly degraded when released into the extracellular fluid. Nevertheless, mammalian DNA and RNA can become potent stimulators of TLR9 and TLR7, respectively, if they are captured by receptors on the surface of immune cells (such as B cell receptors; see the figure) and concentrated in endosomes (*3*, *4*, *13*).

Plants and invertebrates display a modest repertoire of innate immune responses, and this probably limits the damage when immune reactions are inadvertently triggered by self. Vertebrates, however, have evolved adaptive immune responses that exploit the immunoglobulin domain as a platform to build an infinitely diverse array of microbial receptors—antibodies and T cell receptors. Through a combination of

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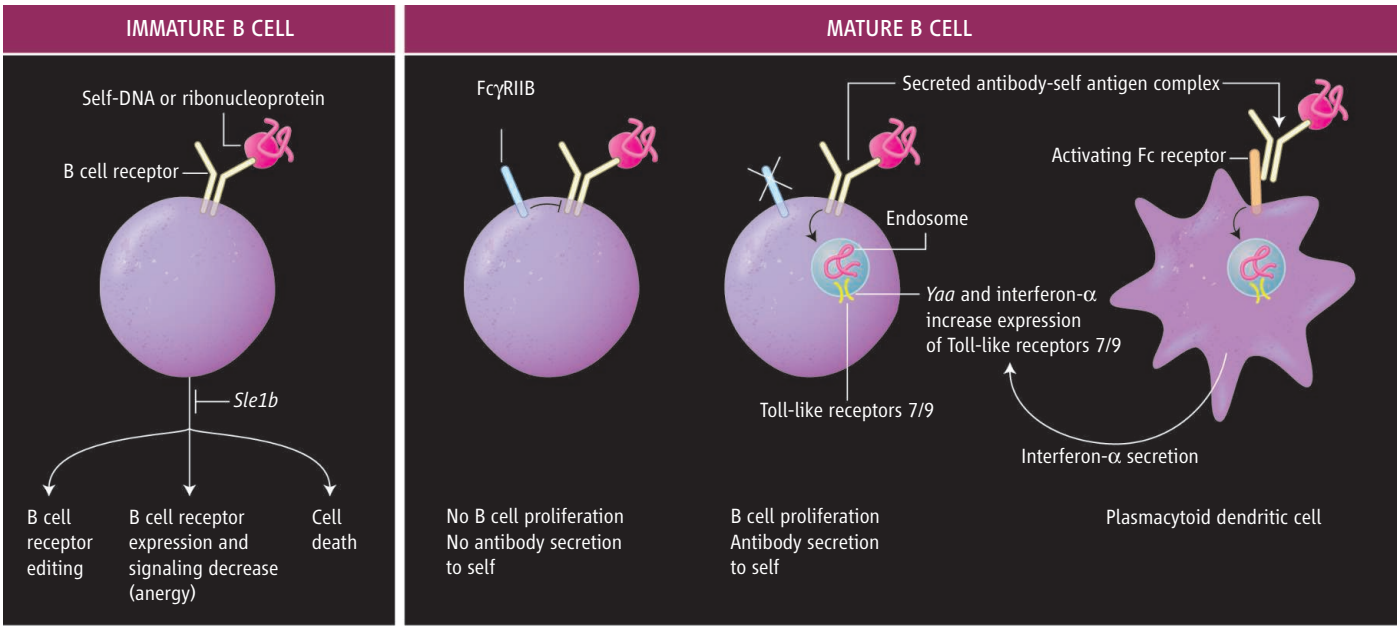

Pathogenesis of lupus autoantibodies. (**Left**) Binding of self-DNA or ribonucleoproteins to B cell receptors on an immature B cell transmits signals that edit the receptor genes and inhibit B cell survival, proliferation, and maturation. These signals are diminished by the *Sle1b* variant of the gene *Ly108*. (**Right**) In mature B cells, the inhibitory receptor (FcγRIIB) normally opposes signals for proliferation that are generated when the B cell receptor binds antigen. FcγRIIB deficiency, coupled with failure to eliminate immature self-reactive B cells due to *Sle1b*, allows self-reactive B cell receptors in mature B cells to signal proliferation and to capture nucleic acids and associated proteins, delivering them to TLR7 or TLR9 in endosomes. This promotes self-reactive B cell proliferation and antibody secretion. *Yaa* duplication of the *TLR7* gene and increased expression of *TLR7* by interferon-α that is secreted from dendritic cells increase this effect. Secreted antibodies complexed with self-DNA or ribonucleoproteins trigger TLR7- or TLR9-expressing dendritic cells to release more interferon-α, creating a potential positive-feedback loop.

somatic gene conversion, DNA rearrangements, and somatic hypermutation of immunoglobulin variable domains in B cells, the mammalian adaptive immune response tailors antibodies with extraordinary affinity and—under normal conditions—exquisite specificity against microbial and not self-antigens.

As indicated in the current studies (*1*, *2*), lupus autoimmunity arises from a conjunction between imperfect self-microbe discrimination by the Toll-like receptor and the antibody systems. When antibodies are formed in immature B cells by random somatic immunoglobulin-gene rearrangement, many are polyreactive and bind charged microbial and self-components—notably DNA, RNA, and their associated highly charged proteins (*14*). These self-binding antibodies are selected against by an ordered series of checkpoints that allow only B cells displaying (as a membrane-bound receptor) nonself-reactive antibodies to proliferate and mature into antibody-secreting cells (*15*). Binding of a self-antigen, such as ribonucleoprotein or chromatin, transmits intracellular selection signals that cause these cells either to die, "edit" the B cell receptor with a second round of gene rearrangement, or become weak (clonal anergy) such that their potential to proliferate decreases (see the figure). Cells that pass these checkpoints can be activated into cell division and antibody secretion when their B cell receptor binds a microbial antigen in lymph nodes or spleen.

The work by Kumar *et al*. culminates a two-decade effort tracking down the many chromosomal loci contributing to inherited lupus in certain mouse strains. One gene locus, *Sle1b*, has been isolated to a small region of chromosome 1 containing the SLAM family of lymphocyte adhesion receptors. Kumar *et al*. show that inherited differences in splicing of messenger RNA encoding one family member, *Ly108*, are sufficient to alter signaling by the B cell receptor. The product of the lupus-susceptible allele lowers the ability of self-reactive B cell receptors to signal clonal anergy and editing, thus increasing the number of self-reactive B cells in the lymph nodes and spleen.

The findings by Pisitkun *et al*. complete an equally long genetic detective story tracking lupus susceptibility in mice to a Y chromosome–linked "autoimmune accelerator" locus dubbed *Yaa*. Like many human and animal quantitative trait loci, the phenotypic effect of *Yaa* is graded and either suppressed or enhanced through complex interactions with other genes. For example, the combination of *Yaa* with *Sle1b* or with a deficiency in a second lupus susceptibility gene, *FcγRIIB* [encoding a B cell receptor for immunoglobulin G (IgG)] dramatically increases the onset and severity of lupus in an inbred strain of mice (C57BL/6) (*16*, *17*). Even though there are few genes on the Y chromosome, the nature of the *Yaa* gene has remained obscure. Pisitkun *et al*. show that *Yaa* is not due to a mutation in a normal Y-chromosome gene but rather is due to a duplication of a segment of X-chromosomal DNA that has been transposed to the Y. This effectively doubles the *TLR7* gene dosage and the responsiveness of B cells to TLR7 ligands. In conjunction with the lupus allele of *FcγRIIB*, this increases the incidence and severity of autoimmunity and redirects it from DNA to an RNA-associated pattern of autoantibodies. From a genetic perspective, the finding has broad significance because variation in copy number is emerging as a major potential source of inherited variation: Almost 4% of the human genome comprises recently duplicated segments, and a substantial fraction is transposed to other chromosomes (*18*).

Elucidation of *Yaa* and its interaction with *Sle1b* and *FcγRIIB*, coupled with evidence that TLR7 and TLR9 signaling deficiency prevents formation of lupus autoantibodies in mice (*3*–*7*), provides a compelling explanation for the pathogenesis of lupus. Breakdown of self-tolerance develops by a succession of defects in the checkpoints that normally control self-reactive cells, much like cancer arises through defects in multiple checkpoints of the cell cycle. One defect is an increase in the fraction of B cells bearing self-reactive B cell receptors. These cells have escaped clonal deletion, anergy, and editing caused by *Sle1b* (*2*), combined with other susceptibility genes in the C57BL/6 mouse strain (*19*). Furthermore, failure of inhibitory receptor signals that normally oppose B cell receptor signaling of proliferation compounds these problems, as exemplified by loss of FcγRIIB function (*1*, *17*).

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Another pivotal step is breakdown in Toll-like receptor discrimination between microbial and self–nucleic acids, which occurs not only in B cells but also in collaborating dendritic cells of the immune system. B cells and plasmacytoid dendritic cells express TLR7 and TLR9, and signaling by these receptors synergizes with either signals from the B cell receptor to activate B cell proliferation (*3*, *4*, *7*, *20*) or with signals from the receptor for IgG on dendritic cells to activate secretion of the cytokine, interferon-α (*13*, *21*, *22*). When B cells bearing B cell receptors against self-RNA, self-DNA, or associated proteins escape deletion, editing, anergy, as well as the inhibitory effects of FcγRIIB, their B cell receptors not only signal proliferation directly but also efficiently capture and deliver self-RNA or self-DNA to endosomal compartments containing TLR7 or TLR9. The combination of B cell receptor and TLR signals provides a T cell–independent self-stimulus for B cell proliferation and autoantibody secretion. The potential for B cell self-stimulation is magnified by the increased sensitivity to TLR7 activation in B cells caused by the *Yaa* gene duplication. Interferon-α produced by plasmacytoid dendritic cells also increases B cell expression of TLR7 and its intracellular signaling adaptor, myd88 (*23*). Initially, the stimulus for interferon production might be a viral infection, but once IgG autoantibodies to ribonucleoproteins or chromatin have begun to be secreted, these circulating immune complexes will sustain and elevate interferon production by plasmacytoid dendritic cells and in turn promote further B cell activation in a positive-feedback cycle.

Toll-driven pathogenesis of lupus now seems firmly established in mice, but it also explains many clinical mysteries: the induction of lupus by drugs (procainamide) that block CpG DNA methylation or mercury compounds that bind to RNA; the therapeutic effect of chloroquine, which blocks TLR7 and TLR9 signaling; the evidence for robust interferon production in some lupus patients; and the observation that attacks of lupus can be precipitated by single-stranded RNA viral infections such as influenza or by ultraviolet light (which induces cell death and the consequential release of self-RNA and self-DNA). Given the fine line that Toll-like receptors tread between microbial and self-stimulation, it will be important to define mechanisms and regulators of TLR7 and TLR9 signaling. These almost certainly will reveal targets for new drugs to specifically treat lupus and candidate genes whose variation in copy number or sequence, like the TLR7 duplication, push the risk of self-stimulating this pathway over the precipice in different people with lupus.

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ANTHROPOLOGY

Autonomous Cultivation Before Domestication

Early Near Eastern crop cultivation was a trial-and-error process. Some crops continued until full domestication, while others were abandoned and later adopted independently by distant societies.

Ehud Weiss, Mordechai E. Kislev, Anat Hartmann

It is widely believed that the primary domesticated crops of the Neolithic—namely, einkorn wheat, emmer wheat, barley, lentil, pea, chick pea, and flax—appeared initially in a core area from which they spread throughout the Middle East (*1*, *2*). Recent archaeobotanical data, however, indicate that predomestication cultivation of some of these species was carried out autonomously in very early sites of the Near Eastern PPNA (Pre-Pottery Neolithic A; ~11,500 to 10,300 calendar years before present). Moreover, the data also suggest that some of these crops did not develop into fully domesticated species because their cultivation was abandoned by the local populations.

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Human domestication of plants can be divided into three stages: "gathering," in which people gathered annual plants from wild stands; "cultivation," in which wild plant genotypes were systematically sown in fields of choice; and "domestication," in which mutant plants with desirable characteristics were raised. Cultivation is the essential stage, as the repetitive cycle of sowing, collecting, and sowing of wild plants gives rise to genotype accumulation that leads to domestication. Given the prevailing view that human selection of domesticated plants was not carried out intentionally, agro-evolution would have to take place over hundreds of years (*3*). The novel characteristics distinguishing domesticated from wild plants are by necessity species-specific. In the Neolithic primary domesticates, these include nonarticulating ears and plump grains in the cereals, pods that do not spontaneously release their seeds (indehiscence), and nondormant seeds in the pulses (i. e., peas, beans, lentils, and other legumes).

Archaeobotanical evidence indicates that different Near Eastern communities throughout prehistory cultivated various local species. Some of these early communities abandoned their plants, which represents a local dead end on the road to domestication. Thus, the initiation of agriculture in one place does not imply that the successfully grown plants would be continuously cultivated, an idea commonly recorded in the literature (*1*). Consequently, the location of the germ plasm of wild-plant founder stocks genetically associated with a particular fully domesticated plant (*1*) are, in reality, just the stocks that led ultimately to the domesticated plant. There might indeed have been earlier predomestication efforts—with different wild stocks—that were abandoned by the ancient society.

Two criteria identify cultivation in PPNA archaeobotanical assemblages. The first is the presence of a conspicuous quantity of seeds in greater amounts than would have been yielded

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