the melt needs to wait for a chain end to diffuse to it through a maze of tubelike constraints around the polymer contour.

But what would happen if there were no ends to be found? Answering this question turns out to be delicate. Roovers found (correctly) that the relaxation times of the ring melts were much lower than those of linear melts of the same molecular weight. But other researchers disagreed. There are several reasons why ring molecules are difficult to study. First, it is essential to synthesize rings that are not interlinked (although such "olympic gels" are themselves interesting as rubbery solids with no molecular cross-links at all). Second, even small amounts of linear polymer contaminants in a melt of rings alter the dynamics, bringing relaxation times rapidly up to linear melt values. Finally, polystyrene, although relatively easy to work with, is composed of very bulky molecules that diminish the effects of entanglement.

These experimental challenges did not prevent theoretical speculation, however. Linear chains in dense melts display the statistical properties of ideal random walks, but a melt of rings should not behave in this way. The topological constraint that the rings are not linked is permanently set from their creation. This constraint in turn biases their conformations so that they grow in size more slowly with molecular weight than do linear chains (*5*). Instead of the snakelike reptation of linear chains, theory suggested that the dynamics of rings should resemble the motion of amoebae: Unentangled loops continually thrust out and retract in the complex hedge of constraints imposed by neighboring molecules (see the figure) (*6*).

Without ready supplies of material to test these intriguing ideas experimentally, the natural recourse has been simulation. Brown *et al.* recently provided strong hints that the earlier theoretical ideas may have been on the right track (*7*). Their simulated rings are indeed more compact in the melt, and diffuse much faster than their linear homologs.

The new catalysts of Bielawski *et al.* may help to test the theoretical models experimentally. The new synthetic route has many advantages: The rings are very unlikely to be linked, linear contamination is small, and the chemistry applies to the well-entangled polyolefin family. Synthesis from deuterated starting material should lead to ring polymers that can be studied with small angle neutron scattering, allowing direct measurements of their dynamics at the molecular level. Best of all, the method should allow synthesis of large amounts of ring polymer.

A minor drawback is that the spread of molecular weights is exponentially distributed and therefore not as narrow as that in principle obtainable from anionic methods. Nevertheless, rheological, diffusion, and scattering experiments on a range of molecular weights of these new materials should provide a test for the scaling-law predictions of the "amoeba dynamics" theories.

Their melt flow properties would, of course, affect the processing of any new plastics made from ring molecules. But the absence of chain ends promises to modify solid state properties as well. The mobility of the disordered glassy regions in solid polymers of low molecular weight has often been linked to the density of chain ends, conceptualized as sources of "free volume." A similar trend is expected from theories of glassy dynamics that invoke entanglement to explain the onset of the glass transition. Similarly, the degree of crystallinity (a strong determinant of strength and toughness) in polyethylene and its cousins must depend on the presence or absence of chain ends. Bielawski *et al.* observe a subtle change in the melting temperature of the new materials, but whether this is due to the lower entropy per chain in the melt, as the authors suggest, must wait for a study over a wider range of molecular weights.

The new polymers may not immediately result in new, competitive products, but they stand every chance of clarifying some unsolved puzzles of polymer science. They are also bound to pose new questions, giving us no cause to cease from our explorations.

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PERSPECTIVES: IMMUNOLOGY

Autoreactive B Cells Migrate into T Cell Territory

Yang-Xin Fu and Ursula Storb

B cells are prevented from producing antibodies against self antigens by the induction of tolerance, which results in deletion of autoreactive B cells. Sometimes autoreactive B cells escape this fate, and produce large amounts of antibodies against self antigens, which they generate through somatic hypermutation of their immunoglobulin (Ig) genes. High levels of autoantibodies generated through somatic hypermutation are found in certain autoimmune diseases, such as systemic lupus erythematosus (*1*, *2*). It has been presumed that autoreactive B cells, like normal B cells, accumulate in the follicles of lymphoid tissues after encountering antigen and begin to proliferate, producing high-affinity autoantibodies and forming germinal centers (*3*). But recent studies using various strains of mice lacking germinal centers suggest that autoreactive B cells can accumulate at other sites in lymphoid tissues (*4*). On page 2066 of this issue, William *et al.* (*5*) working with a mouse prone to developing autoimmune disease report the surprising finding that autoreactive B cells accumulate not in germinal centers of lymphoid tissues but at the T cell zone–red pulp border.

For their study, William *et al.* selected the MRL.Fas^{lpr} mouse strain, which develops systemic lupus erythematosus. They introduced into these mice a transgene encoding the heavy chain of an antibody raised against IgG2a antibodies of the "a" allotype (*5*). The transgenic heavy chain binds to endogenous light chains to create antibodies against "a" allotype IgG2a. The transgenic antibodies behave like the rheumatoid factor found in patients with rheumatoid arthritis, an autoimmune disease. Thus, in "a" mice the antibody is anti-self with the potential to cause autoimmunity. In contrast, in mice with the "b" allotype, no self antigen is present and the transgenic heavy chain is not involved in an autoimmune process.

In the MRL.Fas^{lpr} mouse, B cells encountering an antigen that binds to their surface antibody can alter the specificity

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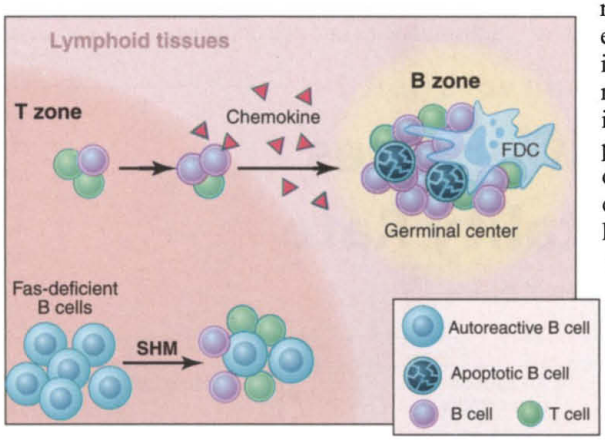
of this antibody through somatic hypermutation. Somatic hypermutation induces point mutations into the variable regions of the Ig heavy and light chain of these B cells (*6*). Usually, germinal centers form in the follicles of lymphoid tissues when B cells produce high-affinity antibodies through somatic hypermutation. These B cells will eventually enter a pool of long-lived memory B cells that circulate in the blood throughout the body. In autoimmune mice (and humans), somatic hypermutation in B cells creates autoantibodies with high-affinity against self antigens. These autoreactive B cells are permitted to survive and presumably are involved in damage to the affected tissues.

Unexpectedly, William *et al.* (*5*) found that in their MRL.Fas^{lpr} mice, autoreactive B cells expressing anti-self antibodies spontaneously accumulated in the T cell–rich zone at the red pulp border of lymphoid tissues. These B lymphocytes underwent somatic hypermutation in this zone, rather than in germinal centers (see the figure). The authors show convincingly that the autoreactive B cells do not simply undergo somatic hypermutation in the germinal centers, and then migrate to the T cell zone. William *et al.* picked multiple clusters of B cells in the T cell zone and found that each cluster contained B cells carrying related point mutations in their Ig genes, but that B cells from different clusters carried unrelated mutations. Somehow, these autoreactive B cells were prevented from entering germinal centers. In contrast, the mice did have germinal centers containing normal B cells producing antibodies against non-self antigens. In addition, MRL.Fas^{lpr} mice with the "b" allotype (carrying the same heavy-chain transgene as the "a" allotype) accumulated B cells in germinal centers in the normal way. Accumulation of autoreactive B cells in the T cell–rich zone of lymphoid tissues depends on the presence of autoantibodies in these cells. Other contributory factors include the presence of autoantigens and a deficiency in either Fas receptor or Fas ligand (molecules required for deletion of autoreactive B cells by apoptosis).

T cells play dual roles in both the generation and deletion of autoreactive B cells. For example, T cell–derived Fas ligand can either positively or negatively regulate the survival of autoreactive B lymphocytes, depending on the circumstances (*7*). Furthermore, B cells require T cell help to undergo somatic hypermutation. Cyster and Goodnow have proposed that in normal individuals, competition for limited niches in lymphoid tissue follicles prevents autoreactive B cells from entering into these regions and becoming part of the long-lived B cell repertoire (*8*). Autoreactive B cells might then be trapped in the T cell zone and would be available to interact with T cells, which would favor further somatic hypermutation and the continued production of autoantibodies. T cells are also crucial for the deletion of autoreactive B cells through various apoptotic pathways. Helper T cells can rescue B cells activated by foreign antigen from follicular exclusion and help these B cells to develop into germinal centers. Helper T cells can also delete autoreactive B cells (*8*), although it is unclear how they distinguish between B cell populations reactive against self-antigen versus those reactive against foreign antigen. Perhaps B cells at different stages of activation have different susceptibilities to T cell–derived ligands. In the germinal center environment, interactions between antigen-specific T and B cells may facilitate the more efficient deletion of autoreactive B cells. In contrast, autoreactive B cells generated outside germinal centers may be more difficult for antigen-specific T cells to recognize and delete.

Why do activated autoreactive B cells in the MRL.Fas^{lpr} mice fail to enter follicles and form germinal centers? The location of B cells depends on a balanced response to competing chemoattractants from adjacent zones (see the figure) (*9*). By responding to distinct sets of chemokines, B cells transit between the B and T cell zones of secondary lymphoid organs, enabling them to sample a variety of antigens. After binding to antigen in a T cell zone, activated B cells normally move to the B cell–T cell boundary and then migrate into follicles where they form germinal centers. Antigen recognition causes T and B cells to undergo a change in responsiveness to chemokines that may help to direct their movements into, or out of, lymphoid follicles. It is possible that autoreactive B cells down-regulate their expression of chemokine receptors or that they exhibit a decrease in responsiveness to chemokines that prevents them from entering follicles and forming germinal centers. Where and for how long B cells engage with antigens and interact with T cells may determine their chemokine receptor expression profile. If antigen-presenting dendritic cells in follicles fail to produce the required B cell specific chemokines, then B cells may be prevented from circulating back to follicles (*10*). In fact, aged MRL.Fas^{lpr} mice exhibit reduced numbers of follicular dendritic cells and a deficiency in expression of adhesion molecules in follicles, which are required for sequestering B cells (*11*). It is possible that autoantigen persistence inside the T cell zone may lead to activation and expansion of autoreactive B cells. However, the reduced numbers of follicular dendritic cells in MRL.Fas^{lpr} mice may lead to a decrease in chemokine production, which results in activated B cells not being attracted to follicles and instead accumulating in the T cell zone.



Autoreactive B cells in the T cell zone. After interacting with antigen, activated B cells normally migrate to B cell follicles in lymphoid tissues where they continue to proliferate and produce antibodies, forming germinal centers. Autoreactive B cells that produce antibody against self-antigen are normally deleted through the Fas receptor/Fas ligand-mediated pathway of apoptosis. In mice deficient in either Fas receptor or Fas ligand, autoreactive B cells cannot be deleted. They do not accumulate in follicles but instead become trapped inside the T cell zone of lymphoid tissues, where they continue to proliferate and undergo somatic hypermutation, producing more autoantibody against self-antigen. This situation also may occur in mice lacking follicular dendritic cells (FDCs) that do not produce the B lymhocyte–specific chemokines that attract B cells to the appropriate follicles of lymphoid tissues.

Several mouse strains have been engineered to lack follicular dendritic cells or germinal centers. However, whether there is an increase in autoreactive B cells in the T cell zones of lymphoid tissues in these mice remains to be determined (*4*). Mice deficient in tumor necrosis factor receptor–1 lack follicular dendritic cells and the chemokines that they produce, and accumulate antibody-producing B cells in the T cell zone of lymphoid tissues (*10*, *12*). However, mice lacking follicular dendritic cells or germinal centers do not exhibit autoimmune disease. It is likely that additional defects in the apoptotic pathway regulating autoreactive B cell death and prolonged exposure to autoantigens will be required before clinical autoimmune

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disease is manifest in these animals (*5*). Crossing mice lacking follicular dendritic cells or germinal centers (or those lacking chemokine/chemokine receptor combinations) with the autoimmune-prone MRL.Fas^{lpr} mice should help to elucidate the relative contributions of autoantigens, chemokines, and their receptors, and anti-apopotic versus proapoptotic signals in the generation of autoreactive B lymphocytes.

The study by William *et al.* (*5*) opens a new vista upon autoimmunity. Their work demonstrates that somatic hypermutation of autoimmune antibodies occurs outside of germinal centers in the autoimmunity-prone MRL.Fas^{lpr} mice. In the absence of the germinal center "checking" mechanism, there is rapid accumulation of high-affinity autoreactive B cells in the T cell zone of lymphoid tissues. The challenge now is to determine how migration to B cell follicles and the formation of germinal centers is prevented in the MRL.Fas^{lpr} mice and whether this is a general phenomenon that will be applicable to other autoantibodies and autoantigens.

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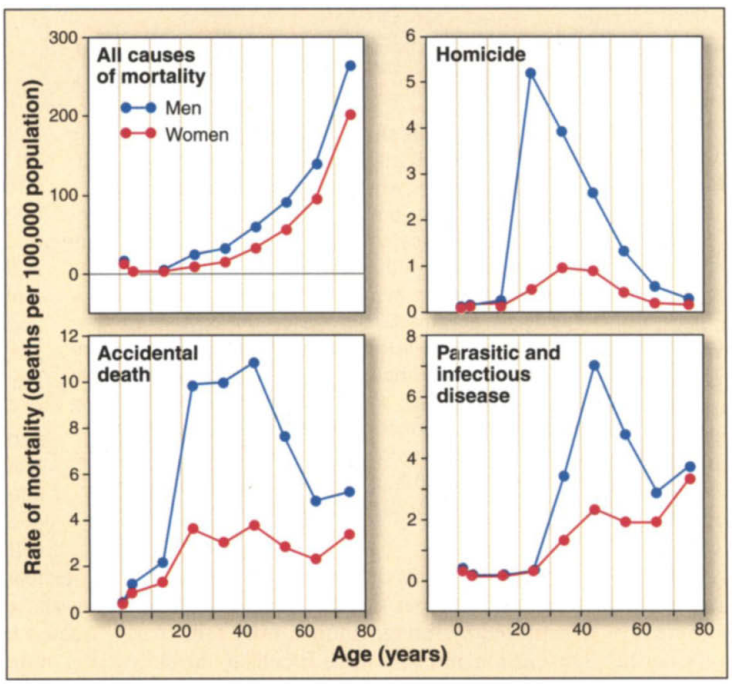
PERSPECTIVES: ECOLOGY AND EVOLUTION

Sex Differences in Mortality Rate

Ian P. F. Owens

Why do men typically die earlier than women in Westernized societies? The traditional explanation has been that men undertake more risky behaviors. Supporting this risk-prone behavior hypothesis are human demographic data showing that men are consistently more likely to die as a result of motor vehicle accidents, homicide, suicide, or accidents caused by firearms (*1*). Although the death rate through homicide in the United States is more than 10 times that in the United Kingdom and Japan, males are still twice as likely as women to be murdered in all three countries (*1*). The way in which the mortality rate changes with age also supports the risk-prone behavior hypothesis: The rise in accidental and violent death among men coincides precisely with the onset of puberty (see the first figure). On page 2015 of this issue, Moore and Wilson (*2*) propose that male-biased mortality may be caused in part by a greater susceptibility of males to infection by parasites, which in turn may be the result of male-male competition to secure mates and territory.



Sex differences in human mortality. The overall mortality rate in males is higher than that in females from puberty onward (**top left**). The other three graphs show sex differences in mortality rate due to homicide, accidental death, and parasitic and infectious diseases. For all three causes, mortality rate is higher in men than in women, but the timing of the onset of male-biased mortality varies across causes. For death through homicide and accidental causes (**top right, bottom left**), the increase in male-biased mortality begins immediately after puberty. For death caused by parasitic and infectious diseases (**bottom right**), the sex difference in mortality rate becomes apparent much later. [Data for 1997 USA population from (*1*) (www.who.int/whois)]

Traditionally, male-biased mortality among nonhuman mammals has also been explained in terms of more risky behaviors by males compared with females. Empirical studies of species in which males fight one another for access to females have shown repeatedly that such male-male competition can prove costly in terms of survival. Likewise, comparative studies demonstrate that the species with the greatest male bias in mortality tend to be those species in which male-male competition is the fiercest (*3*).

Moore and Wilson (*2*) now demonstrate that risky behavior by males is not the full explanation for male-biased mortality among mammalian species. They show that sex differences in mortality correlate with differences in susceptibility to parasitism between males and females. In those species where males die younger than females, the males suffer a disproportionately high rate of parasitism. The authors also show that male-biased parasitism is the general rule among mammals, and that it is most extreme in those species where male-male competition for mates is most severe. Taken together, these findings suggest that male-biased mortality occurs not only as a result of death through risky behavior, but also because males are more susceptible to parasitic diseases.

Human demographic data support the idea that parasites are an important determinant of male-biased mortality. Although sex differences in suicide and homicide grab the headlines, males are also more prone to a range of parasitic and infectious diseases (*1*). In the United States, United Kingdom, and Japan, men are approximately twice as vulnerable as women to parasite-induced death. In Kazakhstan and Azerbaijan, where the overall incidence of parasite-induced death is much higher, men

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