in part with the help of these funds. In 1980 when the bonds matured and interest was due, higher education suffered a blow. University construction budgets were slashed by half and general funds for basic research and education were not increased during the last decade.

For example, Kyoto University and the University of Tokyo were the hardest hit among the national universities. Buildings deteriorated, and research laboratories became outdated, lacking in the latest scientific equipment. At the same time, graduate school enrollment was increasing, especially the number of foreign students, adding an even greater burden to the cramped facilities.

Only in the past 2 years has the severity of the problem been recognized and addressed by the central government, politicians, and leaders of the corporate and financial community as an urgent problem. The Ministry of Education, Science and Culture intends to double its entire budget for higher education and research over the next several years. One plan being considered is to revitalize the research-centered universities by increasing their budgets for basic research.

In 1992 funds for the Grant-in-Aid for General Scientific Research, a program similar to the National Science Foundation in the United States, were increased by 10%. The grant will increase from 52 billion yen in 1989 to 75 billion yen in 1993.

In addition to the national universities' annual construction budget of 80 billion yen, another 20 billion yen per year was approved exclusively for the repair and reconstruction of already existing leading national universities. These budget approvals will contribute immensely to solving many of the problems faced by higher education.

However, that is only part of the solution. Japan has been very successful at providing the average citizen with an outstanding education from elementary school to undergraduate school. But it is time that Japanese society at large begin to recognize the value of a graduate and postgraduate education; only then will our graduate schools improve and scientific research continue to thrive.

To provide incentives and financial support, universities must create more scholarships for graduate students. Currently, most students must take low-interest loans from the government in order to pay for their graduate education.

In the applied science and high-tech industries, doctorate degrees (Ph.D.'s) are not required for employment nor are employees with such degrees rewarded with better salaries or higher job placement. Instead they enter the work force at the same level as someone with an undergraduate or masters degree. Why go to graduate school when you can get the same job and the same salary without a higher degree?

In conclusion, Japan must increase its financial support for basic research and education and for technology education. There is an urgent need to upgrade the quality of graduate schools so that Japan can continue to make significant contributions to the welfare of the world.

Seppuku and Autoimmunity

Tasuku Honjo

An organism must react to and destroy the many foreign antigens it is exposed to in its lifetime. To accomplish this, the immune system of vertebrates is equipped with a powerful genetic mechanism (DNA rearrangement) to amplify its repertoire of antigen receptors. This mechanism is a double-edged sword, however, as it inevitably creates immune cells that react against antigens present in the organism itself and these cells can potentially cause autoimmune diseases.

These self-reactive lymphocytes are usually removed by clonal deletion or inactivation upon interaction of these antigen receptors with antigens in the body (*1*), which triggers programmed cell death (*2*). The principle that the self-sacrifice of a few can save the whole life reminds me of *seppuku* or cutting of the belly, a practice common to the Japanese soldier (samurai) until 130 years ago, when his death was required to save the lives of his retainers or his family pride.

Unfortunately, selection of lymphocytes bearing antigen receptors specific to a particular antigen has been difficult to study in normal animals, because only a few in a million lymphocytes can recognize a given antigen. Recently, transgenic mice expressing autoreactive immunoglobulins (Igs) have been generated so that one can follow the selection of self-reactive B lymphocytes, which in these mice represent the majority of the B cells (*3*, *4*). These studies have clearly shown that autoreactive B cells are either clonally deleted or inactivated (anergized), depending on the type and amount of autoantigen. We have generated transgenic mice expressing an antibody to murine red blood cells (RBCs) (*4*). These mice were derived from strain NZB, which is prone to develop autoimmune diseases (*4*). The number of spleen B lymphocytes, almost all of which express antibody to the RBCs, is reduced to 1 to 10% of the number seen in the spleens of control mice. B cells that escape deletion are anergized. Nonetheless, in spite of this loss of B cells, about 50% of the mice show symptoms of autoimmune anemia, indicative of remaining immune activity against RBCs. Two questions arise: (i) where are these remaining autoantibodies produced? and (ii) why do only 50% of the animals, all of which have the same genetic background, become sick?

The answer to the first question resides in the belly (*5*). All of the transgenic mice contain the same number of B cells expressing the cell surface marker CD5 in the peritoneal cavity as do normal mice, indicating that $CD5^+$ B cells can escape clonal deletion. However, only in the anemic mice are $CD5^+$ B cells producing the autoantibody. Our speculation is that $CD5^+$ B cells in the peritoneal cavity are not deleted because the self-antigen (RBC) is not available in this sequestered compartment of the mouse body. The simplest experiment to test this idea is to inject RBCs into the peritoneal cavity of the transgenic mice. When this is done, exposure to the self-antigen induces apoptotic death of $CD5^+$ B cells in the peritoneal cavity, resulting in a drastic reduction in the $CD5^+$ B cell number. Repeated injection of RBCs into the peritoneal cavity of severely anemic transgenic mice can completely cure their symptoms; anemia disappears and autoantibody production ceases.

Environmental factors may be involved in the variation in symptoms among transgenic individual animals. The most obvious candidate is infection. When we give lipopolysaccharide (LPS) to nonsymptomatic transgenic animals in order to mimic natural infection, all of them become autoimmune and develop severe anemia. Only oral administration of LPS has this effect on peritoneal B cells; intramuscular or intraperitoneal administration does not. We are curious to know how LPS in the gut lumen can stimulate the peritoneal B cells. The gut contains probably the most ancestral, yet still important, part of the immune system and contains $CD5^+$ B cells and $\gamma\delta$ T cells. LPS in the gut lumen appears to activate lymphocytes in the lamina propria of the gut epithelium, cells that can also escape clonal deletion. On the other hand, B cells in Peyer's patches as well as in mesenteric

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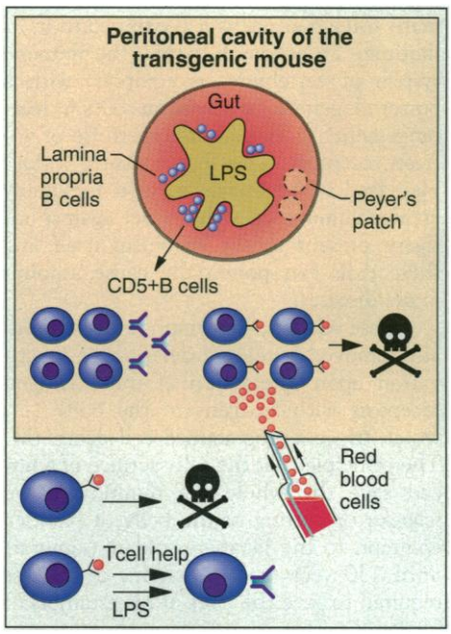
lymph nodes are completely deleted, like those in the spleen (see figure). It is likely that infection in the intestine of our transgenic mice activates lamina propia CD5$^+$ B cells, which may then move into the peritoneal cavity and produce autoantibodies.

Apoptotic death of peripheral B cells by exposure to antigens is not a unique feature of our transgenic mice nor of CD5$^+$ B cells. Mature conventional B cells as well as CD5$^+$ B cells in the peritoneum of normal mice perform *seppuku* when the surface Ig is cross-linked in vivo by intraperitoneal injection of antibody to Ig (6). Such an in vivo experiment is possible only in the peritoneal cavity, which contains just a small amount of natural Ig capable of neutralizing the injected antibody to Ig. In contrast, when the surface Ig molecules of peritoneal B cells are cross-linked in vitro, they are activated to proliferate, as is the case with splenic B cells (7). These contradictory phenomena lead us to one of the classical questions in immunology: how are B cells activated by antigens? Since all antigens are recognized by the same family of antigen receptors, which cannot distinguish by themselves self from foreign antigens, how do B cells know the difference? How can the signal through surface IgM induce apoptosis by self antigens but proliferation by foreign antigens? A likely explanation is that the foreign antigen stimulation can provide, probably via T cells, a second signal that can alter the death-inducing signal through surface Ig (8). The self-antigen cannot do this unless it also receives T cell help by breaking T cell tolerance. To understand such regulatory mechanism, one has to know the nature of the death signal.

Programmed cell death is one of the fundamental mechanisms used in the development of the shape of multicellular organisms. Programmed cell death takes place during embryogenesis of various mammalian organs including the opening of the mouth and anus, establishment of the proper connections of neurons, and the formation of the immune repertoire. Unfortunately, little is known about the molecular mechanism involved. A membrane receptor–like molecule, Fas, was proposed to be involved in the programmed cell death of thymocytes, because mutation of the *fas* gene causes lymphoproliferative disorder (*lpr/lpr*), which leads to autoimmune symptoms in the aged mice. However, the absence of upregulation of *fas* mRNA upon induction of thymocyte death (9) and the localization of the Fas antigen in the thymic epithelial cells (10) make this hypothesis less attractive.

The classical type of programmed cell death requires activation of a set of genes that lead to DNA fragmentation and subsequent apoptotic morphological changes. Subtraction cloning has been used to isolate genes that are activated by the death signal (11). So far only one gene, *PD-1*, has been shown to be specifically expressed after induction by the signal for cell death (9). *PD-1* is a new member of the Ig super gene family and is strongly expressed on the surface of all cell lines undergoing the classical type of programmed cell death. *PD-1* mRNA is found in the thymus but not in the brain, liver, spleen, or kidney. *PD-1* mRNA expression in the thymus is augmented by a stimulus that induces massive thymocyte death in vivo. The PD-1 protein is a membrane receptor that may be able to associate with a tyrosine kinase. An autocrine model for cell death has been proposed in which this membrane protein, together with its yet to be identified ligand, might transduce the final signal and lead to activation of endonucleases and DNA fragmentation (9). The close correlation of *PD-1* mRNA expression with the classical type of programmed cell death makes this hypothesis attractive, but many other possible functions of this protein remain to be excluded.



A mechanism for autoimmunity? (Top) (Left) Activation of peritoneal CD5$^+$ B cells by LPS in the gut lumen of the transgenic mouse and (right) apoptotic death of peritoneal CD5$^+$ B cells by antigen (RBC) injection. **(Bottom)** Two-signal model for B cell activation.

Understanding and eventual treatment of human diseases depend on development of animal models of disease and elucidation of the underlying molecular mechanisms of disorders. In the RBC autoimmune animal model, the development of the disease clearly depends on both genetic and environmental factors as hypothesized for human autoimmune diseases. Detailed studies of the environmental factors causing the onset of autoimmune disease in this transgenic model will provide important clues to the understanding and treatment of human autoimmune diseases. The model also provides a unique concept of autoimmunity: sequestered B lymphocytes that escape clonal deletion by avoiding encounter with self-antigens may subsequently cause autoimmune diseases by polyclonal activation. Molecular mechanisms for programmed cell death and its regulation have been a focus of attention in many fields of biology. Isolation of the *PD-1* gene is likely to be the first step toward isolation of several other genes activated by induction of programmed cell death. The whole scenario of immune tolerance, in which the *seppuku* scene is almost certainly the highlight of the drama, may soon emerge.

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