SPECIAL ARTICLE

Shattuck Lecture — Diversity of the Immune Repertoire and Immunoregulation

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SINCE THE MIDPOINT OF THE 20TH CENTURY, MEDICAL ADVANCES IN ECONOMICALLY developed countries have exceeded all expectations. In 1950, the year I entered medical school, the average life expectancy in the United States was 68 years. By 2000, it was 77 years (80 years for women).[1] In 1957, when I began my fellowship in hematology, there was no combination chemotherapy, the choice of antibiotics was limited, computed tomography and magnetic resonance imaging did not exist, and most neoplasms were incurable. And in 1958, the year I began my research on immunosuppressive drugs, the role of the lymphocyte was unclear, and successful organ and bone marrow transplantation lay in the future.

This is not a comprehensive review, but rather a personal reflection on some aspects of immunology with clinical relevance. My main point is that the immune system's enormous repertoire of antigen receptors allows reactivity not only against pathogens, but also against autoantigens. This potential disadvantage is countered, however, by potent regulatory mechanisms that reduce the risk of harm. Research on these mechanisms has changed clinical practice by uncovering new ways of controlling autoimmune diseases and preventing graft rejection.

CLONAL SELECTION

In 1900, Paul Ehrlich, one of the leading immunologists of the time, published "On Immunity with Special Reference to Cell Life," a detailed account of his receptor theory of the immune response.[2] To convey his ideas, Ehrlich broke with tradition and taboo by showing diagrams of hypothetical molecules — at the time, there was no physical evidence that antibodies existed, and diagrams were regarded as vulgar popularizations of complex matters.[3] Despite fierce opposition, especially to the diagrams, Ehrlich's paper became one of the most highly cited publications in the literature on immunology because it introduced a radically new way of thinking about the immune system.

The essence of Ehrlich's idea, in modern terms, is that antigens bind to preexisting cell-surface receptors (surface immunoglobulins) and thereby stimulate the cell to produce more receptors and to secrete them, in the form of antibodies, into the extracellular fluid.[4] Ehrlich's concept implied that the immune system generates an array of unique receptors before it has any contact with antigens. Like a falling star, this brilliant insight soon vanished, because Ehrlich's contemporaries could not believe that the body has foreknowledge of any compound a chemist could synthesize. Today, Ehrlich's idea is a principal feature of the clonal-selection theory, the basis of modern immunology.[5-8]

The clonal-selection theory asserts that B cells have a proliferative advantage during an immune response if their receptors have a high affinity for the immunogen. When it was introduced in the mid-1950s, the theory shifted the orientation of immunology from chemistry to cells, thereby sparking a revolution in our understanding of how the immune system works. Its implications for clinical medicine were immediately apparent, because it rooted the immune system in clones of lymphocytes, thereby identifying the real targets for the harnessing of unwanted immunity.

THE GENERATION OF DIVERSITY

RECOMBINATION OF VARIABLE-REGION GENES

The antigen receptors displayed by B cells and T cells each have two components: B cells have heavy and light chains, and most T cells have α and β chains (Fig. 1). The human body contains approximately 10^{10} lymphocytes, each with a unique combination of gene segments that specify the variable region, the part of the receptor that binds antigen.[11] Figure 1 shows how, in the developing B cell, random recombination of heavy-chain genes (V_H, D, and J_H) and light-chain genes (V_κ and J_κ) culminates in V_H–D–J_H (heavy chain) and V_κ–J_κ (light chain) coding units.[12] The random shuffling of numerous variable-region genes[10] deals each B cell a distinctive receptor. A similar principle underlies the formation of the T-cell receptor. Adding to variable-region diversity is the insertion of nucleotides (adenine, guanine, cytosine, and thymidine) in a random order into the joints between the D–J_H and V_H–D segments[13] (Fig. 1).

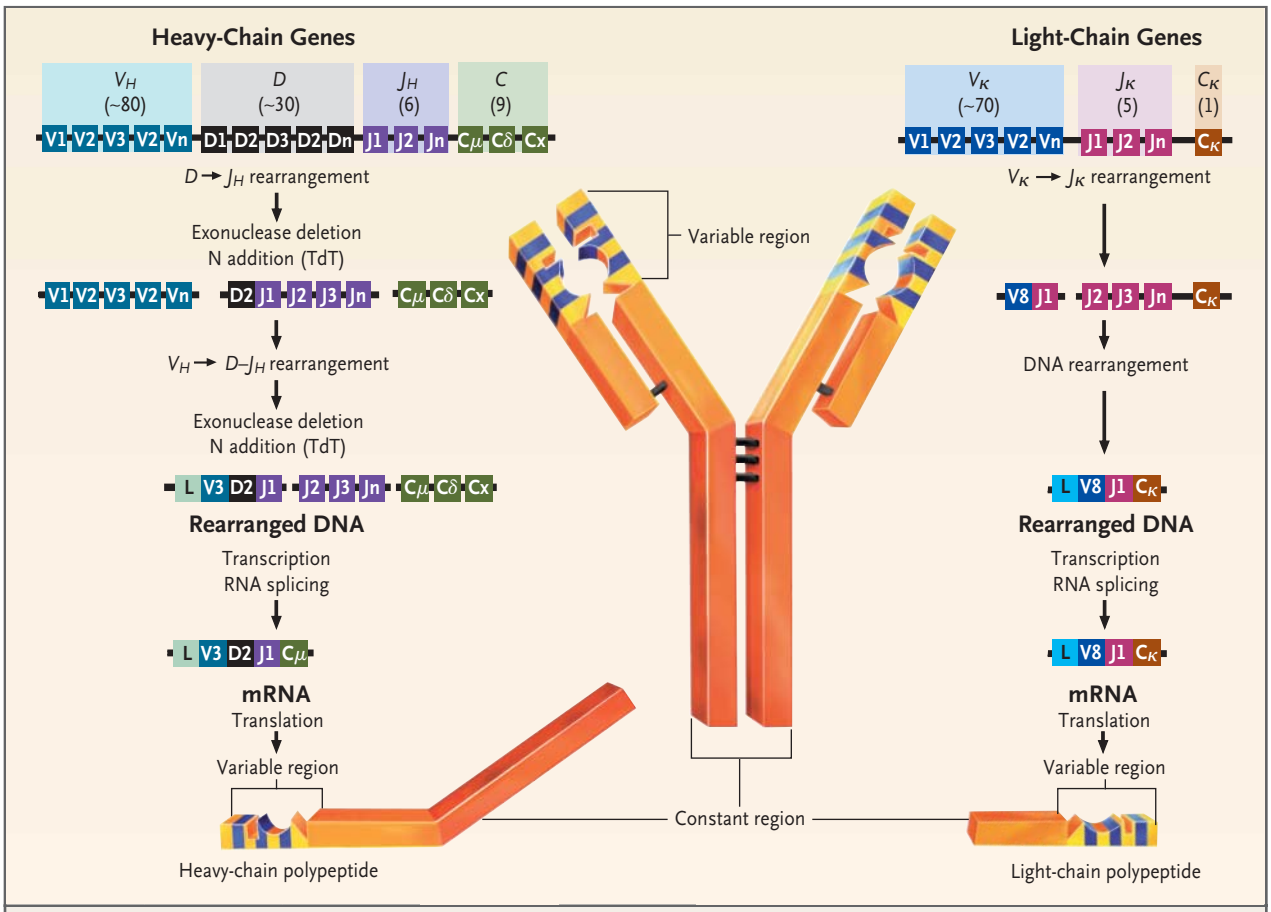

Figure 1. The Generation of Antibody Diversity.

The heavy and light chains of the antibody molecule (center) contain variable and constant regions. The variable region binds antigen, whereas the constant region specifies the isotype of the molecule (IgM, IgG, IgA, IgE, or IgD) — in this case, IgG. The coding unit for the variable region of the heavy chain forms by rearrangement of individual genes from a group of about 125 DNA segments among which are the V_H, D, and J_H segments. The process begins during B-cell development when recombinase enzymes initiate the random joining of one D segment to a J_H segment and an endonuclease excises the remaining D and J_H segments; a similar mechanism joins a V_H gene to the D–J_H unit. After the $D \rightarrow J_H$ and $V_H \rightarrow D$–J_H rearrangement, the enzyme terminal deoxynucleotidyl transferase (TdT) adds up to six nucleotides in random order to the joints between the rearranged genes. The composite V_H–D–J_H trio is then brought together with the DNA segment corresponding to the constant region of the IgM molecule (C_μ) to form the V_H–D–J_H–C_μ coding unit of the heavy chain of an IgM antibody. Next, the immature B cell forms κ or λ light chains by random rearrangement of V_κ and J_κ (or V_λ and J_λ) genes and joining to a light-chain gene of the constant region. The leader sequence (L) in the immunoglobulin mRNA transports the heavy-chain or light-chain polypeptide to the B-cell surface. A similar process generates the antigen receptors of T cells. Modified from Schwartz.[9]

COMPLEMENTARITY-DETERMINING REGIONS

Over 30 years ago, Kabat and Wu identified subregions within the variable region called complementarity-determining regions.[14] Virtually all the variation in populations of antibodies is due to these regions. They form the pocket in the variable region that binds to an antigen with a complementary shape — hence their name. In newly formed B cells, seemingly unrelated ligands (epitopes) can fit into the pocket formed by the complementarity-determining region (Fig. 2). Some fit snugly (have high affinity), some loosely (low affinity), and others not at all. The polyspecificity of antigen receptors and the enormous diversity of the randomly assembled repertoire of receptors explain why many B cells and T cells that have not yet encountered a foreign antigen are "anti-self."[15-17]

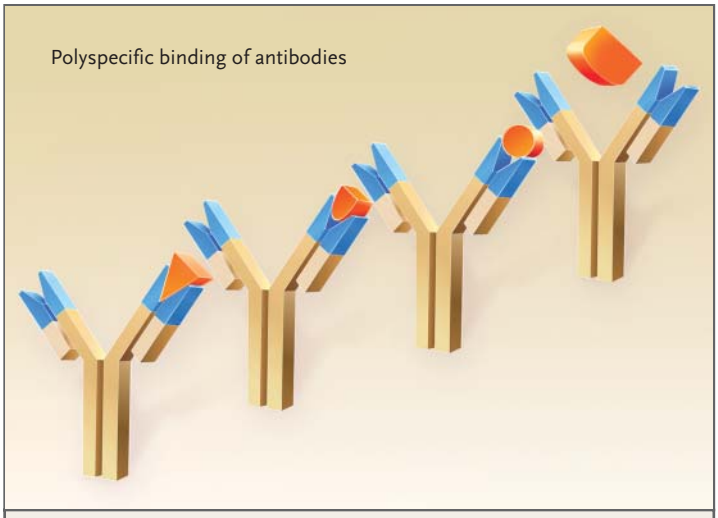

Figure 2. Degeneracy of the Antigen Receptor.

The variable region can accommodate many seemingly unrelated ligands (epitopes [orange]), as long as they fit into the pocket formed by the three complementarity-determining regions. The right-most epitope in the diagram does not fit, whereas others, even those in entirely unrelated molecules, do fit — some loosely (center) and others with a high degree of complementarity (lower left).

SOMATIC MUTATION OF VARIABLE-REGION GENES

The role of the B cell is to produce high-affinity protective antibodies. To succeed in this function, it attempts to increase the affinity of its receptors for the immunizing antigen by mutating its variable-region genes.[18,19] Mutation of *V* genes occurs in the germinal center (Fig. 3A) and requires as yet unknown signals from T cells in the vicinity.[20] Virtually all the mutations affect the complementarity-determining regions; successive affinity-increasing mutations force the evolution of clones of B cells that produce high-affinity antibodies. A master gene, activation-induced deaminase, is essential for both somatic mutation of variable-region genes and the switch of the immunoglobulin isotype from IgM to IgG, IgA, or IgE during the immune response.[21,22]

The variable regions of T cells, by contrast, cannot bind directly to antigen, and their genes do not mutate. Instead, an antigen-activated T cell forms clusters of receptors with high avidity for the immunogen by reorganizing its plasma membrane.[23,24] These receptor-rich membrane microdomains most likely account for the clonal selection of antigen-activated T cells.[25]

HOW T CELLS RECOGNIZE ANTIGENS

DENDRITIC CELLS

The long-armed dendritic cell of lymphoid tissue, skin, and squamous epithelium is the antigen-presenting cell par excellence. It engulfs protein antigens, chops them into peptides, and displays the fragments on its surface by means of major-histocompatibility-complex (MHC) molecules (also called HLA molecules).[26] The site of engagement between the T cell and the antigen-presenting cell has been termed the immunologic synapse,[27] which indeed has some features of the neuronal synapse (Fig. 3B).[28,29]

THE MHC MOLECULE AND SELF PEPTIDES

Antigen receptors activate the T cell when they bind to the peptide clasped within the groove of an MHC molecule (the antigen receptors of B cells do not require presentation of the antigen by MHC molecules). Some of these peptides originate from microbes, but usually they derive from worn-out nuclear and cytoplasmic proteins.[30-32] The MHC molecule, like a garbage truck, carries intracellular junk to the exterior.[33,34] The result is that peripatetic T cells constantly encounter a display of potentially immunogenic self peptides on antigen-presenting cells. Yet, in most cases, T cells ignore them and remain quiescent.

IMMUNOREGULATION BY POSITIVE AND NEGATIVE SELECTION OF T CELLS

T-CELL DIFFERENTIATION IN THE THYMUS

The T-cell precursor migrates from the bone marrow to the corticomedullary zone of the thymus,

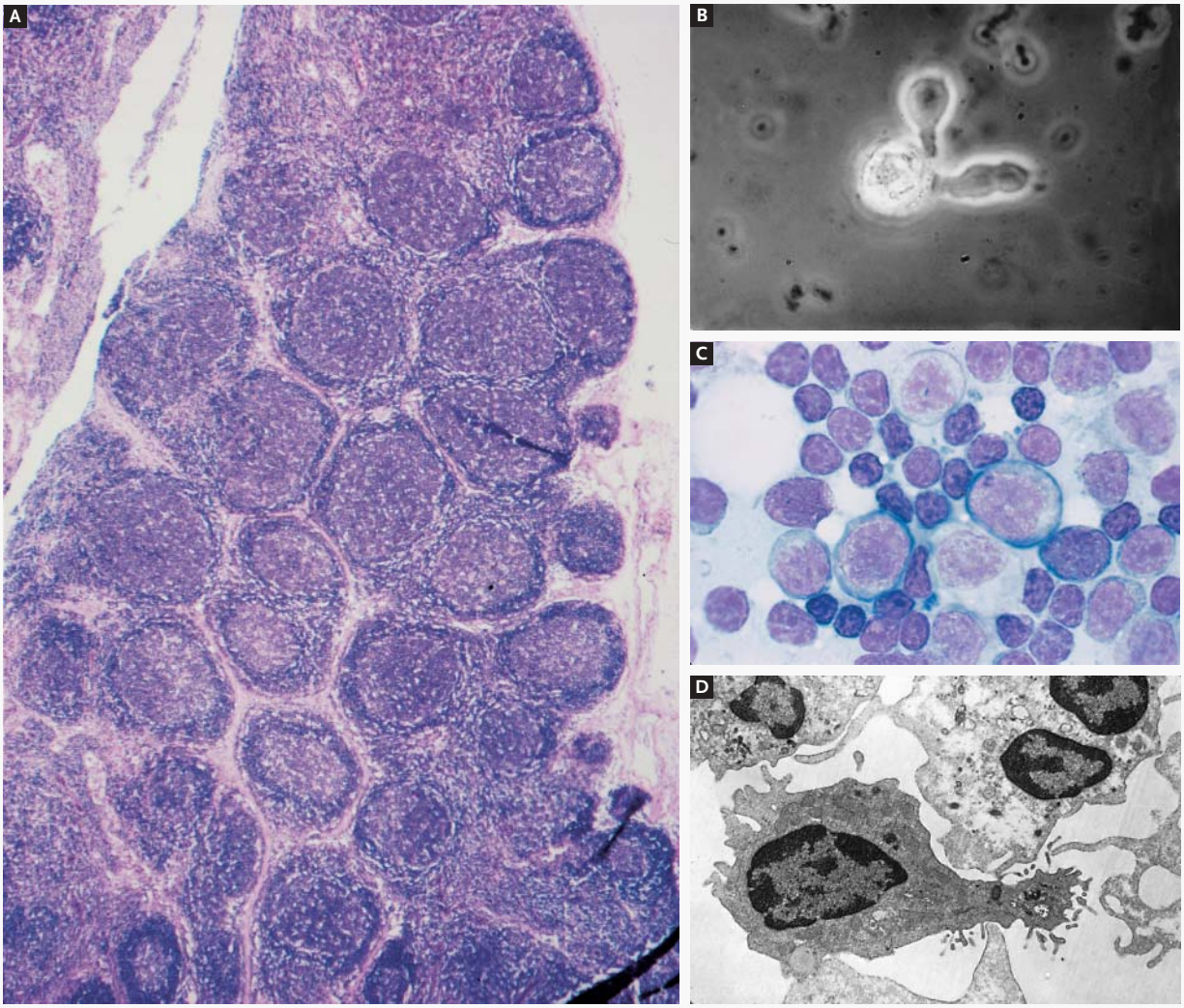

Figure 3. Lymphocytes.

Panel A shows a lymph node draining the site of a subcutaneous injection of a foreign protein. Germinal centers, the light regions surrounded by a rim of blue-stained lymphocytes, are numerous. Panel B shows the immunologic synapse: two T cells have docked onto the surface of an antigen-presenting cell. The dark strip at the tip of the pseudopod of the T cell is the region of the synapse. (Photograph courtesy of Dr. Jack Mitus.) Panel C shows an imprint of a lymph node draining a skin allograft five days after placement of the graft. The many large cells with deep blue cytoplasm are activated lymphoblasts. (Photograph courtesy of Dr. Janine André-Schwartz.) Panel D shows a T cell making its way through a slit between two epithelial cells; there is a characteristic fringed pseudopod. The entrance of such T cells into tissues depends on chemoattractants and adhesion molecules, many of which are targets of new therapies. (Electron micrograph courtesy of Dr. Janine André-Schwartz.)

where it begins to differentiate, rearrange its variable-region genes, and proliferate. This complex program ends in the medullary region of the thymus, from which the mature T cell exits (Fig. 4). In passing through the thymus, more than 98 percent of immature T cells undergo apoptosis. Whether the cell lives or dies depends on the binding affinity of its antigen receptors to peptides within the thymus.[35,36] If the binding affinity is high, the cell dies; if there is no affinity, the cell dies. If the affinity is just right, the cell lives. Survival of the developing T cell because of "just-right" affinity is called positive selection; the "wrong" affinity dooms the cell to death by apoptosis (negative selection).[37]

ECTOPIC AUTOANTIGENS IN THE THYMUS

It is rather amazing that thymic epithelial cells produce and display ectopic autoantigens. Derbinski and colleagues have demonstrated the production by these cells of three islet-cell antigens: glutamic acid decarboxylase (GAD67), insulin, and IA-2.[38,39] These thymic cells also produce the type IV collagen autoantigen of Goodpasture's syndrome,[40] and it is likely that all the peptides they display derive from autoantigens.[41] These self peptides have a central role in orienting the T-cell–receptor repertoire toward self antigens and in eliminating potentially damaging T cells with high affinity to these self antigens.[42] The display of autoantigens in the thymus of mice is influenced by the *aire* gene[43]; thymic epithelial cells of *aire*-deficient mice do not display autoantigens.[44] Remarkably, a variety of autoimmune diseases develop in humans and mice lacking a functional *AIRE* or *aire* gene.[45,46]

DEGENERACY OF ANTIGEN RECEPTORS

Not only do most newly minted T cells have anti-self receptors,[47] but many B cells emerge from the bone marrow with such receptors.[48] However, be-

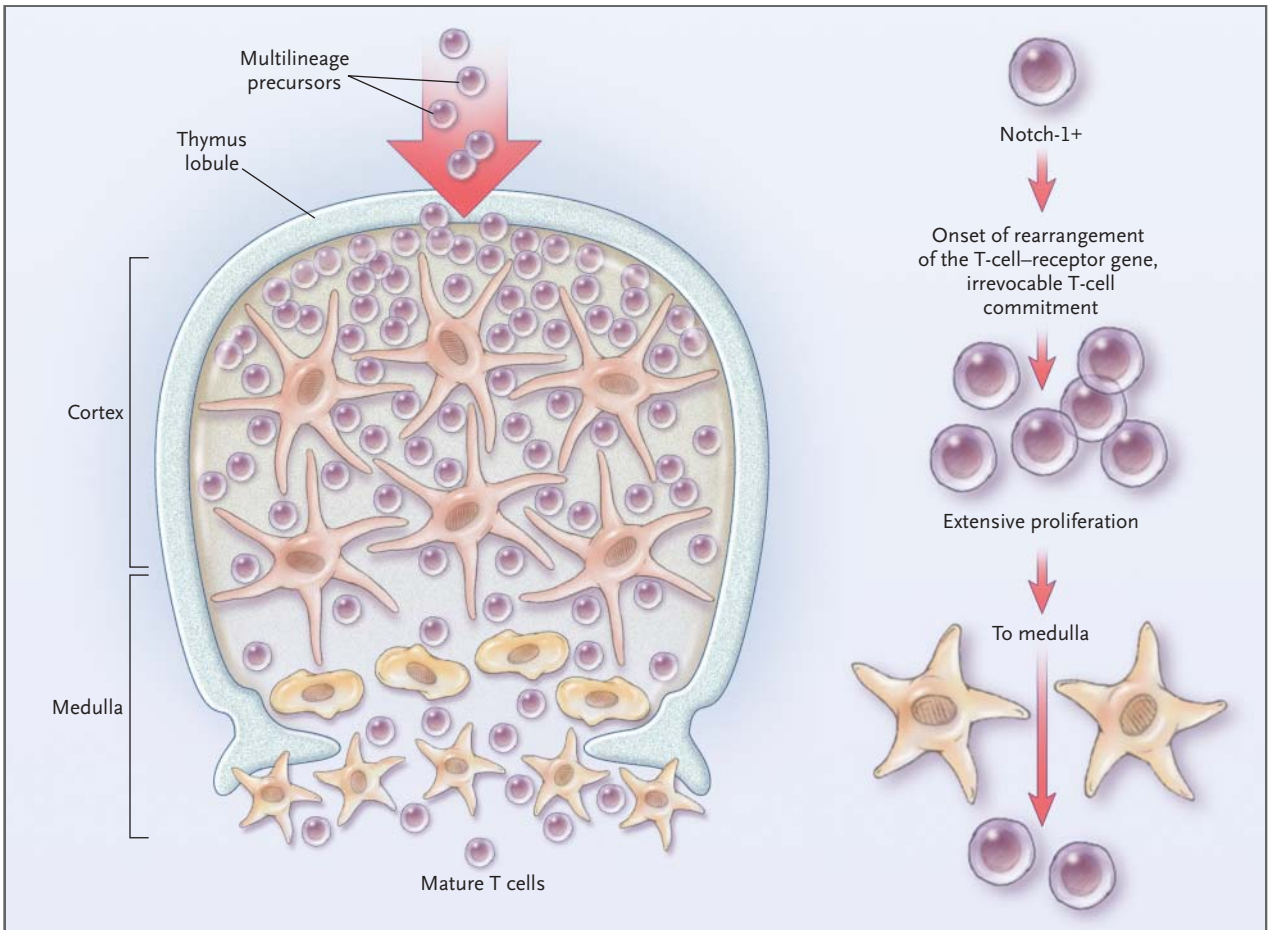

Figure 4. Differentiation and Selection of T Cells within the Thymus.

Cells that will become mature lymphocytes arise in the marrow from a hematopoietic stem cell, which becomes a committed lymphocyte precursor under the influence of a variety of cytokines, growth factors, and specialized nurse cells. These factors trigger biochemical pathways with key roles in determining the fate of the precursors of lymphocytes. Notch-1, a receptor on primitive cells, binds to surface molecules on stromal cells in certain microenvironments and activates a program that directs the primitive cell into the T lineage.

With the Notch pathway activated, the pro–T-cell migrates into the corticomedullary zone of the thymus, where it begins to differentiate, rearrange its variable-region genes, and proliferate. The program ends in the medullary region, and the cell exits as a mature T cell with a unique antigen receptor. In passing through the thymus, more than 98 percent of the developing T cells die by a process of programmed cell death. Whether the cell lives or dies depends on the fit, or binding affinity, between its antigen receptors and peptides within the thymus. These peptides are displayed by HLA molecules on epithelial cells in the cortical zone and dendritic cells in the medullary zone of the thymus.

cause of degeneracy in the binding specificities of their receptors, virgin T cells and B cells can also bind foreign antigens.[49-51] There is, moreover, clear evidence that mutations in variable-region genes convert these polyspecific anti-self–anti-foreign receptors of B cells into specific anti-foreign receptors.[52] The clinical implication here is that immunization can result in the avoidance of autoimmunity, which is a central tenet of the hygiene hypothesis: a "dirty" environment inhibits susceptibility to autoimmune and allergic diseases, whereas a "clean" environment has the opposite effect.[53]

ACTIVATION OF LYMPHOCYTES

Activation of mature T cells requires multiple signals.[54-57] The binding of the T cell's antigen receptor to an HLA–peptide complex activates only one signal. The others, termed costimulatory signals, are usually a bacterial product like endotoxin, cytokines from activated antigen-presenting cells, or adhesion molecules. Adjuvants in vaccines work by engendering costimulatory signals. Under resting conditions, these signals are switched off, thereby minimizing the risk of autoimmunization. Key costimulatory molecules are members of the B7 family (CD80 and CD86), which are displayed by dendritic cells, and CD28, a glycoprotein on T cells.[58] An encounter between B7 and CD28 evokes a signal that helps to activate T cells. If the T cell receives a signal only from its antigen receptor, it enters an unresponsive state called anergy.[59-62]

CONTROL OF T-CELL ACTIVATION

CTLA-4

After activation, several mechanisms restore the quiescent state of T cells. An important regulator, CTLA-4 (CD152), appears on activated T cells and blocks the B7–CD28 interaction by competing with CD28 for B7: the affinity of CD80 for CTLA-4 is 20 times as high as its affinity for CD28.[63,64] As a result, the T cell returns to a quiescent state. CTLA-4 is a promising target in the treatment of autoimmune diseases and prevention of graft rejection.[65,66]

INDUCIBLE COSTIMULATOR

Another regulatory molecule, inducible costimulator, down-regulates pathways that can lead to an autoimmune disease. Inducible costimulator is induced during T-cell activation, and its ligand is a member of the B7 family.[67,68] Numerous animal models and studies in humans have demonstrated that if these regulatory molecules or the transcription factors that control them are defective, the result is an autoimmune syndrome with marked lymphoproliferation.[69]

REGULATORY T CELLS

More than thirty years ago, Gershon and Kondo described a population of T cells that suppress the immune response of mice to foreign antigens.[70] Their report generated considerable excitement, but immunologists lost interest in the phenomenon because of difficulty in reproducing it. Later, it was found that a variety of autoimmune diseases develop in mice whose thymus is removed soon after birth, clearly implying that the thymus produces cells capable of suppressing autoimmunity.[71,72]

These two phenomena were linked by the discovery of a subpopulation of T cells with CD4 and CD25 surface markers (CD25 is the α chain of the interleukin-2 receptor).[73] These T cells have potent inhibitory effects on immune responses to foreign antigens and the development of autoimmunity,[74,75] and they can block the development of autoimmune diseases in mice that have undergone thymectomy.[76] Within this population of T cells, some members are partially anergic and arise after repeated rounds of antigenic stimulation[75]; some exert their effects by direct cell-to-cell contact, others by secretion of the cytokine interleukin-10.[77,78] These cells turned out to be suppressor T cells; their rediscovery is not just a vindication of Gershon's early work but also a major advance with obvious clinical implications for autoimmunity and transplantation.

THE CD2—LFA3 SYSTEM

After activation by antigen, T cells with low-affinity receptors usually die by apoptosis. By contrast, T cells with high-affinity receptors become memory cells, display CD2, and wander through the skin, lymph nodes, and gut in search of antigen[79,80] (Fig. 3D). CD2 binds to LFA3, a ligand on dendritic cells.[81] The CD2–LFA3 system is a new target of a monoclonal antibody that blocks the interaction between the two molecules as a treatment for psoriasis.[82]

RATIONAL MEDICAL CONTROL OF THE IMMUNE SYSTEM

IMMUNOSUPPRESSIVE CHEMICALS

As recently as 1951, the eminent pathologist Arnold Rich was writing about the "mysterious lympho-

cyte."[83] Even so, there was evidence of the involvement of lymphocytes in immunity, and this led William Dameshek and me to the idea that drugs with activity against lymphocytic leukemia could affect the immune response.[84] This hypothesis was supported by the demonstration that the antileukemic compound 6-mercaptopurine suppressed the immune response of rabbits against a foreign protein.[85] In lymph nodes draining the site of a skin allograft in a rabbit, numerous primitive lymphocytes (lymphoblasts) were evident five days after placement of the graft (Fig. 3C).[86] Treatment with 6-mercaptopurine suppressed both the proliferation of lymphoblasts and rejection of the graft.[87] These results were quickly confirmed by others with skin grafts in rabbits[88] and with canine renal transplants[89,90] and then with kidney allografts in humans.[91] From these halting steps, which began 40 years ago, organ transplantation has taken major strides. Almost 14,000 renal allografts were transplanted in the United States in 1999.

Dameshek and I found that 6-mercaptopurine and its analogue azathioprine were also effective treatments for corticosteroid-resistant autoimmune hemolytic anemia, systemic lupus erythematosus, and other immunologic diseases.[87,92,93] Since then, azathioprine has become widely used in the treatment of a wide variety of immunoinflammatory diseases. The drug is rapidly metabolized to the parent compound, and whether it is genuinely superior to mercaptopurine has not been determined. It is now still used along with many other drugs with immunosuppressive properties that have been introduced into the clinic (Table 1).

Table 1. Immunosuppressive Drugs in Clinical Use or Clinical Trials.

Agent	Principal Mode of Action*
Corticosteroids	Inhibition of activation of cytokine and chemokine genes by nuclear factor κB
Mercaptopurine	Inhibits nucleic acid synthesis in activated lymphocytes
Azathioprine	Inhibits nucleic acid synthesis in activated lymphocytes
Mycophenolate mofetil	Inhibits inosine monophosphate and lymphocyte proliferation
Methotrexate	Inhibits dihydrofolate reductase; antiinflammatory
Leflunomide	Inhibits pyrimidine synthesis; antiinflammatory; antiproliferative
Cyclophosphamide	Cross-links DNA; blocks cell division
Cyclosporine	Binds calcineurin, inhibits nuclear factor of activated T cells; early events in T-cell activation†
Tacrolimus (FK506)	Binds tacrolimus-binding protein; inhibits nuclear factor of activated T cells; early events in T-cell activation
Sirolimus	Blocks T-cell proliferation
FTY720	Analogue of sphingosine 1-phosphate; inhibits lymphocyte homing
Rapamycin	Interferes with cyclins; blocks mitogen-activated signals and cell cycle

* Nuclear factor of activated T cells is a transcription factor that regulates production of interleukin-2 and other cytokines; tacrolimus-binding protein is a member of a family of at least 11 proteins, some of which inhibit calcineurin.
† Ligation of the T-cell receptor activates calcineurin, a serine–threonine phosphatase and a member of the family of intracellular regulatory proteins termed cyclophilins. Some cyclophilins can inhibit calcineurin, regulate intracellular calcium flux, and activate the *NFAT* gene. Cyclosporine binds to the cyclophilin Cy^{PA}, thereby inhibiting the phosphatase activity of calcineurin.

MONOCLONAL ANTIBODIES

I can mention here only a few examples of clinically useful monoclonal antibodies. CD52, a small surface protein on T cells and B cells, is the target of Campath-1, a monoclonal antibody with efficacy in the prevention of allograft rejection and the treatment of chronic lymphocytic leukemia[94]; CD20, found only on B cells, is the target of rituximab, now widely used in the treatment of B-cell lymphomas and certain autoimmune diseases.[95] Infliximab, a monoclonal antibody against the inflammatory tumor necrosis factor α (TNF-α), has been found to be effective against rheumatoid arthritis and Crohn's disease.[96,97]

Monoclonal mouse antibodies that are in clinical use in humans can lead to the formation of antimouse antibodies, which can induce allergic reactions and reduce the effectiveness of the mouse antibody. Steps have been taken to solve this problem by genetic engineering (Fig. 5). In a chimeric monoclonal antibody, the variable region is of mouse origin and the constant region is of human origin. Such antibodies are less immunogenic than a conventional monoclonal mouse antibody, but they can still evoke neutralizing antibodies and allergic reactions. Infliximab, a chimeric monoclonal antibody against TNF-α, is active against rheumatoid arthritis, Crohn's disease,[98] and other immunoinflammatory disorders, but antibodies produced during treatment may be a limiting factor in its long-term usefulness. In a humanized antibody, everything in the molecule is of human origin except the three complementarity-determining regions. Rituximab is such an antibody, and it is readily tolerated and can be given repeatedly.

RECOMBINANT FUSION MOLECULES

A protein consisting of CTLA-4 fused with the constant region of IgG is under active investigation in several autoimmune and inflammatory diseases in which proliferating T cells have been implicated.[99]

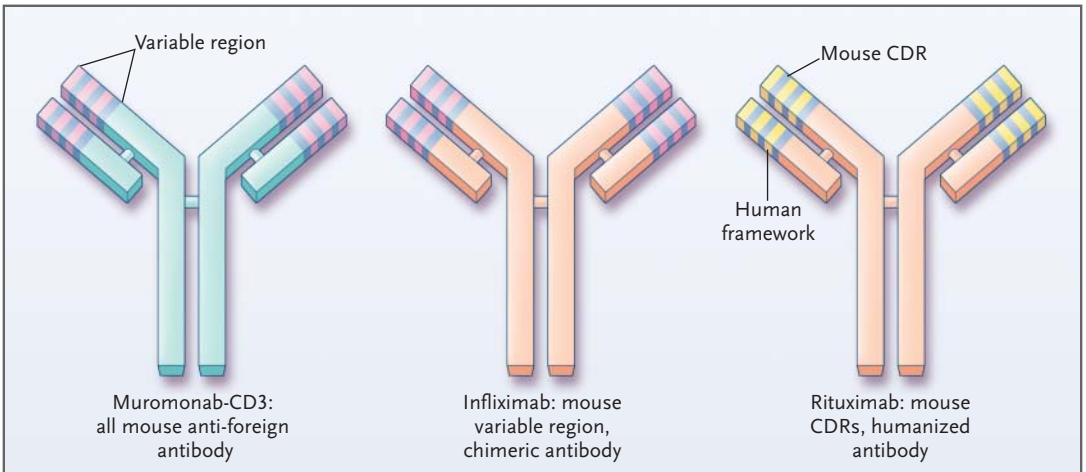

Figure 5. Three Types of Monoclonal Antibodies Now in Clinical Use.

The antibody on the left, muromonab-CD3, an anti–T-cell antibody, is entirely of mouse origin. The middle antibody, infliximab, is a chimeric antibody produced by genetic engineering. It is all of human origin except for the variable region, which is of mouse origin. The humanized antibody on the right, rituximab, is all of human origin except for the antigen-binding portions of the complementarity-determining regions (CDRs).

Alefacept is a recombinant fusion protein consisting of LFA3 (the adhesion molecule on antigen-presenting cells that binds to CD2 on memory T cells) and the constant region of IgG. Most lymphocytes in psoriatic lesions have the CD45RO marker of memory T cells and express large amounts of CD2. Alefacept blocks the binding of CD2 to LFA3 and may even kill the T cells in the lesion.[82,100] Etanercept consists of the extracellular domain of the tumor-necrosis-factor (TNF) receptor joined to the constant region of IgG. Its main effect is to block the receptor, thereby inhibiting the activity of TNF, a potent activator of inflammation with a key role in rheumatoid arthritis. Etanercept has been approved by the Food and Drug Administration for the treatment of rheumatoid arthritis and is under investigation in juvenile rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and a variety of other diseases in which TNF is thought to have a role.[101-103]

CONCLUSIONS

The examples I have selected for discussion show that when basic and clinical sciences are hand-in-hand companions, progress can be extraordinary. We are just beginning to reach the point at which the union of molecular biology, genetic engineering, and genomics will create exceptional opportunities for further advances. It is essential, however, not to allow these dazzling enticements to blind us to our primary goal; our patients must remain the central figures in this endeavor so that progress in immunology leads to true benefit to patients.

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