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The Immune Response to Agents That Cause Acute and Chronic Diseases

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INTRODUCTION

Lymphocytes circulate freely through the blood and lymphatic vessels of the body and are directly responsible for all specific immune responses. Each expresses a receptor molecule capable of binding to a chemical determinant. The lymphocyte population as a whole is divisible into two classes: the T lymphocytes (or T cells), so called because their maturation requires processing in the thymus and the B lymphocytes (or B cells), which are continuously generated in bone marrow and produce antibodies.

The cellular and molecular events that ensure production of specific antibodies after microbial invasion are not well understood. The first notion that made sense was the "clonal selection" theory, which essentially assumed that rules governing clonal expansion of prokaryotic cells might also apply to eukaryotic cells. According to this idea, each immunologic cell is genetically programmed to express a single receptor molecule that binds to a unique chemical determinant on the surface of a virus or bacteria. If the affinity of binding is high, the cell is stimulated to rapidly multiply and give rise to a "clone" of thousands of daughter cells. Each cell within the clone is marked by the same surface receptor and each is capable of secreting a modified form of its surface receptor, antibody, into the bloodstream. Like populations of bacteria, the fittest immunologic cells are selected to multiply. The duration and strength of an individual's immune response to chemical determinant ("antigen") depends on the number of lymphocytes that carry receptors that fit well with that determinant. According to this view, individuals that fail to respond to a foreign protein lack cells bearing receptors that bind strongly to that protein. This view of the immunological system as a defense network required to destroy foreign microbes has had important clinical implications. It has provided the framework for widespread vaccination programs and the development of various antibiotics. The result has been elimination of most acute infectious diseases as a major health problem. Today, the leading causes of disability and death are the chronic and degenerative diseases.

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The concept of the immune system as a collection of cells poised to respond to foreign invaders has not been useful in understanding these new and puzzling disorders. According to the classical view, the immune system is unable to react to the body's own tissues (referred to as "self") because it lacks cells that recognize proteins or sugars displayed on the body's tissues. According to this idea, cells bearing receptor molecules that bind to an individual's own tissues are somehow eliminated very early in development, probably before birth. This theory holds that severe immune reactions against self (autoimmunity) is caused by renegade lymphocytes that have evaded early censorship mechanisms.

These ideas do not fit with recent experimental facts: Lymphocytes from normal people contain many cells that bind to self-proteins. In a test tube, these cells ("B lymphocytes") can be stimulated to produce antibodies that attack host tissues. How are these cells prevented from doing so in normal individuals? Some of the major answers to these questions have come from studies of the class of lymphocytes called T cells.

T CELL SETS

Early experiments showed that T cells could either activate or shut off immune reactions. However, these observations did not explain how T cells mediated these seemingly opposing biologic activities.

Although all T cells look alike under a microscope, studies of their surface proteins revealed that these cells were composed of several "sets" according to selective expression of surface markers. Antibodies to these surface markers were used to separate them and study their functions [3]. Two major types of T cells were defined—one type stimulated immunity ("inducer" T cells) and a second supressed immune responses ("suppressor" T cells) [4,5]. This dissection also revealed that the intensity of inflammatory and antibody reactions is determined precisely by signals emitted by these two types of T cell. Inducer T cells are specialized to secrete a family of peptides that activate a panel of effector cells including antibody-forming cells, mast cells, osteoclasts, inflammatory cells, and precursors of hematopoietic cells.

Additional inducer peptides activate suppressor T cells which dampen the immune response by turning off inducer cells [6]. This "feedback" suppression is essential for well-controlled immune responses and prevention of reactions that destroy host tissues. These observations suggested the possible absence of immune reactions to "self" reflected continuous and active suppression of inducer cells bearing receptors for autoantigens. This was tested in several animal models. Mice depleted of suppressor cells developed a variety of autoimmune diseases; inbred strains of mice that spontaneously develop autoimmune disease each have specific defects in generation of suppressor T cell molecules [7, 8].

These studies established the principle that the genetic program of each specialized cell type combines information for a unique cell surface glycoprotein or "marker" and a particular physiologic function. This principle was rapdily extended to define human inducer and suppressor T cells according to expression of unique surface markers (9–12). Widespread screening of T cell sets in the blood of different patient populations by inducer of suppressor cells is a hallmark of several chronic diseases. Loss of surpressor T cells is currently the best prognostic sign of acute autoimmune attacks. Acute episodes of multiple sclerosis and rheumatoid arthritis in children are

preceded by a loss of T suppressor cells that normally prevent reactions against cells of the nervous system and joint spaces, respectively. For example, virtually all lymphocytes in the joint spaces of patients with rheumatoid arthritis are activated inducer cells; suppressor cells are virtually undetectable. Moreover, these inducer cells secrete large amounts of peptides that activate mast cells and monocytes to produce material that destroys cartilage and bone. Although steroid compounds and prostaglandin inhibitors dampen these mast-cell products, they are not effective at long-term control of this chronic inducer T cell defect [13–18].

T CELL PRODUCTS

The past 10 years has witnessed major conceptual advances in immunology. However, less progress has been made in defining endogenous regulatory molecules secreted by suppressor and inducer T cells. This requires continuously growing homogenous cells that secrete large amounts of protein. For example, homogeneous tumors that secrete immunoglobulins (myelomas) allowed definition of the antibody molecules. Unfortunately, neoplastic T cells have not proved so useful. Many do not grow well in continuous culture and very few express immunologic function.

A more successful approach has come from fusion of normal T cells to T cell lymphomas with polyethylene glycol. Several groups have reported that such fusions result in hybrid cells that suppress or induce antibody responses. However, hybrids resulting from fusion of T cells with T cell lymphomas usually express the phenotype of the tumor-cell partner and tend to lose chromosomes in cell cultures. Biochemical analysis of stable hybrids that express antigen-specific T cell functions has also been hindered by the extremely small amounts of antigen-specific material synthesized by T hybrid cells [19, 20].

The central technical advance in this area has been the development of a general method to produce "carbon copies" of individual murine or human T cells: clones. All of the cells within a clone are derived from a single parent cell; each expresses the characteristics of the parent and all are identical to each other. Inducer and suppressor clones have been separately developed and each continue to secrete proteins that either specifically induce or suppress immunity; both have been grown to very large numbers and can be frozen for storage. Clones of T-suppressor cells have already provided the first cellular source for identification and purification of molecules that suppress immunity to foreign substances. The aim of this effort is to define the structural and genetic basis of the different activities of endogenous immunoregulatory molecules.

The following is a partial summary on the progress made so far.

DEVELOPMENT OF CULTURE CONDITIONS THAT SUPPORT CLONAL GROWTH OF T CELL SETS

The procedure does not require hybridization to tumor cells, and cloned cells can be frozen for storage without loss of function upon thawing and regrowth. These cloned cells express the molecular labels and functional properties of T cells at progressive stages of differentiation [21].

In all cases, a cloning efficiency of 10–85% was achieved, indicating that these clones are a representative sampling of normal T cells. The majority of these cells also have normal karyotypes and carry stable cell-surface markers of either precursor

cells or mature T cell sublines. Each clone can be expanded to large numbers ($> 10^8$) to provide large quantities of material for biochemical and genetic analysis. Some cloned cells from fetal liver and bone marrow express surface glycoproteins associated with thymocyte precursors while others represent clones of mast cells. Another cloned cell type derived from bone marrow resembles precursors of T cells because it acquires surface glycoproteins of more mature T cells after incubation on irradiated thymic monolayers and because these cells migrate to the thymus after intravenous injection into mice.

Clones of mature T cells carry the molecular labels of inducer or suppressor T cells and continue to express these specialized functions (see below). They represent unique material for analysis of molecules and genes responsible for the differentiated functions of regulatory T cell sublines.

MOLECULES SYNTHESIZED BY INDUCER T CELL CLONES

Inducer T cells in normal lymphocyte populations are equipped to recognize a set of cell surface molecules encoded by major histocompatibility complex (MHC) genes as "self-marking" molecules and are activated by variants of these molecules that have been altered by close association with foreign proteins or sugars [22, 23]. Antigen recognition by inducer cells is at least as precise as that displayed by antibody molecules. Insulins from beef and sheep have virtually identical tertiary structures and differ at a single amino acid (residue 9 on the A chain). Clones of inducer cells expanded after immunization with beef insulin are activted to divide by beef insulin but not sheep insulin. These and other data demonstrate that a single inducer T cell can discriminate between proteins differing at a single amino acid [24]. The molecular basis of this discrimination is discussed below.

Although it is clear that T inducer clones bear receptors that allow discrimination between peptides that differ by as little as one or two amino acids, there is less evidence for *secretion* of antigen-binding inducer molecules that might locate and trigger only B cells that bind to antigen. One reason to suspect that such antigen-specific inducer molecules play a central part in B cell stimulation comes from analysis of T-inducer clones that are specifically activated by cow but not sheep insulin. First, peptides synthesized and secreted by these cells bind to cow insulin with a K (or size) of approximately 10^{-9}M, and to sheep to cow insulin with a K of 10^{-6}M [24]. Second, activation of B cells by inducer clones requires an antigen "bridge" between the correct (antigen specific) B cell and the monoclonal inducer peptide. The most likely explanation is that this inducer clone produces antigen-binding molecules that locate and trigger only those B cells which have bound the same antigen and display it on their surface.

After stimulation, inducer T cell clones stimulate division and/or maturation of many other cell types, including B cells, suppressive and cytotoxic T lymphocytes, macrophages, and precursors of hematopoietic cells. Clones of T inducer cells activate one or another of these target cell populations by secretion of a family of peptides. Each peptide is specialized to trigger a particular target-cell type [21, 25–27].

Virtually all inducer clones examined so far secrete a characteristic family of approximately 8–12 polypeptides and these are clearly different from the family of peptides secreted by suppressor T cells [21, 28]. One of these peptides has an apparent

M_r (size) of 30,000 according to sephacryl chromatography and initially fit previous descriptions of factors that stimulate growth of cytotoxic and possibly suppressive T cells. However, further biochemical analysis of this molecule has shown that it is composed of two subunits having MW of 16 Kd and 14 Kd. Mitogenic activity is carried by the 14-Kd chain, while the 16-Kd chain focuses the 14-Kd growth peptide onto T lymphocytes and not other target cells [25].

Inducer molecules that activate B cells to divide and secrete antibodies have also been examined. A polypeptide having an apparrent molecular weight of 45 Kd and pI of 6.0 stimulates B cells to secrete immunoglobulin (Ig) but not to divide [21, 25]. This 45-Kd protein is normally associated with the 14-Kd mitogenic peptide described above. The intact 60-Kd molecule selectively activates B cells to both divide and to secrete Ig and has no effect on other types of lymphocytes [25]. These peptides, as well as other inducer molecules responsible for stimulation of hematopoietic cells, have been purified to homogeneity and antibodies have been made against them to identify expression of these products in cDNA libraries from inducer clones.

DEFINITION OF CELLS AND MOLECULES RESPONSIBLE FOR SPECIFIC IMMUNOLOGIC SUPPRESSION

Cl.Ly23/4 is a sheep red blood cell-specific T-suppressor (Ts) clone that expressed surface receptors for the glycophorin expressed by sheep erythrocytes [28]. Analysis of this T cell clone has suggested that these cells share several major characteristics with antibody-forming B cells. Suppressor T cells and B cells display a similar number of surface receptors that bind to antigen in the absence of major histocompatibilty complex (MHC) products. Both respond to signals from inducer T cells by secretion of antigen-binding proteins. Cl.Ly23/4 in $Ly2^+$ secretes 70-Kd proteins that bind to antigen and mediate suppression. Picogram amounts of purified antigen-binding proteins specifically inhibit an ongoing immune response to the antigen [29].

Degradation of immunoglobulins with proteolytic enzymes has provided important insights into the structural basis of this molecule's biologic activity and specificity for antigen. We used this approach to study the functional organization of the 70-Kd suppressor protein [30]. Although the 70-Kd protein is sensitive to digestion by several different proteases, including pepsin and trypsin, papain yielded the most reproducible cleavage; this enzyme splits almost 100% of the 70-Kd antigen-binding molecule into two subunits, MW 45Kd and 24 Kd. Both were resistant to further degradation and represented 70% to 85% of the fully digested 70-Kd product. Since this was a reproducible characteristic of the protein, we defined the biologic activity of the two cleavage products. The 45-Kd subunit retained suppressive but not binding activity; the 24-Kd peptide lacked suppressive activity but retained specific binding to antigen. Further analysis showed that binding of the intact 70-Kd protein to antigen also results in production of these two 45-Kd and 24-Kd subunits, and these express the same biologic activities as the two peptides obtained after enzyme digestion of the parent molecule. The two subunits can also be distinguished serologically. An antibody made against myeloma proteins that recognizes V_H sequences of immunoglobulins reacts with the 70-Kd parent molecule and the 24-Kd but not the 45-Kd subunit. A rabbit antibody that reacts with several other 70-Kd Ts molecules specific for different antigens binds to the 45 Kd but not the 24 Kd subunit of the Ts molecule [30].

TABLE I. Properties of Two Different Domains of T-Suppressor Molecules

	Molecular Weight	PI	Antigen binding	Suppression		Antibody reactions	
				Specific	Nonspecific	Anti-TSF	Anti-V_H (Ig)
Purified Ts	70 kd	5.0	++	++	−	+	+
Peptide A	45 kd	5.6	−	−	+	+	−
Peptide B	24 kd	?	+	−	−	−	+

The combined activities of the separate 45-Kd and 24-Kd peptides account for the biologic activity of the parent molecule and the two peptides probably represent two distinct domains of the Ts protein (Table I). Although it is formally possible that the 24-Kd peptide is a breakdown product of the 45-Kd peptide, this is unlikely since the isolated 45-Kd peptide is completely resistant to further treatment with papain. Nonetheless, definitive evidence that the 45-Kd and 23-Kd peptides represent independent domains of the 70-Kd parent molecule requires amino acids sequencing and analysis of the mRNA that codes for the 70-Kd molecule.

Papain digestion of Ig chains produces two fragments, Fc and Fab. The Fab monomer (H chain) has an average MW of 22 Kd, carries the antigen-binding site, and contains sequences encoded by V_H genes. The Fc fragment has a mean MW of 50 Kd, mediates the biologic activity of the different classes of immunoglobulins and is encoded by C genes. Each C-gene product displays characteristic "isotypic" determinants which are serologically defined by antibodies. T-suppressor (Ts) molecules purified from cloned T cells also appear to consist of two functionally distinct domains: as judged by papain digestion: a V region (23 Kd) that binds speicfically to antigen but lacks suppressvie activity, and a C region (45 Kd) that does not bind antigen but suppresses antibody responses to a variety of antigens. As noted above, the 45-Kd protein of this molecule appears to share serologic determinants with partially purified Ts proteins which are specific for other antigens [30]. Since these determinants are not detected on T-inducer proteins, they may represent isotypic determinants on T cell molecules that suppress immune responses.

FUNCTION

Picogram amounts of purified 70 Kd Ts protein turns off the entire response to a complex cellular antigen even when administered after immunization to the antigen. More recent experiments have shown that in vivo administration of nanogram amounts of the 70-Kd protein preempts an ongoing antibody response to sheep erythrocytes and possibly to proteins (such as autoantigens) that are covalently attached to these red cells. The structural properties of this molecule suggest the following mechanism to account for its biologic activity: Binding of the 70-Kd molecule to T inducer cells that have bound to correct antigenic determinant is followed by increased sensitivity of the Ts molecule to surface protease on target T inducer cells and release of the 45-Kd subunit. This subunit suppresses both antigen-specific T inducer cells as well as other T inducer cells which have bound to closely associated determinants displayed by the foreign erythrocyte. The net effect of this reaction is suppression of an immune response to a complex foreign cell by a monoclonal Ts molecule specific for one site on the cell. An important feature of this mechanism is that it ensures efficient

suppression of immunity to complex foreign cells or molecules by a relatively small number of Ts clones.

SUMMARY

Recent advances in lymphocyte technology allow production of large amounts of homogenous T cells which create immunoregulatory peptides. This means that it is now possible to define and purify nontoxic peptides that either specifically turn off or turn on immune responses. For example, monoclonal peptides synthesized by inducer cells each activates a different target cell to divide or differentiate. One activates stem cells to differentiate into red cells and white cells [27], another stimulates B cells to secrete antibody [21], and another induces mast cells to divide [26] and perhaps to differentiate. More recent work has shown that some inducer peptides may "fine tune" the immune response: Certain types of inducer clones, for example, selectively stimulate production of IgA. Peptides that mediate the activity of these clones are the subject of intense analysis of because these monoclonal substances offer the possibility of stimulating rapid induction of IgA after infection by microbes that enter through mucosal of the gut, bladder or lungs. This type of antibody (IgA) is the body's key defense against infections by these microbes: Development of a rapid and specific IgA response is the most important factor in the outcome of infections by viruses such as genital herpes type II and infections by intracellular bacterial pathogens that are currently resistant to treatment by antibiotics.

Perhaps the most informative point that has come from these studies is that each peptide that has been isolated from T cell clones exerts powerful regulatory effects on either the intensity or type of the immune response. The hope is that some of these immunoregulatory molecules or their analogs can be used as potent therapeutic agents for some chronic diseases. Since purified inducer and suppressive peptides will be available in large amounts within the next several years, it will not be long before this strategy can be thoroughly evaluated.

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