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Specific Regulation of Immune Responses by Products of T Cell Clones

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THE INTENSITY and type of an immune reaction do not simply reflect the number of antigen-specific cells. The response is regulated by two major types of T cells: inducer cells and suppressor cells. Although these T cells are morphologically indistinguishable, inducer and suppressor T cells express characteristic patterns of glycoproteins at their cell membrane (1). Examination of inducer and suppressor T cells after separation according to these molecular labels had indicated that both express receptors for antigen and that cell supernatants or cell extracts can mimic the antigen-specific functions of intact cells (6). However, analyses of these materials have not defined the genes and molecules responsible for antigen-specific T-cell function.

These questions have been difficult to answer largely because of technical reasons. A biochemical explanation of the function and specificity of antibodies has come from analyses of neoplastic B-cell clones (myeloma cells) that secrete different types of immunoglobulins. Unfortunately, neoplastic T-cell lines 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 can produce hybrid cells that suppress or induce antibody response (10, 11). 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 to date by the extremely small amounts of antigen-specific material synthesized by T-hybrid cells.

By using a method that allows production of many continuously propagatable cloned cells, we have defined both helper and suppressor T-cell clones (8). Cl.Ly23/4 is an SRBC-specific T-suppressor clone that expressed surface receptors for the glycophorin expressed by sheep erythrocytes (4). 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 histocompatibility 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 production of antibody in response to the antigen (5). In addition to the 70 kd protein, 45 kd and 24 kd proteins have been routinely observed under conditions where proteolysis was not carefully controlled (4). Fractionation of biosynthetically-labeled supernatant proteins by size in Sephacryl S-200 frequently demonstrated small amounts of 24 kd peptides that bound specifically to SRBC but did not suppress, as well as 45 kd peptides without binding activity but capable of suppressing PFC responses to all erythrocytes tested (albeit less efficiently than the 70 kd fraction) (5). Interaction of the 70 kd protein with antigen markedly increases its susceptibility to degradation, resulting in breakdown into two major peptides, molecular weight (MW) 45 kd and 24 kd, according to mobility in SDS-PAGE.

Degradation of immunoglobulins with proteolytic enzymes has provided important insights into the structural basis of this molecule's biologic activity and specificity for antigen (2, 9). We used this approach to study the functional organization of the 70 kd suppressor protein (3). 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 the 70 kd antigen binding molecule into two peptide subunits of 45 kd and 24 kd. These subunits were relatively resistant to further degradation and represented 70% to 85% of the digested product after 5 to 120 minutes of digestion. Since this restricted cleavage of the 70 kd protein was a reproducible characteristic of the protein, we defined the biologic activity of the two cleavage products. The 45 kd subunit retained suppressive activity, although activity was 5- to 50-fold less potent than the intact 70 kd peptide; the 24 kd peptide bound specifically to SRBC, but did not suppress. Thus, the two breakdown products obtained after papain digestion displayed the same size and biologic activities as the 45 kd and 24 kd peptides obtained after incubation of 70 kd material with antigen. The two peptides were also distinguished serologically. An antiserum made against myeloma proteins, MOPC 315, that probably recognizes framework regions in V_H sequences reacted with the 70 kd parent molecules and the 24 kd peptide. A rabbit serum prepared against TNP specific T-cell suppressor factor that reacts with determinants on several T-cell

TABLE 1

Properties of two different domains to T-suppressor molecules

	Molecular Weight	PI	Suppression			Serologic Reaction	
			Antigen Binding	Antigen-Specific	Nonspecific	aTSF	aFv
Purified Ts	70 kd	5.0	++	++	−	+	+
Peptide A	45 kd	5.6	−	−	+	+	−
Peptide B	24 kd	?	+	−	−	−	+

suppressor factors specific for different antigens (7), reacted with the 70 kd molecule and the 45 kd, but not the 24 kd subunit.

The combined activities of the separate 45 kd and 24 kd peptides after papain digestion of the 70 kd protein accounted for the biologic activity of the parent molecule and the peptides probably represent two distinct domains of the Ts protein (table 1). However, it is formally possible that the 24 kd peptide is a breakdown product of the 45 kd peptide. This is unlikely, since the ratio between the 45 kd and 23 kd peptides remains approximately equal and constant throughout the entire period of papain digestion (5 to 60 minutes). Moreover, if the peptides resulted from sequential cleavage, the "first" enzymatic cut of the 70 kd protein must result in a 45 kd product that has lost both antigen binding sites and V_H determinants while a "second" cleavage of the 45 kd protein yields a 23 kd peptide which re-expresses both sites. Nonetheless, definitive evidence that the 45 kd and 23 kd peptides represent independent regions of the 70 kd parent molecule requires amino acid sequencing or peptide mapping of the three molecules.

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, carries the biologic activity of different classes of immunoglobulins, and is encoded by C genes. Each C gene product displays characteristic "isotypic" determinants and can be serologically defined by antibodies. T-suppressor (Ts) molecules purified from cloned T cells also appear to consist of two functionally distinct domains formed after cleavage by papain: a V region (23 kd) that binds specifically to antigen but lacks suppressive activity and a second C region (45 kd) that does not bind antigen but suppresses antibody responses to a variety of antigens. The 45 kd protein of this molecule appears to share serologic determinants with partially purified Ts proteins that are likely to be specific for other antigens (3). Since these determinants are not detected on proteins synthesized by cloned inducer T cells, they may represent isotypic determinants on T-cell molecules that suppress immune responses.

Picogram amounts of a purified monoclonal 70 kd protein suppress the entire antibody response to a complex cellular antigen (4, 5). The structural properties of this molecule suggest the following mechanism to account for "global" suppression by single T-cell molecule: binding of the 70 kd protein to Th cells that display the correct antigenic determinant is followed by increased sensitivity of the Ts molecule to surface protease on target T-helper cells and release of the 45 kd subunit. This subunit suppresses both antigen-specific target Th cells as well as other Lyl Th 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 protein by Ts molecules specific for one or several sites on the foreign protein. An important feature of this mechanism is that it ensures efficient regulation of immunity to a large array of foreign molecules by a relatively small number of Ts clones.

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