PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 171, 127–145 (1982)

The Antigen Receptor of Thymus-Derived Lymphocytes: Progress in the Characterization of an Elusive Molecule (41488)

JOHN J. MARCHALONIS[1] AND JEFFREY C. HUNT

Department of Biochemistry, Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina 29425

Abstract. Thymus-derived lymphocytes (T cells) show remarkable specificity in their capacity to recognize non-self antigens and this recognition must serve as the initial step in the differentiation of immunologically competent T cells into antigen-specific effector cells including helpers, suppressors, and cytotoxic lymphocytes. The problem of determining the molecular nature of the receptor for antigen on these cells is a challenging area of investigation, and considerable insight into the serological and molecular properties of this receptor has recently been obtained using antibodies directed against immunoglobulin combining site regions as probes for the detection and isolation of the T-cell molecules. This review stresses results obtained within the past 3 years and (1) addresses the expression of immunoglobulin variable region determinants on T-cell receptors and factors, (2) presents a serological and molecular comparison of the structure of T-cell antigen-specific regulatory factors with those of receptors, and (3) presents a theoretical discussion of the genetics of antigen-specific T-cell factors and receptors. A pattern is emerging which indicates that T-cell receptors and some factors have a combining site which is related to immunoglobulin heavy chain variable regions. These molecules apparently do not bear determinants specified by the major histocompatibility complex (MHC), but express Ig-related variable regions and constant regions unique to T-cell products. The genes encoding these antigen-specific molecules (receptors, helper and suppressor factors) apparently are associated with the immunoglobulin heavy chain gene cluster. The intact V_H-related T-cell molecules have a subunit mass of approximately 68,000 daltons and can form disulfide-bonded dimers. Studies using proteolytic enzymes, coupled with antigenic and functional analyses, indicate that the molecule is composed of domains resembling those of immunoglobulin heavy chains, although the T-cell molecule does not bear classical heavy chain isotypic determinants. The formation of active suppressor or helper factors often requires association of V_H-related molecules with MHC-encoded proteins.

Thymus-derived lymphocytes (T cells) exhibit an exquisite specificity in their capacity to recognize antigen, and this recognition must serve as the initial step in the differentiation of immunologically competent T cells into antigen-specific effector cells such as helpers, suppressors, and cytotoxic lymphocytes. The problem of determining the molecular nature of the surface receptor for antigen on T cells is a challenging area of investigation and has been one of the major unresolved issues in modern immunology. It has been possible to gain considerable insight into the serological and molecular properties of this receptor using antibodies directed against combining site regions of immunoglobulins as probes for its detection, and for its isolation using immune affinity chromatography of T-cell receptor molecules. Antigen-specific T-cell immunoregulatory molecules such as helper and suppressor factors also have been shown to express determinants serologically related to variable regions of immunoglobulin heavy chains. A number of comprehensive reviews have been written regarding the problem of the T-cell receptor for antigen (1–6). In this brief review, we will focus predominantly upon data generated in the past 3 years and consider the following major issues regarding the T-cell receptor for antigen: First, the evidence supporting the existence of T-cell products

[1] To whom all correspondence should be addressed.

0037-9727/82/100127-19$01.00/0

related to immunoglobulin variable regions will be reviewed. Second, the relationship between T-cell receptors and T-cell released effector factors will be analyzed. Third, recent data regarding the characterization of isolated receptors and factors will be reviewed. Finally, based upon the molecular characterization data presently available, we will describe tentative models for the polypeptide structure of the T-cell receptor which is related to immunoglobulin heavy chain variable regions, and discuss possible models for the arrangement of genes encoding variable and constant regions of T-cell receptors and their relationships to immunoglobulin heavy chain V and C genes.

Existence of T-Cell Receptors and Factors Serologically Related to Immunoglobulin Variable Regions. Although a number of studies performed in the early 1970s indicated that some antisera directed against immunoglobulin determinants reacted with T-cell products and could be used in the isolation of immunoglobulin-related T-cell surface markers (6–11) and factors (12, 13), the location of these determinants on the immunoglobulin-related molecule was not established. In retrospect, it would appear that the original anti-immunoglobulin sera that were used to isolate immunoglobulin-related T-cell products must have done so because of a cross-reaction with variable region determinants because the ability of such sera to recognize determinants on T-cell molecules was not related to class-specific markers (6). Furthermore, subsequent studies established that such antisera were reactive with determinants lying in the Fd fragment of purified heavy chains (15), and also were associated with either interaction determinants formed by combination of V_H and V_L (16) or with restricted variable region framework determinants (17). Two types of antisera have been very useful in establishing a relationship between serological properties of the T-cell receptor and those of the combining site region of antibodies. The first type was the production of antibodies directed against idiotypic determinants on specific antibodies; the second type of reagent consisted of antisera directed against V_H framework determinants or to "non-idiotypic V_H-related determinants" which are localized to some presently unspecified area of the variable region of heavy chains. Table I gives a partial listing of characterized idiotypic determinants which have been detected either on the surfaces of T cells or an antigen-specific T-cell factors. *More than 13 defined idiotypic determinants have been found to be shared between antibodies and T cells or T-cell products exhibiting corresponding specificity.* A range of idiotypic specificities have been detected which include (a) specificity for defined low-molecular-weight haptens such as NP and the arsonate hapten, (b) specificities directed against naturally occurring proteins such as hen egg-white lysozyme, (c) specificities directed against synthetic polypeptides such as GAT and (T,G)A—L, (d) specificities for polysaccharides, and (e) specificities directed against MHC alloantigens in mice and rodents. Moreover, idiotypes cross-reactive with those of human myeloma immunoglobulins have been detected on the surface of certain peripheral T cells in man and on isolated idiotype-bearing receptors (40, 41). The question whether or not the idiotype-bearing surface molecules or factors detected were synthesized by the T cells has been answered affirmatively by a number of approaches (3, 4, 28, 30), and recent studies involving the production of T-cell hybridomas *in vitro* also strengthens the conclusion that T cells can synthesize and express idiotype related molecules (22, 31, 42, 43). The range of defined specificities and idiotypes of T cells suggests that, first, the recognition repertoire of antigen-specific T cells is diverse, and, second, that the antigen-specific products of T cells are serologically related in their combining site region to that of antibodies of corresponding specificities. It might be argued that the second property could arise by chance; that is, all proteins which bind a defined ligand with a certain affinity might be expected to have a similar geometry in the combining site and therefore have similar serological (or "idiotypic") properties. This argument

TABLE I. PARTIAL LIST OF IDIOTYPES SHARED BETWEEN T CELLS AND ANTIBODIES OF CORRESPONDING SPECIFICITIES

"Common name" of idiotypic marker	Specificity	Reported occurrence on	
		T cells	T-cell factors
NP	(4-Hydroxy-3-nitrophenyl)acetyl	Suppressor T-cell hybrid; specific T hybridomas (104)	Antigen-specific isolated receptor (4, 18, 19)
	Timothy allergen	Suppressors (20)	Antigen-specific (20) helper factor
HEL	Hen eggwhite lysozyme	Suppressors (21)	
(T,G)-A—L	Tyramyl-glutamyl-alanyl-lysine	Helper hybridomas (22)	Specific helper factor (23)
tyr(TMA)	L-Tyrosine-*p*-azophenyl-trimethylammonium	Antigen-binding T cells (24)	
ARS(Ar)	*p*-Azophenylarsonate	Antigen-binding T cells (25, 26)	Specific suppressor factors (27); biosynthetically labeled receptors (28, 29)
R 5,936	B6 anti-CBA antibodies (MHC specific)	Alloreactive T blasts (30)	Antigen-specific isolated receptor (30)
GAT	L-Glutamic acid ^{60}-L-alanine ^{30}-L-tyrosine^{10}		Specific suppressor factor (31, 32)
	Human γ globulin		Specific suppressor factor (33)
TEPC 15	Phosphoryl choline	Helpers (34, 35); delayed-type hypersensitivity (36)	
A5A	Streptococcal polysaccharide A	Helpers (4, 18) suppressors (4, 18)	
Nase	Staphylococcal nuclease	Helpers (37)	
	Directed against combining sites of rat anti-MHC alloantibodies	Alloreactive T cells (5)	Receptor (38, 39)
Idiotypes of human myeloma immunoglobulins	Unknown (40); anti-horse α_2-macroglobulin (41)	Peripheral T cells (40, 41)	Isolated idiotype-bearing receptors (40, 41)

is essentially one of convergence in which molecules lacking common ancestral genes might have evolved similar structures because of common function. The second explanation would be one of direct evolutionary homology. Since hundreds of millions of years of evolutionary time were occupied in the generation of a variable gene genetic system to generate antibody diversity, it is reasonable to expect that antigen-specific lymphocyte products including antibodies and T-cell receptors would express combining sites encoded by the variable region genes.

Possible examples indicating convergence have been reported, e.g., a sharing of idiotype between C-reactive protein and the phosphoryl choline-binding immunoglobulin HOPC 8 (44), the unexpected cross-reaction between antibodies to the TEPC 15 V_K light chain and the Thy-1 alloantigen (45), and the finding of a ubiquitous lymphocyte-associated protein which apparently shares the Ar cross-reactive idiotype as well as Ia antigenic determinants (46). However, *the more reasonable conclusion at this time is that the T-cell receptor molecules and factors express combining site determinants encoded by genes related to those of immunoglobulin heavy*

chain variable regions. This conclusion follows from the diversity of V_H-related idiotypes expressed by T cells. It appears possible that a sharing of "idiotype" might appear once or twice by chance; but it appears inherently improbable that such an event would happen at least 14 times. A second point which indicates that the combining sites of antigen-specific T-cell products most probably resemble immunoglobulin variable region-related idiotypes is that T cells recognize immunoglobulin idiotypes, whether expressed on cells or on antibody molecules (47, 50). Current data show that helper T cells and their products are specific for antigen and express the idiotype of the corresponding antibody (19, 23, 30), whereas suppressor T cells can be either specific for antigen and express idiotype (primary suppressors (27, 32)) or can react with idiotype, rather than with antigen (secondary suppressors (47–50)). Since idiotypes are usually formed as conformational determinants which require interaction between V_H and V_L (51–53), the fact that T cells and T-cell molecules (especially products of secondary suppressor T cells) can react with conformational determinants further suggests that the T-cell receptors, like antibody combining sites, recognize three-dimensional shapes. This issue is worth considering because it has been reported that T-cell antigen receptors differ from antibodies in recognizing short, linear stretches of amino acids in denatured proteins, as shown in immune response gene effects expressed in macrophages (54). The property of recognizing short linear stretches of amino acids resembles properties of proteases (55), rather than those of antibody.

The above data support a prima facie case for a sharing of idiotypic combining site determinants between antigen-specific T cells and their products, and antibodies of corresponding antigen specificities. In addition, although T-cell receptors and factors have not been found to express any of the known immunoglobulin constant regions, they have been shown to share a number of other properties with those of defined immunoglobulin variable regions. As of this time, there is persuasive evidence for a relationship between antigen-specific T-cell products and heavy chain variable regions, but the evidence for a relationship with light chain variable regions is much less certain. This particular problem is difficult to resolve because many of the idiotypic determinants studied above require interaction with the proper light chain variable region to form the combining site. Other properties shared between T-cell receptors and immunoglobulin heavy chain variable regions are as follows: association with allotype (30, 56), heterocliticity and fine structure for hapten binding (56), and nonidiotypic V_H-related determinants (57–60). Cramer *et al.* (56) maintain that the V_H regions of T-cell antigen-specific receptors share both framework and complementarity determining regions with V_H as expressed by B cells and antibodies.

T-cell hybridomas have been constructed which produce suppressor factors specific for the protein keyhole limpit hemocyanin and express a membrane-associated antigen-specific receptor. The specific suppressor factors bear an antigenic determinant detected using rabbit antisera made against the V_H region of the murine myeloma protein MOPC 315 (61). A point which will be considered below with respect to antigen-specific T-cell factors is the association of such functional molecules with products of the major histocompatibility complex; a finding which has been observed in the case of keyhole limpit hemocyanin-specific suppressor factors produced by hybridomas, and in the case of the (T, G)-A—L specific helper factor and the GAT-specific suppressor factor described in Table I above.

The present data indicate that *the spectrum of V_H-related molecules expressed by T cells is most probably not identical to that of the entire V_H pool, but may represent a restricted subset of this pool.* Evidence for this follows from the restricted expression of molecules related to the TEPC-15 idiotype by T cells (35), and from the expression of the NP idiotype by helper T cells and their factors. Apparently only V_H is required for the NP-idiotype of T cells,

whereas the serum antibody requires both the presence of the proper V_L and the proper V_H (56). In our hands, we find that some human T-cell tumor lines of amplifier phenotype express a V_H-related determinant which comprises about 5% of the total human V_H pool and most probably is defined by amino acid sequence lying between residues 23 and the end of the heavy chain variable region (J. J. Marchalonis, J. C. Hunt, G. R. Vasta, unpublished observations).

Table II presents a comparison between properties of T-cell variable regions and variable regions of antibodies or B cells. As described above, a number of idiotypic determinants shared with antibody molecules have been described for murine T cells and T-cell products, and idiotypic receptors have been described on human T cells. The rabbit differs from man and mouse in expressing allotypes in the variable region, and rabbit T-cell receptors have been described which bear the V_H allotype (62). It is worthwhile to note at this point that all investigators do not find V_H-related receptors on rabbit T cells. Jensenius *et al.* (63) do not find evidence for V_H (a allotype) markers occurring in the absence of light chains (b allotypes) by quantitative immunoassay, although they find large ($>10^4$ molecules/cell) numbers of Ig molecules in purified T-cell populations. They interpret their results to establish that V_H molecules found on T cells must represent B-cell contamination, and assert that all positive results obtained by other workers in any system must be due to either contamination of preparations with B cells or to use of poorly characterized antisera. Unfortunately, these workers present no data regarding the properties of an alternative recognition molecule. It is possible that the V_H determinant expressed on rabbit T cells and their products (62) is not a major a-allotype marker and that some antisera, thus, might not detect it. Furthermore, the rigid assumption that a-allotypes should be found on T cells only the complete absence of b-allotypes is unwarranted because the conformation of free V_H is different from that observed in the native state where it is noncovalently associated with V_L structures.

The murine T-cell receptor for the hapten NP resembles the NP-specific serum antibody of the same strain in showing a heteroclitic response in which the hapten nitroiodophenyl binds better than the original NP immunogen. Heterocliticity, like idiotype, is dependent upon the structure of the complementarity determining regions (hypervariable regions) within the antibody variable regions. In addition to idiotypic and heteroclitic properties, murine T cells and T-cell factors also express determinants which are associated with framework residues on murine heavy chain variable regions (56). Other determinants which can be localized to the heavy chain variable re-

TABLE II. COMPARISON BETWEEN PROPERTIES OF T-CELL AND ANTIBODY (B CELL) VARIABLE REGIONS

Feature of T-cell "variable" region	Association with antibody variable regions
(1) Idiotype (mouse, man)	V_H/V_L interaction (52); V_H (56); complementarity determining regions (56)
(2) Allotype (V_H)	Framework sequences (78) variable D-J-C_μ interaction (78); rabbit
(3) Heterocliticity ("fine structure"); mouse	Complementarity determining regions (56), e.g., anti-NP reacts better with NIP than with NP
(4) Framework, e.g., rabbit anti-mouse V_H	Framework sequences (79)
(5) Nonidiotype; restricted determinant	V_H nonidiotype, location unknown: mouse (79), man (58–60)
(6) Genetic linkage to C_H allotypes	Genetic linkage to C_H allotypes (5, 56, 80)

gion and which are not idiotypic have been described for T-cell products of mouse and man (57–59). Genes specifying idiotypic T-cell variable regions have been found to be genetically linked to allotypes of immunoglobulin constant regions (64–66) in a parallel fashion to that well established for V_H and C_H genes encoding immunoglobulins.

This section, which is based upon the serological properties of the antigen-specific T-cell receptor, indicates that T-cell antigen receptors possess a variable region exhibiting remarkable similarity to antibody variable regions. However, it is still possible that T-cell variable regions are not identical to the V_H structures expressed by antibodies. T-cell variable regions may represent a subset of the total V_H pool, or they may represent molecules similar to the primitive variable regions in evolution, rather than being directly homologous to antibody V_H structures in higher species. Moreover, although some T cells have been shown to produce messenger RNA for the constant region of μ chain, no evidence has yet been published describing V/D/J/CH rearrangements in T cells (67–69).

Receptors Versus Factors. *Although antigen-specific soluble effector factors which mediate either helper or suppressor function might share the variable region combining site determinants with the antigen-specific T-cell receptor and with the corresponding antibody, it does not necessarily follow that the constant regions or effector portions of the soluble molecules would be identical to those of the T-cell surface antigen receptor.* By analogy with immunoglobulins, it would be expected that the antigen-specific molecules of T cells would represent distinct classes (isotypes) depending upon their function. Owen and her colleagues (64–66) have found evidence for allotypes which apparently are associated with constant regions of T-cell-derived molecules. These form a family of three genes; one encoding a suppressor factor, one specifying a helper T-cell product and the other encoding the surface receptor found on immature thymic T cells (64–66). Genetic studies have shown that these genes are linked to genes specifying murine immunoglobulin constant regions and that they map downstream from the locus specifying Cα chains. A difference between receptors and factors has been defined by functional analyses; namely, *recognition defined as binding of antigen in solution is not dependent upon the MHC background of the T cells (70), whereas helper factors show a strong dependence upon the Ia (I-A) background (23) and some, but not all, antigen-specific suppressor factors require a functional association with products of I–J subregion (31, 71, 72).*

It is useful to consider the amounts of T-cell antigen-specific membrane receptors and soluble factors and to compare these quantities with other characterized membrane proteins found in lymphoid cells. Lymphocyte surface receptors usually comprise approximately 0.1 to 1% of membrane protein. This figure has been obtained for histocompatibility antigens (73), the Thy-1 alloantigen (74), and V_H-related receptors on primate T cells (75). The amount of a particular lymphocyte surface receptor computes to approximately 10,000 per cell. Factors can function at extremely small protein concentrations. For example, the monoclonal T-cell suppressor factor specific for GAT described by Krupen *et al.* (31) occurs at a calculated concentration of only 0.013 ng per mouse. This estimate represents the amount of active idiotype-bearing factor produced by a small fraction of the heterogeneous murine T-cell pool. The yield recovered from the monoclonal hybridoma line allows an estimation of the amount produced per cell. Krupen *et al.* (31) isolated two micrograms of GAT-specific suppressor factor from 6 liters of culture fluid. Assuming that the cells reached a reasonable density of 10^6/ml, and, using their molecular weight estimate of 24,000 for the specific factor, each cell on the average is calculated to release approximately 8000 molecules. This amount is consistent with those noted above for isolation of membrane receptors and is also congruent with previous observations that antigen-specific T-cell receptors are "shed"

or released from the cell surface via a metabolic process (76) which differs from the secretory process carried out by activated B cells or plasma cells. By contrast, a single plasma cell can secrete more than one million immunoglobulin molecules within an hour (77). *These calculations illustrate the difficulty inherent in obtaining large amounts of T-cell receptor because monoclonal T-cell lines express and release only the same amounts of receptor which are associated with normal T cells.* The hope of obtaining an immortalized T-cell line secreting large quantities (comparable to Ig secretion by plasma cells) has not yet been realized, although numerous antigen-specific monoclonal T-cell lines have been generated and studied (91).

Despite the relatively low yields of T-cell receptors and factors which can be isolated even from monoclonal T-cell lines, a remarkable amount of serological and molecular information has been generated for T-cell receptors and factors in recent years as summarized in Table III. This table presents data only on T-cell products which have been isolated by immune affinity chromatography and characterized to some degree by techniques such as polyacrylamide gel electrophoresis. The first five listings might be classified as T-cell surface-associated receptors which bear either idiotypic markers or nonidiotypic V_H determinants. In these five separate cases, no MHC-associated products have been detected on the isolated receptor, and a common theme is evident in that molecules of approximately the size of heavy chain (50–70,000 d) are consistently isolated. The remaining items in the table consist of factors which can be classified functionally as either antigen-specific helper or suppressor molecules which have been isolated either from sensitized normal T cells or from T-cell hybridomas. "IgT" helper factors have been described in the mouse (12) and the rat (13). The nature of the immunoglobulin-related determinant on these molecules is not clear, but these helper factors do not carry MHC determinants. Other helper T-cell factors have been shown to carry both I-A and V_H-related determinants (23), or unspecified immunoglobulin determinants such as those detected using chicken antibodies directed against the murine μ chain (81). Lonai *et al.* (82) have generated murine T-cell hybridomas producing helper factor directed against chicken gammaglobulin, and this factor expresses V_H framework determinants detected using rabbit antiserum against the V_H of MOPC 315, as well as I-A-associated determinants. A number of suppressor factors have recently been described; some of these consist of polypeptide chains of approximate mass 68,000–70,000 d and lack MHC components (84–89). On the other hand, the 68,000-d suppressor factor specific for keyhole limpit hemocyanin which is produced by a specific T-cell hybridoma described by Taniguchi *et al.* (61) apparently consists of two subunits. One of these has an approximate M_r of 45,000 d and bears V_H determinants whereas the other has an approximate mass of 25,000 and bears I-J markers. Evidence indicates that these subunits can be covalently linked by disulfide bonds in the "secreted" form, or noncovalently associated in factors extracted by cell lysis. The GAT-specific suppressor factor isolated by Krupen *et al.* (31) consists of a single chain of M_r 24,000 which is reported to carry both idiotypic and I-J markers. Although there has been considerable interest in the production and specificity of factors directed against the arsonate hapten because of the existence in A/J mice of a cross-reactive idiotype, the status of factors in this system is not clear. Both normal sensitized T cells (25, 26) and monoclonal T-cell hybridomas bearing the cross-reactive idiotype have been described (29), but confusion has resulted in regard to the nature of factors produced. One group reports the isolation, by affinity chromatography on the arsonate hapten, of a single chain molecule of mass 92,000 d which does not express V_H or MHC determinants (90), and another group reports the isolation of the single chain of approximate mass 62,000 daltons which expresses both idiotype and I-J determinants (defined using alloantisera; 29). However, the latter molecule is found in all lymphocytes (46). In our investiga-

TABLE III. PROPERTIES OF ISOLATED T CELL RECEPTORS AND FACTORS

Source	Specificity	V_H determinants	MHC	Function	Organization
Rat alloreactive T cells (5)	Alloantigens	Idiotype	NIL	Receptor	Polypeptide of M_r 70,000; can form disulfide-bonded dimers (38, 39)
Mouse alloreactive T cell (30) Lyt-1$^+$ 2,3-	Alloantigens	Idiotype (5936) allotypes (Ig-1^b)	NIL	Receptor	Single, nonglycosylated polypeptide chain; M_rs observed: 50,000; 62,000 and 75,000; "domains"
Marmoset T-cell line/ amplifier phenotype (58)	Unknown	Restricted V_H determinant shared with human myeloma heavy chains	NIL	Receptor (?)	Polypeptide of M_r 68,000–70,000; can form disulfide-bonded dimers
Human peripheral T cells	Unknown	Idiotype (40, 41); nonidiotypic V_H determinant (58–60)	NIL	Receptor (?)	Polypeptide of M_r 70,000
Sensitized mouse helper T cells (19, 56)	NP	Idiotype (V_H associated)	NIL	Receptor	M_r 150,000 (gel filtration; heavy chains (M_r 50,000), light chains (M_r 25,000)
Murine helper T cells (12)	Keyhole limpit hemocyanin (KLH)	Unspecified Ig determinants probably V_H framework (17)	NIL	Helper	M_r approx. 180,000; disulfide-bonded heavy chains (M_r 70,000)/light chains (M_r 25,000), disulfide linked (?)
Murine antigen-specific T-cell hybridoma (61)	KLH	V_H framework	I-J	Suppressor factor	M_r 68,000; I-J bearing chain (M_r 25,000) disulfide ("secreted") or non-covalently ("extracted") linked to V_H-bearing chain (M_r 45,000)
GAT-specific murine T-cell hybridomas (31)	GAT	GAT idiotype	I-J	Suppressor	Single chain M_r 24,000 carrying Id and I-J markers
CG-specific murine T-cell hybridomas (82, 83)	Chicken γ globulin	V_H framework	I-A	Helper	Major component M_r 60–70,000; minor components M_rs 35,000, 30,000; higher components (140,000; 300,000) (83)
Murine suppressor T cells (84–86)	DNP or TNP	Not tested	NIL	Suppressor delayed-type hypersensitivity	Single chain M_r 68,000
Murine suppressor T cells (87–89)	Sheep erythrocyte glycophorin	F_V	NIL	Suppressor	Single chain M_r 68,000
Murine T-cell hybridoma of suppressor phenotype (29)	ARS	Id	I-J	?	Single chain M_r 62,000; ubiquitous molecule (29)
	ARS	NIL	NIL	?	Single chain, M_r 92,000 (90)
Stimulated murine peripheral T cells (26)	ARS	Id	?	?	Single chain M_r 68,000 (28); also reacts with chicken antibody to murine Fab fragment

tions of an idiotype-bearing molecule biosynthetically produced by stimulated murine peripheral T cells, we have isolated, under reducing conditions, a single chain of 68,000 d which also reacts with chicken antibody directed against the Fab fragment of murine immunoglobulin (26, 28). At this point in time, it can only be said that the results in the arsonate system are inconclusive and further analysis of antigen binding molecules is required, particularly because of the report that a widely distributed molecule of unknown function apparently binds arsonate and expresses a cross-reactive idiotype (46).

Evidence obtained from murine (71) and primate systems (58, 59) indicates that a subpopulation of normal peripheral T cells (approximately 30% of PTL) expresses V_H-related determinants (58, 59), and that this subpopulation also tends to express I region markers (71, 92). The studies summarized above indicate that the capacity of a T cell or a released molecule to combine with antigen results from the presence of a V_H-related component, not from the presence of an MHC marker. Because we have at hand human and lower primate T cell lines which express V_H-related surface components as well as HLA-Dr (the human equivalent of Ia markers), we carried out studies designed to determine whether or not the I region and V_H-bearing components existed as a functional unit on the cell surface. Investigations involving direct isolation of either V_H-bearing (58) or HLA-Dr-bearing components (92) indicated that anti-V_H antibodies isolated components of approximately 70,000 daltons, whereas monoclonal anti-Ia isolated components of 28,000 and 32,000 d. We did not find evidence for a covalent or a strong noncovalent association between these two components in the form in which they are released into the culture fluid or as they are expressed on membrane fragments. In codistribution analyses using double immunofluorescence, we found a lack of congruity between HLA-Dr and V_H products (D. DeLuca and J. J. Marchalonis, unpublished observations). Although we cannot exclude the transient association of components of MHC and V_H systems, we did not find any evidence indicating a strong linkage between the two sets of surface components.

At this point in time, it is reasonable to conclude that the antigen-specific recognition moiety on the T-cell receptor and released effector factors is a V_H-related marker. A good deal of evidence now suggests that this component most probably has an intact subunit mass of 68–70,000 d. This component binds antigen, and bears idiotype. Evidence also indicates that it (by itself) can bind to the surface of macrophages (1), presumably by some sort of constant region structure which can bind to a macrophage receptor (which is analogous to an Fc receptor). The association of V_H-bearing products with MHC products in the generation of helper or suppressor factors appears to be involved in situations which require cell/cell interaction, and might also be expected to depend upon the type of suppressor factor under consideration. For example, helper and suppressor factors would be expected to have different constant regions adapted for their particular effector functions, which would imply the existence of different MHC associations and different types of cell/cell interactions. Furthermore, it might be expected that primary suppressor factors (which bear idiotype and are antigen specific) could differ from secondary suppressors (which are anti-idiotypic) which differ in their combining site specificity also could differ in MHC restrictions. This situation is analogous to that of antibody heavy chains where structurally and functionally distinct heavy chains (e.g., γ chain and ϵ chain) can share the same V_H structure.

Tentative Model for Receptor and Factor Structures. A number of significant issues remain to be resolved regarding the nature of T-cell receptors and factors. Among the most prominent of these are as follows: *The valence or number of combining sites of intact T-cell antigen receptor in the absence of denaturing solvents is unresolved.* Since the isolated T-cell factors will neutralize hapten-derivatized bacteriophage, the number of combining sites on the molecule or molecular complex must

be at least two (93), and the size of these isolated receptors as estimated by gel filtration is approximately 150,000, a value which would correspond to a dimer of heavy chains. *The requirement for light chain variable regions with the T-cell receptor is unclear.* Many of the antisera directed against idiotypes or V_H region determinants which react with T-cell receptor structures have a strong dependence upon the association of V_H and V_L for detection (51–53). In some cases, polypeptide chains resembling light chains have been isolated and partially characterized (1, 6, 14), although these molecules have been shown to be serologically distinct from standard κ or λ chains (17, 94) and they usually express a nominal molecular weight slightly higher than that usually observed for light chains. Third, the *detailed molecular properties of T-cell variable regions and their similarity to immunoglobulin variable regions both at the polypeptide and nucleic acid levels remains to be established.* This is a fundamental problem, and we will consider it in detail below. Even though antigen-specific T-cell products express a variable region serologically related to Ig V_H, the exact degree of homology can be answered only by detailed sequence analysis of the polypeptide and its gene.

Despite these questions to be resolved, a consensus regarding the properties of the V_H-related T-cell receptor is emerging from characterization studies being performed in many laboratories which were cited in Table III above. In particular, there is general agreement that T-cell receptors (and factors) bear serologically detectable variable regions and constant regions. Constant regions apparently unique to T-cell products have now been detected using alloantisera (64–66, 95, 96), xenoantisera (97), and hybridoma antibodies (98) produced against isolated T-cell products of man and rodent species. The T-cell constant regions are distinct from the immunoglobulin isotypic determinants, but evidence now exists for the presence of a family of related isotypes of T-cell products (64–66, 95). These will be considered in detail below. It has recently become possible to isolate (by immune affinity chromatography) sufficient quantities of T-cell products (approximately 100 μg) to allow initial molecular characterization studies. Table IV presents a comparison of the amino acid compositions of V_H-related T-cell products isolated from a monoclonal T-cell hybridoma producing GAT-specific suppressor factor (31), a long-term *in vitro* grown marmoset T-cell line of amplifier phenotype (75, 99), and an idiotype bearing murine T-cell product (30). The molecules show an overall similarity, particularly in acidic amino acids, basic amino acids, and in the hydrophobic amino acids isoleucine, leucine, tyrosine, and phenylalanine. They are very similar in the hydroxylic amino acid threonine.

Sufficient quantities of the V_H-bearing T-cell products (receptor and certain factors) have been isolated to allow characterization studies using standard techniques of protein chemistry in order to compare the structures of the T-cell molecules with one another and with classical immunoglobulin chains. A number of serologically and functionally characterized fragments have recently been generated

TABLE IV. COMPARISON OF AMINO ACID COMPOSITIONS OF V_H-RELATED T-CELL PRODUCTS

Amino acid	Residues/100 residues		
	70-N2(τ)[a]	GAT-TsF[b]	Tcr[c]
Asx	12.7	9.0	10.1
Thr	5.1	5.1	5.4
Ser	10.5	7.9	6.7
Glx	14.8	18.3	12.8
Pro	6.7	N.D.	5.0
Gly	N.D.	12.8	6.5
Ala	10.5	7.1	8.1
Val	6.2	5.5	6.7
Met	0.7	1.0	2.5
Ile	3.1	2.5	3.0
Leu	7.5	7.0	9.5
Tyr	2.6	4.0	3.0
Phe	3.4	3.3	4.2
His	3.2	2.5	3.2
Lys	8.5	7.4	7.4
Arg	4.7	6.6	3.6

[a] Data of J. J. Marchalonis, J. C. Maxwell, and C. Schwabe (unpublished observations) for V_H-related product of the *in vitro* marmoset amplifier T-cell 70-N2.

[b] Data of Krupen *et al.* (31) for GAT-specific suppressor factor from murine T-cell hybridoma.

[c] Data of Rubin *et al.* (30) for the idiotype-bearing allospecific murine T-cell receptor.

(30, 39, 75, 86, 89) by cleavage with specific proteolytic enzymes or with CNBr. Figure 1 presents data from this laboratory showing the intact 68,000-d product of a human T-cell line YT4E (Fig. 1A, lane 1) and the cleavage products generated by tryptic proteolysis of this T-cell product (Fig. 1B, lane 1) and of the corresponding product of the amplifier T-cell line 70-N2 (Fig. 1B, lane 2). Major fragments in the range 20–25,000 d are generated as is a major fragment of approximate mass 45–47,000 d. In addition, a number of higher-molecular-weight fragments are observed. The fragments in the molecular weight range 20–25,000 d react with antisera directed against V_H determinants; the major fragment of approximate mass 45,000 d is not precipitated by anti-V_H reagents. These V_H-related products of two separate T-cell lines are serologically related, but are not identical to one another. In addition (Fig. 1B), their tryptic fragments give similar but not identical patterns. Our data and those recently generated by other laboratories (30, 39, 86, 89) are summarized in Fig. 2 which gives a schematic diagram illustrating observed fragments of V_H bearing T-cell receptors. The τ chain undergoes a fragmentation pattern which indicates the presence of domains of approximate mass 12,000 d (30, 75). A V_H-bearing fragment (of approximately 24,000 d) comparable to an Fd fragment of heavy chain has been observed (75), as has a fragment of about 45–47,000 d (75, 89) which expresses effector functions similar to that shown by the Fc fragment of heavy chains (89). Notably, the Fc-like fragment of suppressor factors apparently expresses nonspecific suppressor activities (89). In addition, by following cleavage using CNBr with immune affinity chromatography it has been possible to isolate an antigenic fragment of approximate mass 12,000 d which most probably corresponds to the T-cell heavy (τ) chain variable region (75). In the cases which have been studied, the isolated τ chains have had blocked N-terminuses (1, 30, 75), a result which might indicate some relationship to either the V_HI or V_HII subgroups of heavy chains. Rubin reports that his murine idiotype bearing T-cell receptor lacks detectable carbohydrate (30), but this question has to be resolved for other T-cell receptors and factors. Using papain digestion, we (J. C. Hunt, J. J. Marchalonis, unpublished observations) have recently isolated a fragment of approximate mass 7800 d which is hydrophobic, as assessed by elution behavior from reverse phase columns (by high-performance liquid chromatography) and amino acid composition analysis. This peptide reacts with antisera directed against Fab region and V_H region determinants. It is possible that this fragment represents the disulfide-bonded loop of the variable region, and studies are in progress to establish its identity. The structure illustrated here is based upon

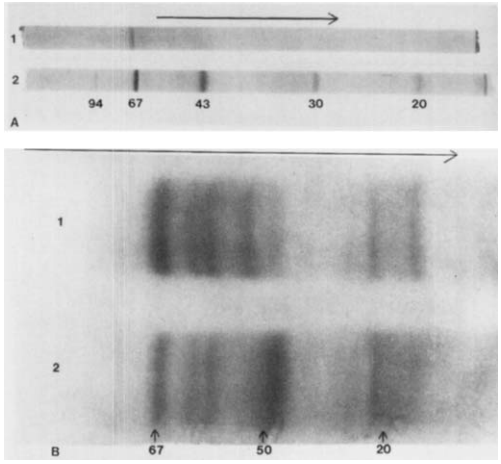

FIG. 1. Analysis by polyacrylamide gel electrophoresis under reducing conditions of intact (A) V_H-associated T-cell product and fragments produced by tryptic proteolysis of the molecule (B). (A) Lane 1: the 68,000-d component produced by the human *in vitro* grown T-cell leukemia line YT4E. This component was isolated from formic acid-solubilized membrane preparations by immune affinity chromatography using goat antisera directed against the Fab monomer fragment of a human IgM myeloma protein. Lane 2: molecular weight standards having masses as indicated on the gel. (B) Lane 1: tryptic fragments of the 68,000-d V_H-related product of the human T-cell lymphoma line YT4E. Lane 2: tryptic fragments of the 68,000-d V_H-related molecule produced by the marmoset *in vitro* T leukemia line of amplifier phenotype 70-N2. V_H-related products were isolated and digested as described in ref. (75).

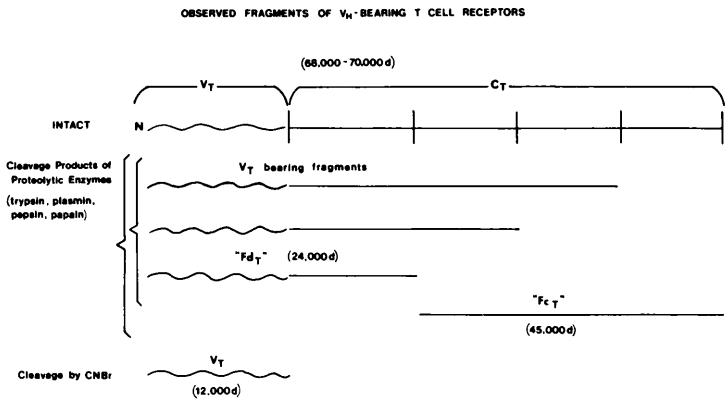

FIG. 2. Schematic diagram illustrating the proposed domain structure of V_H-bearing T-cell receptors and the organization of observed antigenic and functional fragments produced by proteolysis or chemical cleavage. This diagram pertains only to the V_H-bearing, non-MHC chain. It is likely that I-region products can be associated as separate chains with molecules of this nature in the formation of active factors or a possible recognition/activation complex on the cell surface. Documentation regarding these fragments is given in the text.

studies of receptors and the suppressor factors which are of approximate mass 68–70,000 d and lack strongly associated MHC products. It is possible that either I-A or I-J region products are associated with functional T-cell factors either as a second polypeptide associated through noncovalent linkage or through disulfide bonds. It is frequently found that products of separate chromosomes can form noncovalent multimers (hemoglobins) or disulfide-bonded assemblies (immunoglobulins). However, the question of a single polypeptide (31) expressing both MHC-associated regions (chromosome 6 in man) and immunoglobulin-associated regions (V_H; chromosome 14 in man) is a most interesting and unique one and requires further detailed analysis.

Possible Relationships between T-Cell Receptor and Immunoglobulin Genes. *Although the serological data marshalled above on idiotypes and other V_H markers indicates that antigen-specific T-cell receptors possess a combining site region showing remarkable similarity to immunoglobulin variable regions (Tables I and II above), a paradox remains because no definitive reports have yet appeared which establish that T cells possess genes consisting of rearranged V_H, D, J, and immunoglobulin CH regions (67–69).* It is now generally accepted that T cells do not express standard immunoglobulin heavy chain constant region isotypes, but it might intuitively be expected that the heavy chain expressed by T cells would show the same sort of V_H, D, J, C rearrangement that immunoglobulins do. This arrangement has not been demonstrated, and it may be that either the V_H-like products of T cells are not encoded by immunoglobulin V_H genes, or that a different arrangement or type of rearrangement has occurred in the generation of T-cell products. One important result of the serological studies which should be stressed here is that there are indications that the repertoire of V_H structures expressed by T cells most probably represents a subset of the total V_H population (35, 36), J, J. Marchalonis, J. C. Hunt, G. R. Vasta, A. C. Wang, unpublished observations). An important series of recent experiments directly germane to this question revealed constant region allotypes on products of T suppressor cells (64, 96), T helper cells (65, 96), and on T-cell primitive receptors (66). Owen and her colleagues have described a series of alleles for T-cell markers which map downstream from the C_α locus of the immunoglobulin chain cluster. Furthermore, Tokuhisa and Taniguchi (96) have also recently described two distinct allotypic determinants on the antigen-

specific suppressor and enhancing T-cell factors that are encoded by genes linked to the immunoglobulin heavy chain locus. Figure 3 presents a hypothetical model that delineates alternatives which might account for the observed observations. It has been proposed that the constant region of the T-cell receptor heavy chain, the τ chain, might be very similar to the primitive heavy chain in immunoglobulin evolution (1, 100) and therefore the C_τ gene might be located between the V_H cluster and the group of D genes. This location (position I), with either a different set of D-like or J-like genes or a lack of them, would account for present observations that V, D, J, and C rearrangements have not been found in T cells. Another major site on the immunoglobulin heavy chain chromosome for the location of T-cell receptor genes is shown in position II which follows from the studies of Owen and her collaborators (64–66). The constant regions of the suppressor, amplifier, and thymocyte antigen receptor are located to the right of the standard immunoglobulin isotype genes. This arrangement has been questioned because, if standard V, D, J, and C rearrangement occurred within the T cells, it would be predicted that the entire collection of immunoglobulin constant region genes would be deleted in T-cell maturation. This is not the case; in fact, T cells have $C\mu$ genes (101). It is possible, however, that a subset of V_H genes was duplicated and translocated to a position between the standard C_H genes and the C_τ cluster. Because of the enormous interest and the vigor of the attack on the genetic location of T-cell receptor genes, it is anticipated that the exact solution to this problem will be found in the near future.

Conclusions. The problem of the molecular nature of the T-cell receptor for antigen is one of the major unresolved issues in contemporary immunology. A consensus is developing regarding the existence of recognition structures related to immunoglobulin heavy chain variable regions on the surface of certain functional T cells and on many of the properties of isolated receptor (and factor) structures. A molecule of approximate subunit mass 68,000 d has been isolated from certain primate and rodent T cells and has been subjected to controlled proteolysis using various proteolytic enzymes and standard chemical cleavage methods. This molecule can be degraded into a major fragment of M_r 24,000, which bears the V_H determinant and binds antigen, and to a fragment of approximate mass 45–47,000 d which lacks V_H determinants

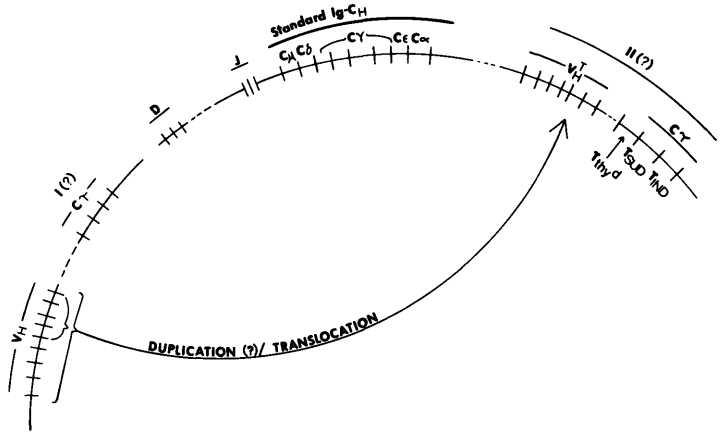

FIG. 3. Hypothetical model depicting possible arrangements of T-cell receptor variable and constant region genes within the immunoglobulin heavy chain gene cluster. The constant region of the T-cell heavy chain is designated C_τ. The markers $T_{thy}{}^d$, T_{sud}, and T_{ind} are the T-cell allotypic constant region markers defined by Owen and her colleagues (64–66). I (?) and II (?) indicate possible positions at which the C_τ genes can be located.

and carries out nonspecific effector functions. In addition, larger V_H-bearing fragments as well as subfragments of the V_H have been isolated and partially characterized. Although this molecule does not share constant region determinants with immunoglobulin heavy chains, the present data suggest that the molecule resembles heavy chains in being formed of domains of approximate mass 12,000 d. Genetic mapping studies indicate that the V_H structure associated with T-cell receptors maps with immunoglobulin V_H, although the genes encoding T-cell V_H structures most probably represent a subset of the total V_H pool available to immunoglobulin heavy chains. It is also possible that the T-cell variable regions and constant regions represent direct lineal descendants of primitive immunoglobulins in evolution and, therefore, would show a closer sequence homology to immunoglobulins of lower vertebrates rather than to those of man and rodents. Further amino acid sequence data and nucleic acid sequence data are required to test this hypothesis. The T-cell receptor molecule apparently possesses a blocked N-terminus. Although amino acid compositions have now been obtained for three V_H-bearing T-cell molecules, amino acid sequence data are lacking. Major issues which remain to be resolved are the direct demonstration using nucleic acid probes that the T-cell V_H genes lie within the immunoglobulin V_H cluster and the location of the constant region genes of the antigen-specific T-cell receptor. The genes that specify the factors likewise remain to be precisely mapped, although it is likely that antigen-specific receptors and factors share the same V_H pool.

One of the major features which is often taken to distinguish "recognition of antigen" by T cells from that of antibodies and B cells is that the T-cell recognition process is usually considered to show restrictions imposed by the major histocompatibility complex whereas antibodies show no such constraints. Based upon the MHC restriction in T-cell recognition, it has been proposed that T cells either express one surface receptor capable of recognizing both MHC products and nominal antigens (single receptor), or two distinct receptors (dual recognition hypothesis), one recognizing antigen and the other recognizing MHC products. Antigen-specific T-cell hybridomas expressing two specificities for nominal antigen and two MHC backgrounds have been constructed in the attempt to determine whether or not V_H and MHC products assort independently in T-cell function (102, 103). The present results are inconclusive, however, because one group finds conjunct association (102), but the other provides evidence of independent functional distribution suggesting dual recognition (103). The molecular data reviewed here suggest that the V_H-bearing/non-MHC-associated structure most probably binds antigen on the cell surface and is the antigen recognition unit in T-cell factors, whereas an association between this molecule and I-A- or I-J-specified products might be required for the production of active factors involved in cell/cell interactions. Substantial progress has been made in the molecular characterization of the elusive T-cell antigen receptor, although a formidable task remains in determining the exact mechanisms of antigen-driven activation of T cells and cell/cell collaboration.

Original work presented here was supported by Grant AI 17493 from the National Institute of Allergy and Infectious Diseases. We thank Mrs. Joan Maxwell for expert technical assistance. We thank Drs. M. Cramer, R. E. Cone, P. Lonai, B. Rubin, H. Wigzell, M. Taniguchi, T. Tada, and T. Kishamoto for providing us with preprints of unpublished manuscripts.

1. Cone RE. Molecular basis for T lymphocyte recognition of antigen. Prog Allergy 29:182–221, 1981.
2. Marchalonis JJ. The T cell antigen receptor: The minimal hypothesis revisited. Immunol Today 3:10–17, 1982.
3. Tada T, Okumura D. The role of antigen-specific T cell factors in the immune response. Advan Immunol 28:1–87, 1979.
4. Rajewsky K, Eichmann K. Antigen receptors of T helper cells. Contemp Topics Immunobiol 7:69–12, 1977.
5. Binz H, Wigzell H. Antigen-binding, idiotypic T-lymphocyte receptors. Contemp Topics Immunobiol 7:113–177, 1975.
6. Marchalonis JJ, Cone RE. Biochemical and

biological characteristics of lymphocyte surface immunoglobulin. Transplant Rev **14**:3–49, 1973.
7. Moroz C, Lahat N. Surface immunoglobulin of mouse thymus cells and its *in vitro* biosynthesis. Cell Immunol **13**:397–406, 1974.
8. Boylston AW, Watson SR, Anderson RL. Mouse T cell tumor immunoglobulin. I. Antigenic properties and effects on T cell responses. Immunology **31**:827–835, 1976.
9. Putnam D, Clagett J, Storb U. Immunoglobulin synthesis by T cells. Quantitative aspects. J Immunol **124**:902–912, 1980.
10. Singh G, Bazin H, Ladoulis C. *In vitro* synthesis and release of immunoglobulins by thymocytes of inbred rats. Mol Immunol **16**:755–766, 1979.
11. Hammerling U, Pickel HG, Mack C, Masters D. Immunochemical study of an immunoglobulin-like molecule of murine T lymphocytes. Immunochemistry **13**:533–538, 1976.
12. Feldmann M, Cone RE, Marchalonis JJ. Cell interactions in the immune response *in vitro*. VI. Mediation by T cell surface monomeric IgM. Cell Immunol **9**:1–11, 1973.
13. Taniguchi M, Tada T. Regulation of homocytotropic antibody formation in the rat. X. IgT-like molecule for the induction of homocytotropic antibody response. J Immunol **113**:1757–1769, 1974.
14. Moseley JM, Marchalonis JJ, Harris AW, Pye J. Molecular properties of T lymphoma immunoglobulin. I. Serological and general physical chemical properties. J Immunogene **4**:233–248, 1977.
15. Marchalonis JJ, Warr GW, Santucci LA, Szenberg A, von Fellenberg R, Burckhardt JJ. The immunoglobulin-like T cell receptor IV. Quantitative cellular assay and partial characterization of a heavy chain cross reactive with the Fd fragment of serum mu chain. Mol Immunol **17**:985–999, 1980.
16. Warr GW, Marton G, Szenberg A, Marchalonis JJ. Reactions of chicken antibodies with immunoglobulins of mouse serum and T cells. Immunochemistry **15**:615–622, 1978.
17. Cone RE, Rosenstein RW. Isolation of T cell membrane proteins (IgT) with antisera to non-isotypic determinants of immunoglobulins: Evidence that IgT "light" chains are not identical to B cell kappa chains. Mol Immunol **18**:67–77, 1981.
18. Eichman K. Expression and function of idiotypes on lymphocytes. Advan Immunol **26**: 195–254, 1978.
19. Cramer M. T cell factors-T cell receptors. In: Steinberg CM, Lefkovitz I, eds. The Immune System: Basel, Karger, Vol. I: pp311–315, 1981.
20. Malley A, Brandt CJ, Deppe LB. Preparation and characterization of the antiidiotypic properties of rabbit anti-timothy antigen B helper factor and antimouse timothy IgE antisera. Immunology **45**:217–225, 1982.
21. Harvey MA, Adorini L, Miller A, Sercarz EE. Lysozyme-induced T-suppressor cells and antibodies have a predominant idiotype. Nature (London) **281**:594–596, 1979.
22. Eshhar Z, Apte RN, Lowry I, Ben-Neriah Y, Givol D, Mozes E. T cell hybridoma bearing heavy chain variable region determinants producing (T, G)-A—L-specific helper factor. Nature (London) **286**:270–272, 1980.
23. Mozes E, Haimovich J. Antigen specific T cell helper factor cross reacts idiotypically with antibodies of the same specificity. Nature (London) **278**:56–57, 1979.
24. Prange CA, Fiedler J, Nitecki DE, Bellone CJ. Inhibition of T-antigen-binding cells by idiotypic antisera. J Exp Med **146**:766–778, 1977.
25. Lewis GK, Goodman JW. Purification of functional determinant-specific idiotype-bearing murine T cells. J Exp Med **148**:915–924, 1978.
26. Warr GW, DeLuca D, Marchalonis JJ. The immunoglobulin-like T cell receptor. III. Binding of the arsonate hapten by idiotype-bearing T cells and antibody is blocked by avian antibody to $(Fab')_2$. Mol Immunol **16**:735–738, 1979.
27. Nisonoff A, Greene MI. Regulation through idiotypic determinants of the immune response to the p-azophenylarsonate hapten in strain A mice. In: Fougereau M, Dausset J, eds. Progress in Immunology IV. New York, Academic Press, pp58–80, 1980.
28. Marchalonis JJ, Warr GW, Hunt JC, Wang AC, Decker JM, DeLuca D. Murine and primate T cell-derived proteins related to immunoglobulin combining site-region determinants. In: Janeway C, Sercarz EE, Wigzell H, eds. Immunoglobulin Idiotypes. New York, Academic Press, pp501–507, 1981.
29. Pacifico A, Capra JD. T cell hybrids with arsonate specificity. I. Initial characterization of antigen specific T cell products that bear a cross-reactive idiotype and determinants encoded by the murine major histocompatibility complex. J Exp Med **152**:1289–1301, 1980.
30. Rubin B, Suzan M, Kahn-Perles B, Bayor C, Schiff C, Bourgois A. Isolation and biochemical characterization of idiotype-bearing T-cell receptors which express private allotypes and/or isotypes: A review. Bull Inst Pasteur **78**:305–346, 1980.
31. Krupen K, Araneo BA, Brink L, Kapp JA, Stern S, Weider KS, Webb DR. Purification and characterization of a monoclonal T-cell suppres-

sor factor specific for poly (LGlu^{60} LAla^{30}LTyr^{10}). Proc Nat Acad Sci USA **79**:1254–1258, 1982.

32. Germain RN, Ju ST, Kipps TJ, Benacerraf B, Dorf ME. Shared idiotypic determinants on antibodies and T-cell derived suppressor factor specific for the random terpolymer L-Glutamic acid ^{60}-L-Alanine^{30}-L-Tyrosine^{10}. J Exp Med **149**:613–622, 1979.
33. Chaouat G. Human gammaglobulin tolerance: Shared idiotypy of T-cell suppressor factor and antibodies. Ann Immunol (Inst Pasteur) **131**:91–95, 1980.
34. Julius MH, Cosenza H, Augustin AA. Evidence for the endogenous production of T cell receptors bearing idiotypic determinants. Eur J Immunol **8**:484–491, 1978.
35. Benca R, Quintans J, Kearney JF, Flood PM, Schreiber H. Studies on phosphorylcholine-specific T cell isotopes and idiotype-specific immunity. Mol Immunol **17**:823–831, 1980.
36. Sugimura K, Kishimoto T, Maeda K, Yamamura Y. Demonstration of T15 idiotype-positive effector and suppressor T cells for phosphorylcholine-specific delayed-type hypersensitivity response in CBA/N or (CBA/N X Balb/C)F_1 male mice. Eur J Immunol **11**:455–461, 1981.
37. Nadler PI, Miller GG, Sachs DH, Hodes RJ. The expression and functional involvement of nuclease-specific idiotype on nuclease-primed helper T cells. Eur J Immunol **12**:113–120, 1982.
38. Binz H, Wigzell H. Shared idiotypic determinants on B and T lymphocytes reactive against the same antigenic determinants.V. Biochemical and serological characteristics of naturally occurring, soluble antigen-binding T-lymphocyte-derived molecules. Scand J Immunol **5**:559–571, 1976.
39. Binz H, Wigzell H. T cell receptors with allomajor histocompatibility complex specificity from rat and mouse. Similarity of size, plasmin susceptibility and localization of antigen-binding region. J Exp Med **154**:1261–1278, 1981.
40. Lea T, Forre Ø, Michaelsen TE, Natvig JB. Shared idiotypes on human peripheral blood B and T lymphocytes. J Immunol **122**:2413–2417, 1979.
41. Preud'Homme JL, Klein M, Labourne S, Seligmann M. Idiotype-bearing and antigen-binding receptors produced by blood T lymphocytes in a case of human myeloma. Eur J Immunol **7**:840–846, 1977.
42. Kishimoto T, Suemura M, Sugimura K. Okada M, Nakanishi K, Yamamura Y. Characterizations of T cell-derived immunoregulatory molecules from murine or human T hybridomas. In: Feldmann M, Schrier MH, eds. Lymphokines, Vol 5, Monoclonal T Cells and Their Products. New York, Academic Press, pp129–160, 1982.
43. Kapp JA, Araneo BA, Clevinger BL. Suppression of antibody and T cell proliferative responses to L-glutamic acid 60-L-alanine 30-L-tyrosine 10 (GAT) by a specific monoclonal T cell factor. J Exp Med **152**:235–240, 1980.
44. Volanakis JE, Kearney JF. Cross-reactivity between C-reactive protein and idiotypic determinants on a phosphocholine-binding murine myeloma protein. J Exp Med **153**:1604–1614, 1981.
45. Pillemer E, Weissman IL. A monoclonal antibody that detects a V_K-TEPC15 idiotypic determinant cross-reactive with a Thy-1 determinant. J Exp Med **153**:1068–1079, 1981.
46. Clark AF, Capra JD. Ubiquitous nonimmunoglobulin p-azobenzenearsonate-binding molecules from lymphoid cells. J Exp Med **155**:611–616, 1982.
47. Sherr DH, Ju ST, Dorf ME. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. XII. Fine specificity of anti-idiotypic suppressor T cells (TS_2). J Exp Med **154**:1382–1389, 1981.
48. Minami M, Okuda K, Furusawa S, Benacerraf B, Dorf ME. Analysis of T cell hybridomas. I. Characterization of H-2 and Igh-restricted monoclonal suppressor factors. J Exp Med **154**:1390–1402, 1981.
49. Sy MS, Nisonoff A, Germain RN, Benacerraf B, Greene MI. Antigen- and receptor-driven regulatory mechanisms VIII. Suppression of idiotype-negative, p-azobenzenearsonate-specific T cells results from the interaction of an idiotype-specific second order T suppressor cell with a cross-reactive-idiotype-positive, p-azobenzenearsonate-primed T cell target. J Exp Med **153**:1415–1525, 1981.
50. Bona C, Paul WE. Cellular basis of regulation of the expression of idiotype. I. T cell suppressor cells specific for MOPC 460 idiotype regulate the expression of cells secreting anti-TNP antibodies bearing 460 idiotype. J Exp Med **149**:592–600, 1979.
51. Sher A, Lord E, Cohn M. Reconstitution from subunits of the hapten binding sites and idiotypic determinants of mouse anti-phosphorylcholine myeloma proteins. J Immunol **107**:1226–1234, 1971.
52. Schiff C, Boyer C, Milili M, Fougereau M. The idiotypy of the MOPC 173 (IgG_{2b}) mouse myeloma protein: Characterization of syngeneic, allogeneic and xenogeneic anti-idiotypic antibodies. Contribution of the H and L chains to the idiotypic determinants. Eur J Immunol **9**:831–841, 1979.
53. Marchalonis JJ, Warr GW, Smith P, Begg GS,

Morgan FJ. Structural and antigenic studies of an idiotype-bearing murine antibody to the arsonate hapten. Biochemistry **18**:560–565, 1979.

54. Rosenthal AS. Determinant selection and macrophage function. Immunol Today **3**:33–34, 1982.
55. Barrett, AJ, McDonald, JK. Mammalian proteases: A glossary and bibliography. Vol I, Endopeptidases. New York, Academic Press, 1980.
56. Cramer M, Reth M, Grutzmann R. T cell V_H versus B cell V_H. In: Janeway C, Sercarz EE, Wigzell H, eds. Immunoglobulin Idiotypes. New York, Academic Press, pp429–439, 1981.
57. Ben Neriah Y, Givol D, Lonai P, Simon MM, Eichmann K. Allotype-linked genetic control of a polymorphic V_H framework determinant on mouse T-helper cell receptors. Nature (London) **285**:257–259, 1980.
58. Marchalonis JJ, Warr GW, Rodwell JD, Karush F. Surface component of primate thymus-derived lymphocytes related to a heavy chain variable region. Proc Nat Acad Sci USA **77**:3625–3629, 1980.
59. Førre Ø, Michaelsen T, Natvig J. Nonidiotypic V_H antigens can be detected on human lymphocytes. Clin Immunol Immunopathol **22**:436–441, 1982.
60. Michaelsen TE, Lea T. Sheep, rabbit and chicken antisera against a human V_H fragment: Reactivity with immunoglobulins and lymphocytes. Immunology **45**:751–759, 1982.
61. Taniguchi M, Saito T, Takei I, Kanno M, Tokuhisa T, Tomioka H. Suppressor T cell hybridomas and their soluble products. In: Feldmann M, Schrier MH. Lymphokines, Vol. 5, Monoclonal T Cells and Their Products. New York, Academic Press, pp77–117, 1982.
62. Krawinkel U, Cramer M, Mage RG, Kelus AS, Rajewsky K. Isolated hapten-binding receptors of sensitized lymphocytes II. Receptors from nylon wool-enriched rabbit T lymphocytes lack serological determinants of immunoglobulin constant domains but carry a locus allotypic marker. J Exp Med **146**:192, 1977.
64. Jensenius JC, Crone M, Koch C. The still elusive T cell receptor. On the possibility of a common V-gene pool for B- and T-cell-antigen receptor molecules. Scand J Immunol **14**:693–704, 1981.
64. Owen FL, Riblet R, Taylor BA. The T suppressor cell alloantigen Tsu^d maps near immunoglobulin allotype genes and may be a heavy chain constant-region marker on a T cell receptor. J Exp Med **153**:801–810, 1981.
65. Owen FL, Spurll GM. Evidence for a T cell constant region gene family: Characterization of cell surface antigens by immunoprecipitation with alloantisera and monoclonal antibodies. In: Janeway C, Sercarz EE,Wigzell H, eds. Immunoglobulin Idiotypes. New York, Academic Press, pp419–428, 1981.
66. Owen FL, Spurll GM, Panageas E. $Tthy^d$, a new thymocyte alloantigen linked to Igh-1. Implications for a switch mechanism for T cell antigen receptors. J Exp Med **155**:52–60, 1982.
67. Kronenberg M, Davis MM, Early PW, Hood LE, Watson JD. Helper and killer T cells do not express B cell immunoglobulin joining and constant region gene segments. J Exp Med **152**:1745–1761, 1980.
68. Kurosawa Y, vonBoehmer H, Haas W, Sakarno H, Trauneker A, Tonegawa S. Identification of D segments of immunoglobulin heavy-chain genes and their rearrangement in T lymphocytes. Nature (London) **290**:565–570, 1981.
69. Williamson AR. Genes coding for T-lymphocyte receptors. Immunol Today **3**:68–72, 1982.
70. Marchalonis JJ, Molecular interactions and recognition specificity of surface receptors. Contemp Topics Immunobiol **9**:255–288, 1980.
71. Tada T, Hayakawa K, Okumura K, Taniguchi M. Coexistence of variable region of immunoglobulin heavy chain and I region gene products on antigen-specific suppressor T cells and suppressor T cell factor. A minimal model of functional antigen receptor of T cells. Mol Immunol **17**:867–875, 1980.
72. Taussig MJ. Review. Antigen-specific T-cell factors. Immunology **41**:759–787, 1980.
73. Robb RJ, Strominger JL, Mann DL. Rapid purification of detergent-solubilized HLA antigen by affinity chromatography employing anti-β_2-microglobulin serum. J Biol Chem **251**: 5427–5428, 1976.
74. Zwerner RK, Barstad PA, Acton RT. Isolation and characterization of murine cell surface components. I. Purification of milligram quantities of Thy-1.2. J Exp Med **146**:986–1000, 1977.
75. Marchalonis JJ, Hunt JC, Maxwell J, Wang AC. Antigenic polypeptide fragments of a receptor related to the Fab fragment of human immunoglobulin from thymus-derived lymphocytes. Proc Nat Acad Sci USA **79**:4733–4736, 1982.
76. Cone RE, Marchalonis JJ, Rolley RT. Lymphocyte membrane dynamics. Metabolic release of cell surface proteins. J Exp Med **134**:1373–1384, 1971.
77. Marchalonis JJ, Nossal GJV. Electrophoretic analysis of antibody produced by single cells. Proc Nat Acad Sci USA **61**:860–867, 1968.
78. Mage RG. A new look at the biological and genetic significance of rabbit heavy chain allotypes. Ann Immunol (Inst Pasteur) **130C**:105–114, 1979.

79. Ben-Neriah Y, Wuilmart C, Lonai P, Givol D. Preparation and characterization of anti-framework antibodies to the heavy chain variable region (V_H) of mouse antibodies. Eur J Immunol 8:797–801, 1978.
80. Rubin B, Hertel-Wulff B, Kimura A. Alloantigen-specific idiotype-bearing receptors on mouse T lymphocytes. I. Specificity characterization and genetic association with the heavy chain IgG allotype. J Exp Med 150:307–321, 1979.
81. Howie S, Feldmann M. *In vitro* studies on H-2-linked unresponsiveness to synthetic polypeptides. III. Production of an antigen-specific T helper cell factor to (T,G)-A—L. Eur J Immunol 7:417–421, 1977.
82. Lonai P, Puri J, Hammerling U. H-2-restricted antigen binding by a hybridoma clone that produces antigen-specific helper factor. Proc Nat Acad Sci USA 78:549–553, 1981.
83. Lonai P, Arman E, Savelkoul HPC, Friedman V, Puri J, Hammerling U. Factors, receptors and their ligands: Studies with H-2 restricted helper hybridoma clones. In: Fathman G, Fitch F, eds. Isolation, Characterization and Utilization of T Lymphocyte Clones. New York, Academic Press, in press.
84. Cone RE, Rosenstein RW, Murray JH, Iverson GM, Ptak W, Gershon RK. Characterization of T-cell surface proteins bound by heterologous antisera to antigen-specific T-cell products. Proc Nat Acad Sci USA 78:6411–6415, 1981.
85. Rosenstein RW, Murray JH, Cone RE, Ptak W. Iverson GM, Gershon RK. Isolation and characterization of an antigen specific, T-cell factor associated with the suppression of DTH. Proc Nat Acad Sci USA, in press.
86. Cone RE, Murray JH, Rosenstein RW, Ptak W, Iversen GM, Gershon RK. The use of heteroantisera to T cell antigen binding proteins as probes for T cell receptors. In: Janeway C, Sercarz EE, Wigzell H, eds. Immunoglobulin Idiotypes. New York, Academic Press, pp457–482, 1981.
87. Fresno M, McVay-Boudreau L, Nabel G, Cantor H. Biologic properties of a purified antigen-specific suppressive glycopeptide. Transplant Proc 13:1124–1127, 1981.
88. Fresno M, McVay-Boudreau L, Nabel G, Cantor RH. Antigen-specific T lymphocyte clones. II. Purification and biological characterization of an antigen-specific suppressive protein synthesized by cloned T cells. J Exp Med 153:1260–1274, 1971.
89. Fresno M, McVay-Boudreau L, Cantor H. Antigen-specific T lymphocyte clones. III. Papain splits purified T suppressor molecules into two functional domains. J Exp Med 155:981–993, 1982.
90. Goodman JW, Lewis GK, Primi D, Hornbeck P, Ruddle NH. Antigen-specific molecules from murine T lymphocytes and T cell hybridomas. Mol Immunol 17:933–945, 1980.
91. Tada T, Nonaka M. T cell hybridomas and their products: An overview. In: Fitch F, Fathman G, eds. Isolation, Characterization and Utilization of T Lymphocyte Clones. New York, Academic Press, in press.
92. Neubauer RH, Marchalonis JJ, Strand BC, Rabin H. Surface markers of primate B and T lymphoid cell lines defined by antibodies to human lymphocyte antigens. J Immunogenet 9: 209–221, 1982.
93. Cramer M, Krawinkel U. Immunochemical properties of isolated hapten-specific T cell receptor molecules. In: Pernis B, Vogel HJ, eds. Regulatory T Lymphocytes. New York, Academic Press, pp39–55, 1980.
94. Moseley JM, Beatty EA, Marchalonis JJ. Molecular properties of T-lymphoma immunoglobulin III. Peptide composition of the light chain. J Immunogenet 6:19–28, 1979.
95. Kontiainen S, Feldmann M. Structural characteristics of antigen-specific suppressor factors: Definition of "constant" region and "variable" region determinants. Thymus 1:59–79, 1979.
96. Tokuhisa T, Taniguchi M. Two distinct allotypic determinants on the antigen-specific suppressor and enhancing T cell factors that are encoded by genes linked to the immunoglobulin heavy chain locus. J Exp Med 155:126–139, 1982.
97. Marchalonis JJ, Wang AC. A marmoset T-lymphocyte protein related to defined human serum immunoglobulin and fragments. J Immunogenet 8:165–175, 1981.
98. Feldmann M, James R, Culbert E, Todd I, Makidono R, Cecka JM, Koniainen S. Uses of antifactor antisera and monoclonals in the analysis of T cell products. In: Janeway C, Sercarz EE, Wigzell H, eds. Immunoglobulin Idiotypes. New York, Academic Press, pp483–492, 1981.
99. Marchalonis JJ, Strelkauskas AJ. A marmoset T lymphoma which functions as a human amplifier T cell. J Immunogen 8:477–483, 1981.
100. Marchalonis JJ, Warr GW, Vasta GR, Ledford BE. Molecular recognition of "non-self" determinants: The existence of a superfamily of recognition molecules related to primordial immunoglobulins. In: Gorczynski RM, ed. Receptors in Biological Systems. New York, Marcel Dekker, in press.
101. Kemp DJ, Harris AW, Cory S, Adams JM. Ex-

pression of the immunoglobulin C_μ gene in mouse T and B lymphoid and myeloid cell lines. Proc Nat Acad Sci USA 77:2876–2880, 1980.

102. Kappler JW, Skidmore B, White J, Marrack P. Antigen-inducible, H-2-restricted, interleukin-2-producing T cell hybridomas. Lack of independent antigen and H-2 recognition. J Exp Med 153:1198–1214, 1981.
103. Lonai P, Bitton S, Savelkoul HFJ, Puri J, Hammerling GJ. Two separate genes regulate self-Ia and carrier recognition in H-2 restricted helper factors secreted by hybridoma cells. J Exp Med, in press.
104. Tada T, Suzuki G, Hiramatsu K. Some comments on the antigen-receptors expressed on functional T cell hybridomas. In: Feldmann M, Schrier MH, eds. Lymphokines, Vol 5. Monoclonal T Cells and Their Products. New York, Academic Press, pp119–128, 1982.

Received June 21, 1982. P.S.E.B.M. 1982, Vol. 171.