# ADVANCES IN
# Immunology

EDITED BY

FRANK J. DIXON

VOLUME 41

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# Immunology

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*La Jolla, California*

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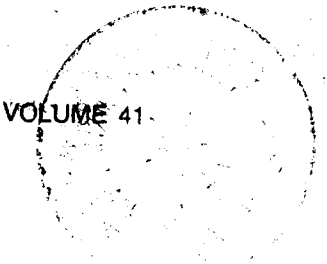


ACADEMIC PRESS, INC.
**Harcourt Brace Jovanovich, Publishers**
Orlando San Diego New York Austin
Boston London Sydney Tokyo Toronto

ACADEMIC PRESS, INC.
Orlando, Florida 32887

*United Kingdom Edition published by*
ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 61-17057

ISBN 0–12–022441–0 (alk. paper)

PRINTED IN THE UNITED STATES OF AMERICA

87 88 89 90 9 8 7 6 5 4 3 2 1

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# Cell Surface Molecules and Early Events Involved in Human T Lymphocyte Activation

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## I. Introduction

The activation of human thymus-derived (T) cells is the result of ligand–receptor interactions. Under physiologic conditions, such ligand–receptor interactions occur at the interface of the plasma membranes of an antigen-specific T cell and an antigen presenting cell (APC) or target cell. These antigen-specific and non-antigen-specific ligand–receptor binding events result in the transduction of these events into intracellular biochemical signals in the form of "second messengers." Ultimately, such intracellular biochemical signals influence specific targeted genes receptive to these signals which can become transcriptionally active or inactive. The summation of these events is the expression of the phenotype of an activated T cell. The diverse manifestations of T cell activation include the production of lymphokines, the appearance of new cell surface proteins (which include growth factor receptors), the acquisition of cytolytic effector function, and, as a consequence of the production of growth factors and their receptors, proliferation. In this review, we will focus primarily on the structures and function of the cell surface molecules of the human T cell which appear to initiate activation. Where appropriate, data referring to the murine system will be drawn upon. The events subsequent to initial activation events, i.e., the interaction of interleukin 2 (IL-2) with its receptor and the resultant proliferative response, will not be addressed in this review.

## II. Cell Surface Molecules Involved in T Cell Activation

The study of the T cell surface molecules involved in T cell activation has been facilitated through the use of homogeneous cell populations, such as T cell clones, hybridomas and leukemic lines, and the availability of monoclonal antibodies (mAb) which define antigenic epitopes expressed on an

array of distinct molecules. By virtue of the agonist or antagonist properties of mAb reactive with these molecules, many of these cell surface molecules are felt to play a role in T cell activation, either in antigen-induced activation or in alternate pathways of activation. Most of these molecules are thought to function as cell surface receptors. The T cell antigen receptor must play a central role in antigen-driven T cell activation and has been most intensively studied. The ligands of these other putative receptors remain to be identified or confirmed.

Some of these receptors can play a primary role in activation (the T cell antigen receptor, T11, Thy-1), initiating distinct biochemical events, which alone, following ligand interaction, can lead to T cell activation under appropriate conditions. Triggering of these receptors results in an increase in the concentration of cytoplasmic free calcium ($[Ca^{2+}]_i$), one of the intracellular events which is generally felt to be required for T cell activation. Other receptors appear to function as accessory molecules [Tp44, T1, interleukin-1 (IL-1) receptor], which when stimulated, are able to synergize with stimuli provided by the T3/Ti complex. These receptors have little effect upon T cell activation when stimulated alone. Still other molecules have been implicated as receptors involved in increasing the overall avidity between the T cell and the APC (or target cell) (LFA-1, T4, T8). The following will attempt to summarize the structure and evidence supporting the role of some of these cell surface molecules in T cell activation. However, we will not attempt to exhaustively review all molecules involved in activation.

## A. T Cell Antigen Receptor

Activation of the T cell induced by an antigen on the surface of an APC must involve an interaction with the T cell antigen receptor. This receptor subserves two functions in antigen-induced activation: (1) a recognitive function in which a specific antigen is recognized in the context of the appropriate major histocompatibility complex (MHC) molecules, and (2) an effector function in which the recognitive event is transmitted across the plasma membrane to the interior of the cell, with the resultant appearance of intracellular second messengers. A fundamental understanding of the structure of the receptor is useful in order to begin to understand the basis for these two functions.

### *1. Structure of the T Cell Antigen Receptor*

The T cell antigen receptor was identified independently in several laboratories by the generation of mAb, which reacted with unique clonally distributed antigenic epitopes on T cell lines, hybridomas, or clones (clonotypic determinants) (Allison *et al.*, 1982; Meuer *et al.*, 1983a; Haskins *et al.*, 1983). These antibodies react with disulfide-linked heterodimer glycoproteins (Ti)

of 80–90 kDa. These heterodimers are composed of an acidic Ti-α chain of 43–54 kDa and a more basic Ti-β chain of 38–44 kDa (Reinherz *et al.*, 1983; Kappler *et al.*, 1983a,b). Peptide mapping studies suggested each chain has both constant and variable domains (Reinherz *et al.*, 1983; Acuto *et al.*, 1983; Kappler *et al.*, 1983b). Both chains are integral membrane proteins, have two to six N-linked glycosylation sites, and have an intracytoplasmic tail of five amino acids at the carboxy-terminus (McIntyre and Allison, 1984; Yanagi *et al.*, 1984; Sim *et al.*, 1984). The relatively short cytoplasmic tail of these chains suggests that they are by themselves not responsible for transmembrane signaling events.

A detailed understanding of the structure of the human Ti has come from study of the complementary DNA (cDNA) clones and genomic clones of the Ti-α and β chains (Yanagi *et al.*, 1984; Sim *et al.*, 1984). The human Ti-α and -β chains have limited homology to immunoglobulin genes, suggesting a common evolutionary origin (Yanagi *et al.*, 1984; Sim *et al.*, 1984; Hood *et al.*, 1985). Both Ti-α and -β chains are assembled from gene segments which undergo rearrangements and expression during T cell ontogeny (Royer *et al.*, 1984; 1985; Yoshikai *et al.*, 1984; Raulet *et al.*, 1985; Collins *et al.*, 1985). Analogous to immunoglobulin heavy chain genes, Ti-β chains are assembled from recombinational events involving variable (V), diversity (D), joining (J), and constant (C) gene segments (Siu *et al.*, 1984). The Ti-α chain genes are similarly assembled from V, J, and C segments, but, to date, no D segments have been identified (Yoshikai *et al.*, 1985). Thus, the diverse antigen-reactive repertoire of T cells can be accounted for, in part, from the joining of different V, J, and D gene segments as well as combinatorial associations between the Ti-α and -β chains. Transfection studies and cell fusion studies have suggested that the Ti-α and -β chains are sufficient to confer antigen and MHC specificity upon the T cell (Dembic *et al.*, 1986), although primary sequence studies suggest that neither Ti-α nor -β chains are solely responsible for antigen or MHC specificity (Fink *et al.*, 1986). Thus, the evidence strongly implicates Ti heterodimer in the antigen/MHC-specific recognitive events.

More preliminary evidence, however, suggests that Ti-α and -β chains may not be the only chains involved in antigen recognition. In the course of attempts to isolate the Ti-α chain, another cDNA, the Ti-γ chain, was isolated (Saito *et al.*, 1984). This gene, once thought to be prefentially transcribed in cytolytic cells (Kranz *et al.*, 1985), has now clearly been found to be expressed in helper T cells as well (Zauderer *et al.*, 1986). The Ti-γ chain gene, like the α and β chain genes, undergoes rearrangement utilizing V and J region segments linked to constant region segments (Hayday *et al.*, 1985; LeFranc *et al.*, 1986). Interestingly, the Ti-γ chain is the first of the Ti chains to rearrange and to be expressed during T cell ontogeny (Raulet *et al.*, 1985;

Haars *et al.*, 1986) and, thus, has been proposed to be important in thymocyte selection (Raulet *et al.*, 1985; Garman *et al*, 1986). Until recently, the protein product of the Ti-γ chain had not been identified. However, recent studies suggest that it is expressed as a 55-kDa glycoprotein on an small, unusual subpopulation of human peripheral T cells which fail to express T4 (CD4) or T8 (CD8) antigens (Brenner *et al.*, 1986; Weiss *et al.*, 1986c; Lanier and Weiss, 1986). It may exist as a non-disulfide-linked heterodimer or as a single chain in association with T3 (see below). It has also been detected on the surface of $T4^-/T8^-$ thymocytes felt to represent the most immature population of the thymus (Bank *et al.*, 1986; Lanier and Weiss, 1986).

On human T cells, the Ti heterodimer or the protein product of the Ti-γ chain gene is associated with three invariant peptides which comprise the T3 (CD3) antigenic complex. T3 consists of at least three distinct integral membrane proteins: The T3-δ chain, a 22-kDa glycoprotein; the T3-ε chain, a 21-kDa nonglycosylated protein; and the T3-γ chain, a 26- to 28-kDa glycoprotein (Borst *et al.*, 1982, 1983a; Kanellopoulos *et al.*, 1983). The cDNAs encoding these three chains have been isolated and sequenced (van den Elsen *et al.*, 1984; Gold *et al.*, 1986; Krissansen *et al.*, 1986). The expression of these T3 genes is limited to T cells. T3-δ and T3-γ chains exhibit substantial homology to each other, but not to other known proteins (Krissansen *et al.*, 1986). Homologous chains have been identified in the murine system (Allison and Lanier, 1985; Samelson *et al.*, 1985; Oettgen *et al.*, 1986). However, additional chains have also been identified in the mouse. These include the ζ chain, a disulfide-linked homodimer or heterodimer of 32 kDa, with monomers of 14–17 kDa (Samelson *et al.*, 1985; Oettgen *et al.*, 1986), and a more recently isolated p21, a disulfide-linked dimer of 42 kDa with 21-kDa subunits, which is phosphorylated on tyrosine residues with activation by antigen (Samelson *et al.*, 1986). It is likely that homologues to the T3-ζ chain and p21 will be identified in the human. Thus, the T3 complex may consist of seven chains. All three of the cloned chains of T3 contain between 40 and 80 cytoplasmic residues (van den Elsen *et al.*, 1984; Gold *et al.*, 1986; Krissansen *et al.*, 1986). This observation, together with the findings discussed below regarding the agonist properties of T3 mAb and the phosphorylation of T3 chains, are consistent with the notion that T3 plays a role in transmembrane signaling events.

Several lines of evidence suggest that the antigen receptor exists as a molecular complex composed of Ti and T3, or, in the case of the protein product of the Ti-γ chain, Ti-γ and T3. The physical association of the Ti-α/β chain heterodimer was demonstrated by comodulation (Meuer *et al.*, 1983a), coimmunoprecipitation (Reinherz *et al.*, 1983; Borst *et al.*, 1983b), and the chemical cross-linking (Allison and Lanier, 1985; Brenner *et al.*, 1985). T3

has been linked to the protein product of the Ti-γ chain by coimmunoprecipitation (Weiss *et al.*, 1986c) and chemical cross-linking (Brenner *et al.*, 1986; Bank *et al.*, 1986). Evidence suggests that the association between T3 and Ti is obligatory in that mutants of the T cell leukemic line Jurkat, which lack Ti-β chain transcripts, contain T3 proteins trapped intracellularly (Weiss and Stobo, 1984; Ohashi *et al.*, 1985). Reconstitution of the Ti-β chain by transfection into one such mutant resulted in the reexpression of Ti and T3 (Ohashi *et al.*, 1985). Although the close association of T3 and Ti is suggested by such studies, the exact nature of this association is not clear. Under many conditions of immunoprecipitation, T3 and Ti do not coprecipitate (Allison *et al.*, 1982; Haskins *et al.*, 1983; Samelson *et al.*, 1983; Weiss and Stobo, 1984). In a cross-linking study, the T3-γ chain was chemically cross-linked to the Ti-β chain, suggesting a close association between these chains (Brenner *et al.*, 1985). A shortcoming of this study is the observation that neither the Ti-α and -β chains nor the chains of T3 were cross-linked to each other. One striking observation has been made from the sequence analyses of the component chains of Ti and T3. All three of the Ti chains, α, β, and γ, of mouse and man contain an unusually placed highly charged basic lysine residue within the putative transmembrane domain (Yanagi *et al.*, 1984; Sim *et al.*, 1984; Saito *et al.*, 1984), whereas the three chains of T3 contain conserved acidic residues of aspartic or glutamic acids within their hydrophobic putative transmembrane domains (van den Elsen *et al.*, 1984; Gold *et al.*, 1986; Krissansen *et al.*, 1986). It has been suggested that these charged amino acids may play a role in the association between T3 and Ti. Collectively, these observations support a model of the T cell antigen receptor as a multisubunit complex composed of five to nine chains consisting of T3 and Ti.

### 2. *Role of the T3/Ti Complex in Activation*

The T cell antigen receptor must play a role in antigen-induced T cell activation. However, the direct study of the role of the receptor binding to the antigen is hampered by the inherent difficulty in studying cell–cell interactions and the ill-defined structure of antigen associated with MHC molecules. The use of monoclonal antibodies reactive with Ti or T3, which can function as agonists or antagonists, has facilitated the study of the role of the T3/Ti complex in T cell activation. Thus, such antibodies can serve as probes to elucidate the function of the receptor, mimicking the effects of antigen, without the participation of other cell surface molecules which may interact during T cell–APC interactions. Although this approach has limitations, since the effects of agonist mAb may not fully mimic the effects of antigen–antigen receptor interactions, it provides a first approximation toward the study of the function of the antigen receptor.

A large number of studies have revealed that mAb reactive with T3 could function as polyclonal agonists in inducing resting human peripheral blood T cells within peripheral mononuclear cells to secrete the lymphokines IL-2 or interferon-γ (IFN-γ) (von Wussow *et al.*, 1981; Chang *et al.*, 1982; van Wauwe *et al.*, 1984), to express IL-2 receptors (Meuer *et al.*, 1984a; Schwab *et al.*, 1985; Tsoukas *et al.*, 1985; Ledbetter *et al.*, 1986), or to proliferate (van Wauwe *et al.*, 1980; Chang *et al.*, 1981). These antibodies have also been used to activate T cell clones and tumor lines to produce lymphokines or kill targeted bystander cells (Meuer *et al.*, 1983b; Weiss *et al.*, 1984a; Kranz *et al.*, 1984; Mantzer *et al.*, 1985). Similarly, clonotypic Ti mAb and Ti mAb reactive with nonpolymorphic determinants of Ti, such as mAb WT31, can activate T cells in a manner analogous to T3 mAb (Kappler *et al.*, 1983a; Meuer *et al.*, 1983b; Tax *et al.*, 1983; Kaye and Janeway, 1984; Weiss and Stobo, 1984). It is of interest that under appropriate conditions of antibody immobilization, all anti-T3 or anti-Ti mAb described, with one exception, can function as agonists (Lanier *et al.*, 1986). This implies that, in contrast to the T11 molecule (discussed below), perturbation of several distinct sites on the T3/Ti complex can lead to appropriate triggering of the complex. The potency of T3 and Ti mAb suggests that occupancy of relatively few receptors is sufficient to activate T cells (Chang *et al.*, 1982; Kaye and Janeway, 1984). T3 and Ti mAb are also capable of functioning as antagonists, under some circumstances, to block the interactions between T cells and antigen-presenting cells or target cells (Chang *et al.*, 1981; Meuer *et al.*, 1983a; Haskins *et al.*, 1983; Lancki *et al.*, 1983; Samelson *et al.*, 1983). Thus, the use of T3 and Ti mAb has proved to be a powerful tool to examine the role of the T cell antigen receptor in activation.

The conditions required for activation of T cells by T3 or Ti mAb are dependent upon the particular manifestation of T cell activation examined. For instance, expression of the IL-2 receptor (IL-2R) has less stringent requirements than T cell proliferation. Hence, IL-2R expression can be induced by T3 or Ti mAb under conditions in which no proliferative response is observed (Schwab *et al.*, 1985; Wakasugi *et al.*, 1985; Tsoukas *et al.*, 1985; Ledbetter *et al.*, 1986). Therefore, production of IL-2 is more stringently regulated than the expression of the IL-2R. Since both the growth factor and its receptor must be produced in order for T cell proliferation to occur, T cell proliferation is primarily limited by the production of IL-2. This view must be qualified by the recent findings that there may be IL-2 independent pathways of T cell proliferation (Moldwin *et al.*, 1986). BSF-1 is produced by T cells and can support the growth of some T cell clones (Smith and Rennick, 1986; Mosman *et al.*, 1986; Yokota *et al.*, 1986; Fernandez-Botran *et al.*, 1986). It is not clear what the requirements are for BSF-1 production or for its role in T cell proliferative responses to antigen.

Regardless of the growth factors by which T cell proliferation is mediated, the induction of T cell proliferation by anti-T3 or anti-Ti mAb is dependent upon accessory cells (AC) (Chang *et al.*, 1982; Tax *et al.*, 1983; Landegren *et al.*, 1984). In the case of human PBM, these AC are contained within the adherent cell population (Tax *et al.*, 1983). At least two functions of these AC have been demonstrated. One function is dependent upon an interaction of the Fc portion of the T3 mAb and the Fc receptor on these AC (Tax *et al.*, 1983, 1984; Landegren *et al.*, 1984; Smith *et al.*, 1986; Wakasugi *et al.*, 1985; Ceuppens *et al.*, 1985). This function of AC can be bypassed by immobilization of the T3 mAb onto Sepharose beads or onto the surface of culture dishes (Tax *et al.*, 1984; Ceuppens *et al.*, 1985). This suggests that the formation of a cross-linked matrix of antibody and T3 may be critical in activation requirements. Alternatively, as has been suggested, the immobilization of the T3 mAb may be important in preventing receptor internalization which might result in blunting the stimulatory response (Manger *et al.*, 1985; Ledbetter *et al.*, 1986). Indeed, as reviewed below, transmembrane signaling by soluble and immobilized anti-T3 or anti-Ti has been shown to differ. Whereas neither soluble nor immobilized anti-T3 induce IL-2 production or proliferation of highly purified T cells, only immobilized anti-T3 is able to induce IL-2R expression (Wakasugi *et al.*, 1985; Ledbetter *et al.*, 1986). It is likely that immobilized T3 mAb more closely mimics the Ti/T3 interaction with antigen/MHC on the surface of the T cell and APC.

The second function of the AC is revealed by the failure of highly purified resting T cells to proliferate to immobilized anti-T3 or mitogenic lectins (Schwab *et al.*, 1985; Williams *et al.*, 1985; Ledbetter *et al.*, 1986; Manger *et al.*, 1986; Weiss *et al.*, 1986a). The requirements for the activation of purified freshly isolated resting T cells and previously stimulated T cell clones or lines appear to differ (Manger *et al.*, 1985; Meuer and zum Buschenfelde, 1986). Immobilized anti-T3 or anti-Ti alone is sufficient to activate T cell clones to produce IL-2 and to proliferate (Meuer *et al.*, 1983b, 1984a; Manger *et al.*, 1985). Similarly, the T cell leukemic line HUT 78, which phenotypically resembles a previously activated T cell, produces IL-2 in response to immobilized but not soluble T3 mAb (Manger *et al.*, 1985). In contrast, the Jurkat cell line, like resting highly purified T cells, fails to respond to immobilized anti-T3 (Manger *et al.*, 1985; Williams *et al.*, 1985; Ledbetter *et al.*, 1986; Weiss *et al.*, 1986a). These findings suggest that resting T cells require an additional stimulus, provided by AC, which is not observed with T cells previously activated. Relatively small numbers of AC can provide this additional stimulus; hence, the notion that a soluble mediator is involved has emerged.

Four ligands that bind to the surface of the T cell can mimic the effect of AC in providing this second function: mAb reactive with T1, T11, or Tp44.

as well as IL-1. These will be discussed separately below. In addition, it should be noted that both functions of the AC can be provided by phorbol myristate acetate (PMA), a potent activator of protein kinase C (pkC) (Hara *et al.*, 1985; Ledbetter *et al.*, 1986; Weiss *et al.*, 1986a). The role of PMA and pkC in T cell activation will be discussed at length later in this review.

Thus, mAb reactive with T3 or Ti can function as polyclonal activators of T cells in a manner analogous to that of anti-Ig and B cells. However, simple ligand binding to the T cell antigen receptor does not appear to be sufficient for activation. In view of the fact that T cells do not respond to soluble antigen, but, rather, react with cell-bound antigen, the AC dependence of anti-T3 mAb may be quite consistent with the physiologic situation.

In addition to its role in antigen-induced T cell activation, the T3/Ti complex appears to be important in the activation of T cells by the T cell-specific mitogenic lectins phytohemagglutinin (PHA) and concanavalin A (Con A). Both lectins bind to large numbers of T cell surface glycoproteins (Henkart and Fisher, 1975; Sitkovsky *et al.*, 1984); however, the cell surface molecules responsible for the ability of these lectins to stimulate T cells have been undefined. Biochemical analyses of solubilized cell surface proteins have demonstrated that Con A can bind to the T3 chains but not Ti, whereas PHA can interact with the Ti heterodimer but not the isolated T3 chains (Kanellopoulos *et al.*, 1985). Thus, among the many cell surface glycoproteins bound by these lectins are component chains of the T3/Ti complex. Indeed, both Con A and PHA can induce cocapping of T3 (Kanellopoulos *et al.*, 1985). Simple demonstration of binding to components of the T3/Ti complex does not establish that the T3/Ti complex mediates the relevant activation signal induced by these mitogens. Evidence supporting the role of the T3/Ti complex in PHA- and Con A-induced T cell activation is the observation that Jurkat mutants which fail to express the T3/Ti complex lose the capacity to produce IL-2 in response to either PHA or Con A (Weiss *et al.*, 1984b; Weiss and Stobo, 1984). Moreover, reconstitution of the T3/Ti expression in one of these mutants by transfection resulted in the restoration of the PHA and Con A responsiveness of this cell (Ohashi *et al.*, 1985; Weiss *et al.*, 1986b). These results are in contrast to those suggesting that the T11 (CD2) molecule may function as the relevant PHA receptor. In these studies, anti-T11 mAb were used as antagonists (O'Flynn *et al.*, 1986). The explanation for this discrepancy is not clear but may reflect differences in experimental approach. The evidence that the T3/Ti complex plays a role in mitogenic lectin-induced T cell activation is compelling.

### B. T11 (CD2, Leu5, LFA-2)

The T11 molecule is a 50-kDa glycoprotein on the surface of all T cells and thymocytes (Howard *et al.*, 1981; Kamoun *et al.*, 1981). This molecule func-

tions as the sheep erythrocyte receptor on human T cells. As many as six distinct epitopes have been defined by mAb reactive with T11: 9.6/$T11_1$, D66, 35.1, $T11_2$, $T11_3$, and 9.1 (Meuer *et al.*, 1984b; Martin *et al.*, 1983; Brottier *et al.*, 1985; Yang *et al.*, 1986). Certain epitopes of T11 are not expressed on resting T cells, $T11_3$ and 9.1, but can be induced by other mAb reacting with the T3/Ti complex or other epitopes of T11 (Meuer *et al.*, 1984b; Yang *et al.*, 1986). Interest in this molecule has been stimulated by the finding that such mAb can function as agonists or antagonists in inducing T cell activation.

Initial studies revealed that an anti-T11 mAb, OKT11, could inhibit lectin and anti-T3-induced lymphokine production T cell proliferation and the lytic activity of cytolytic T cell clones (CTL) (Palacios and Martinez-Maza, 1982; Sanchez-Madrid *et al.*, 1982; Wilkinson and Morris, 1984; Moretta *et al.*, 1985b). This led to the proposal that the T11 molecule might function in immune responses by delivering negative signals (Palacios and Martinez-Maza, 1982). Supporting a negative signal role for the T11 molecule is a recent study demonstrating diminished levels of IL-2 transcripts in stimulated T cells preincubated in the presence of mAb 9.6, reactive with T11 (Tadmori *et al.*, 1986).

Several studies from independent laboratories have demonstrated that certain combinations of anti-T11 mAb can activate T cells, as measured by proliferation or IL-2 production (Meuer *et al.*, 1984b; Brottier *et al.*, 1985; Yang *et al.*, 1986). Similarly, non-antigen-specific cytolytic activity of antigen-specific CTL and natural killer (NK) clones can be induced by appropriate anti-T11 mAb (Siliciano *et al.*, 1985). Individual anti-T11 mAb are insufficient in inducing T cell activation. Only certain combinations of appropriate mAb are able to induce the activation of T cells. Whereas mAb reactive with $T11_2$ + $T11_3$ or 9.1 + 9.6 can activate T cells in an AC-independent manner (Meuer *et al.*, 1984b; Yang *et al.*, 1986), D66 + 9.6 or D66 + $T11_1$ depend upon the presence of Fc receptor-bearing AC (Brottier *et al.*, 1985). Certain T11 mAb can activate resting T cells in the presence of PMA without the addition of a second anti-T11 mAb (Holter *et al.*, 1986). As some combinations of antibodies reactive with different epitopes of T11 do not activate T cells (i.e., $T11_1$ + $T11_3$), simple cross-linking of molecules does not appear to account for the ability of certain combinations of mAb to activate. In contrast to the T3/Ti complex, stimulation of T cells via T11 appears to be exquisitely epitope dependent and requires relatively high (probably saturating) amounts of stimulating mAbs (Meuer *et al.*, 1984b). The ability to stimulate T cells in the absence of AC with appropriate combinations of anti-T11 mAb would appear to exclude the participation of other cell surface molecules in this model of T cell activation. Thus, appropriate triggering of the T11 molecule appears to be able to provide a primary activation signal in

resting T cells which is distinct from that induced by the antigen receptor in view of the AC independence of this pathway.

The T11 molecule is nonpolymorphic. Therefore, it is not likely to play a major role in antigen binding. The physiologic function of this molecule is not clear. The activation of T cells via stimulation of the T11 molecule has been termed the alternative pathway of human T cell activation to distinguish it from the antigen-dependent T3/Ti mediated pathway (Meuer *et al.*, 1984b). As it is functional in thymocytes, it has also been proposed to play a role in thymocyte ontogeny (Fox *et al.*, 1985). Recent studies have suggested that LFA-3 may represent the physiologic ligand of T11 (Springer *et al.*, 1987). This 55- to 70-kDa glycoprotein is widely expressed on tissues. Antibodies reactive with LFA-3 inhibit a wide variety of T cell-dependent functions. A role for LFA-3 and T11 interactions in the thymus has also been proposed. Binding studies suggest a direct interaction between LFA-3 and T11.

The interaction between the T3/Ti complex and the T11 molecule is of some interest. Stimulation of resting T cells via the T11 pathway does not require interaction with the T3/Ti complex. However, prior modulation of the T3/Ti complex inhibits the ability of T11 mAb to activate T cells (Meuer *et al.*, 1984a; Fox *et al.*, 1986). Conversely, modulation of T11 has little effect upon T3/Ti-induced activation. T11 mAb are able to activate NK cells which do not express T3 molecules (Siliciano *et al.*, 1985). As no physical interaction between T11 and T3/Ti has been demonstrated, the explanation of these findings is not clear. However, one possibility is that in addition to their antigen removal effects, the mAb used in such modulation studies may have physiologic effects upon the cell. Of further interest, however, is a recent report suggesting that anti-T3 and anti-T11 mAb can synergize in inducing proliferation in cultures prepared with highly purified T cells (Yang *et al.*, 1986). Thus, in addition to independent pathways of activation, these two pathways may interact under certain conditions.

### C. Thy-1

Thy-1 is included in this review because of its unique structure and the evidence which had accumulated to support its role as a receptor involved in T cell activation in the murine system. The Thy-1 molecule is a 25- to 30-kDa glycoprotein with two allelic forms expressed on mouse thymocytes, peripheral T cells, fibroblasts, epithelial cells, and neurons (Reif and Allen, 1966a,b, 1984). The cDNAs encoding murine and human Thy-1 genes have been cloned and sequenced and exhibit some sequence homology to immunoglobulin genes (Evans *et al.*, 1984; Seki *et al.*, 1985). The murine Thy-1 gene is located on the ninth chromosome and encodes the 112 amino acid polypeptide chain (Blankenhorn and Douglas, 1972; Cohen *et al.*, 1981). A most interesting structural feature of Thy-1 is the finding that the predicted

membrane anchoring region of the molecule does not span the membrane, but is instead truncated and covalently linked to the membrane lipid phosphatidylinositol (Tse *et al.*, 1985; Low and Kincade, 1985). This feature is of particular interest as Thy-1 can function as a receptor involved in activation, but has no described associated molecule and cannot communicate with intracellular effector molecules via a transmembrane of cytoplasmic domain.

Early work with heterosera demonstrated that antibodies reactive with Thy-1 could be mitogenic for murine T cells (Smith *et al.*, 1982). Subsequently, only certain mAb reactive with Thy-1, used individually, could induce IL-2R, IL-2, or IFN-γ production and be mitogenic for murine T cells, whereas other mAb could not (Gunter *et al.*, 1984; MacDonald *et al.*, 1985). However, most anti-Thy-1 mAb failed to induce T cell activation when used alone (Kroczek *et al.*, 1986a). This difference in the agonist effects of these mAb was interpreted to correlate with the distinct epitopes recognized by agonist versus nonagonist antibodies (Kroczek *et al.*, 1986a). However, T cell proliferation was observed if cross-linking of Thy-1 was induced using a rabbit anti-mouse Ig in combination with nonactivating Thy-1 mAb in the presence of PMA (Kroczek *et al.*, 1986a). This response was independent of AC or the epitope of Thy-1 with which the mAb reacted. The requirement for cross-linking Thy-1 is reinforced by the observation that combinations of two Thy-1 mAb, reactive with distinct noncompeting epitopes, were also effective in inducing T cell activation if used in the presence of PMA (Kroczek *et al.*, 1986a). Why some anti-Thy-1 mAb are able to function as agonists in the absence of additional cross-linking antibodies or PMA is not clear. It is of interest that the antigenic epitope recognized by one of these mAb, which by itself can activate T cells, is lost following transfection of Thy-1 into human T cells, murine B cells, or fibroblasts (Kroczek *et al.*, 1986b). Thy-1 has been transfected into the human T cell line Jurkat, and Thy-1 mAb can activate this line in the presence of PMA (Gunter *et al.*, 1986). This important study demonstrates that transfer of the Thy-1 molecule alone is sufficient for the active cell surface receptor. Clarification of the mechanisms of Thy-1-induced activation should be possible in such a transfection system. Although the physiologic ligand of Thy-1 has not been identified, the potent effects of anti-Thy-1 mAb would suggest a potential for involvement in murine T cell activation.

### D. Receptors Which May Provide Accessory Signals in T Cell Activation

#### *1. Tp44*

Several reports have implicated the cell surface molecule Tp44 as potentially playing an important role in T cell activation. The only mAb reactive

with Tp44, 9.3, identifies an 80- to 90-kDa disulfide-linked homodimer composed of 44-kDa subunits which is expressed on the surface of all T4 and ~50% of T8 human T cells (Hansen *et al.*, 1980; Yamada *et al.*, 1985). A murine homologue of this molecule may have recently been identified (Nagasawa *et al.*, 1986). Based on modulation studies of normal T cells and studies of mutants of the Jurkat cell line which fail to express the T3/Ti complex, no physical association between Tp44 and T3 exists (Hara *et al.*, 1985; Moretta *et al.*, 1985a; Weiss *et al.*, 1986a).

Initial studies demonstrated that 9.3 could inhibit the cytolytic activity of CTL (Fast *et al.*, 1981). However, more evidence has accumulated demonstrating agonist properties of 9.3. The addition of 9.3 mAb to T cell cultures has demonstrated two distinct effects. In the first, 9.3 can play a primary role in inducing T cell proliferation (Hara *et al.*, 1985; Moretta *et al.*, 1985a). In monocyte-depleted cultures, the addition of PMA was required to observe proliferation (Hara *et al.*, 1985). Addition of 9.3 mAb to monocyte-depleted cultures failed to induce IL-2 production or IL-2R expression, whereas abundant IL-2 production and IL-2R expression was observed in cultures containing monocytes or PMA (Hara *et al.*, 1985). Thus, the effects of 9.3 mAb mimic the effects of anti-T3 or anti-Ti mAb, although in one study the kinetics of the response to 9.3 were delayed compared to anti-T3 (Moretta *et al.*, 1985a). The dependency upon the T3/Ti complex for activation by 9.3 has been addressed by modulation of the T3/Ti complex and the study of a Jurkat cell mutant which failed to express the T3/Ti complex (Hara *et al.*, 1985; Moretta *et al.*, 1985a; Weiss *et al.*, 1986a). The modulation experiments performed in different laboratories led to conflicting results regarding this dependency; however, the finding that the Jurkat mutant could still be activated by 9.3 plus PMA supports the notion that activation via the Tp44 molecule is independent of the participation of the T3/Ti complex. Although the ligand of Tp44 is unknown, it is clear that this molecule can be involved in delivering primary activation stimuli.

A second accessory function has been demonstrated for Tp44. Addition of 9.3 mAb can substitute for one of the functions of adherent cells in the response to anti-T3, anti-Ti, or T cell mitogenic lectins. If 9.3 is added to cultures of purified T cells in the presence of cross-linked anti-T3, T cell proliferation is observed (Ledbetter *et al.*, 1985; Martin *et al.*, 1986; Weiss *et al.*, 1986a). Thus, 9.3 substitutes for the second function provided by AC, alluded to above, which may involve a soluble factor. In a similar manner, 9.3 can synergize with anti-T3, anti-Ti, or the lectin PHA in inducing Jurkat to produce IL-2 (Martin *et al.*, 1986; Weiss *et al.*, 1986a). Interestingly, 9.3 cannot reconstitute the response to soluble antibody or calcium ionophore by purified T cells or Jurkat (Weiss *et al.*, 1986a). Thus, it does not fully replace the function of AC.