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Thy-1: More than a Mouse Pan-T Cell Marker

S. M. Mansour Haeryfar and David W. Hoskin

Thy-1 (CD90) is a small GPI-anchored protein that is particularly abundant on the surface of mouse thymocytes and peripheral T cells. T cell proliferation and cytokine synthesis in response to Thy-1 cross-linking by specific mAb suggests a role for Thy-1 in mouse T lymphocyte activation. However, a physiological ligand or counterreceptor for murine Thy-1 in the lymphoid compartment has not yet been identified. Thy-1 cross-linking, in the context of strong costimulatory signaling through CD28, results in an activating signal that can at least partially substitute for TCR signaling during mouse T cell activation. Remarkably, Thy-1 cross-linking also results in the potent costimulation of T cells activated through the TCR. This novel dual signaling capacity suggests a possible role for Thy-1 in the maintenance of T cell homeostasis in the absence of TCR triggering, as well as potentiating Ag-induced T cell responses. The Journal of Immunology, 2004, 173: 3581–3588.

Thy-1 was discovered over four decades ago during the search for heterologous antisera against mouse leukemia cells. The serendipitous observation that thymocytes from AKR mice could be lysed with alloantisera raised against AKR thymocytes in H-2-compatible C3HeB/Fe mice was ascribed to the existence of two allelic variants of a then-novel Ag named \( \theta \) due to its presence on mouse thymocytes (1). \( \theta \)-AKR and \( \theta \)-C3H were subsequently renamed Thy-1\(^a\) and Thy-1\(^b\) and, eventually, Thy-1.1 and Thy-1.2, respectively, in part because AKR/Cum mice were later found to possess the \( \theta \)-C3H allele, whereas as a substrain of AKR mice, they were expected to be \( \theta \)-AKR\(^a\) (2).

Thy-1 was initially described as a differentiation marker expressed predominantly in the mouse brain and thymus (3). Later studies revealed differences between thymocytes and lymphocytes from other lymphoid tissues in terms of their susceptibility to cytolyis by anti-thymocyte alloantisera, resulting in the adoption of Thy-1 as a marker for thymus-derived lymphocytes (4). The abundance of Thy-1 in mouse brain allowed \( \theta \)-specific antiserum to be produced by immunizing rabbits with mouse brain. Rabbit anti-mouse brain (RAMB) \(^3\) antiserum differed from murine \( \theta \)-specific alloantisera in that it reacted with both C3H and AKR thymocytes (5). The finding that RAMB antiserum could induce spleen and lymph node cell proliferation was the first indication of Thy-1 involvement in T cell activation (6), which was later confirmed in studies using the first anti-Thy-1 mAb (clone G7) with T cell-activating properties (7).

A number of important discoveries have resulted from research on Thy-1. Structural studies on Thy-1 contributed to the foundation of the Ig gene superfamily (8), and also led to the first biochemical description and subsequent characterization of a vertebrate GPI anchor (9). More recently, Thy-1 has been used as a marker for lipid rafts in murine T cells owing to its GPI anchorage and consequent localization within these specialized microdomains (10). Amazingly, the precise biological function of Thy-1 is not yet clear.

Structure, tissue distribution, and interspecies variations

Thy-1 has been conserved throughout evolution, suggesting an important function for this molecule. Thy-1 homologs have been described in many species, including squid, frogs, chickens, mice, rats, dogs, and humans (8, 11). The locus for murine Thy-1 maps to chromosome 9 and includes two alleles termed Thy-1\(^a\) and Thy-1\(^b\), which code for Thy-1.1 and Thy-1.2 glycoproteins, respectively. Thy-1.1 and Thy-1.2 differ solely in amino acid position 89, which is occupied by arginine in Thy-1.1 and by glutamine in Thy-1.2 (8). Thy-1.2 is expressed by most mouse strains, whereas Thy-1.1 is an alloantigen of the AKR/J and PL strains. In humans, the gene coding for Thy-1 is located in chromosome 11q22.3 (12).

The core protein of 25-kDa rodent Thy-1 consists of 111 or 112 aa, and is \( N \)-glycosylated at three sites (11). In contrast, human Thy-1 contains only two glycosylation sites (12). Thy-1 is one of the most heavily glycosylated membrane proteins with a carbohydrate content up to 30% of its molecular mass (11). The composition of Thy-1 carbohydrate moieties varies considerably between different tissues in the same animal, and even among cells of the same lineage at different stages of ontogeny. For instance, galactosamine is present only on brain Thy-1, whereas galactosamine is present only on brain Thy-1.

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\(^{3}\) Abbreviations used in this paper: RAMB, rabbit anti-mouse brain; PI-PLC, phosphatidylinositol-specific phospholipase C; DAG, diacylglycerol; GPI-AP, GPI-anchored protein; PKC, protein kinase C; PTK, protein tyrosine kinase; PFN, perforin; GzmB, granzyme B; DC, dendritic cell.
Thy-1 is also expressed by endothelial cells, smooth cells, myoblasts, epidermal cells, and keratinocytes (3, 11). In variety of other cell types including thymocytes, peripheral T studied thus far (11). In the mouse, Thy-1 is also found on a membrane-spanning proteins.

Thy-1 lacks a transmembrane region, and is tethered to the outer leaflet of the lipid bilayer by a GPI moiety that is sensitive to cleavage by phosphatidylinositol-specific phospholipase C (PI-PLC) (9). However, Thy-1 sensitivity to PI-PLC often varies, depending on cell type as well as the maturation and activation state of the cell. Although a substantial proportion of Thy-1 is released from thymocytes exposed to PI-PLC, very little Thy-1 is cleaved by PI-PLC from CTL-L cells (15). Sensitivity of Thy-1 to PI-PLC also varies among subpopulations of thymocytes (16). Consistent with the notion that sensitivity to PI-PLC can be modulated, Thy-1 becomes resistant to PI-PLC after mitogenic stimulation of T cells (17). It is conceivable that variations in the GPI anchor per se or possible interactions of Thy-1 with other cell surface proteins may interfere with the action of PI-PLC. Interestingly, the GPI anchor is important in determining the conformation of Thy-1, because cleavage of the GPI anchor causes a loss in Thy-1 reactivity with several mAbs (18). Approximately 40–60% of Thy-1 is estimated to be highly mobile in the plane of the cell membrane with a diffusion coefficient of $2–4 \times 10^{-9}$ cm$^2$/s in murine thymocytes, lymphoma cells, and fibroblasts (19). This value is $\sim 10$ times greater than diffusion coefficients reported for many membrane-spanning proteins.

Thy-1 is present on brain cells and fibroblasts of all species studied thus far (11). In the mouse, Thy-1 is also found on a variety of other cell types including thymocytes, peripheral T cells, myoblasts, epidermal cells, and keratinocytes (3, 11). In humans, Thy-1 is also expressed by endothelial cells, smooth muscle cells, a subset of CD34$^+$ bone marrow cells, and umbilical cord blood- and fetal liver-derived hemopoietic cells (20, 21). Thy-1 expression in the nervous system is predominantly neuronal, but some glial cells are also Thy-1$^+$, especially at later stages of their ontogeny (22). Thy-1 expression in the brain is developmentally regulated. Thy-1 levels in the neonatal rat brain, as well as the developing human brain, are low in comparison with adult brain (23, 24). During the first few weeks of postnatal development, Thy-1 levels increase dramatically in parallel with histological and physiological maturation of the brain.

Lymphoid tissues of different species show a great deal of variation in Thy-1 expression. Thy-1 is probably the most abundant glycoprotein of murine thymocytes, with about one million copies per cell covering up to 10–20% of the cell surface (25). Mouse cortical thymocytes express higher levels of Thy-1 than medullary thymocytes, whereas lymph node cells possess considerably less Thy-1 ($\sim 200,000$ copies/cell) in comparison with thymocytes (11). A similar inverse relationship between levels of Thy-1 expression and T cell differentiation is seen in rats, although rat Thy-1 is lost at an earlier stage of T cell maturation (26). In humans, Thy-1 expression is restricted to only a small population of cortical thymocytes (27).

Thymopoietin, thymosin, PGs, nerve growth factor, IL-1, TNF, PMA, Ca$^{2+}$ ionophore, and diacylglycerol (DAG) are among the agents that can induce Thy-1 expression in different cell types (21, 28–30). Thy-1 expression is also up-regulated during mouse and human NK cell activation (31). Stimulation of resting T cells with anti-CD3 mAb does not noticeably affect cell surface expression of Thy-1 (our unpublished data), although Thy-1 mRNA expression increases 2-fold when murine T cells transition from a naive to effector/memory phenotype (32). Thy-1 expression in different tissues is typically determined serologically using anti-Thy-1 Abs, some of which cross-react with other subcellular entities (33). Serological detection of Thy-1 where Thy-1 expression is not expected should therefore be interpreted with caution and confirmed at the mRNA level. In addition, when defining Thy-1$^+$ and Thy-1$^-$ cell populations, as well as Thy-1 induction on different cell types, it is important to differentiate shedding and cell-to-cell transfer of Thy-1 from transcriptional and/or posttranscriptional up-regulation. Spontaneous in vitro shedding of cell surface Thy-1 has been reported (34). A soluble form of Thy-1 also exists in vivo, perhaps released by the action of endogenous phospholipases (35). Thy-1 may also participate in a process by which certain GPI-anchored proteins (GPI-APs) (e.g., CD55 and CD59) are transferred from one cell membrane to another (36).

**Thy-1 immunobiology**

**General physiological function(s).** The exact biological role(s) of Thy-1 has remained controversial, if not mysterious. Thy-1 has been proposed to mediate cell-cell interactions (8), a concept supported by the finding that both RAMB and anti-Thy-1 mAbs partially inhibit E-rosette formation by mouse thymocytes (37). Thy-1 also promotes the adhesion of thymocytes to thymic epithelium (38), and electron micrographs taken from the thymus of Thy-1$^{-/-}$ mice show the presence of atypical contacts between thymic cells (25). Given the abundance of Thy-1 in the cell-cell contact areas of the brain and lymphoid compartments (10, 39), it is possible that Thy-1 serves a similar function in both the nervous and immune systems. In fact, Thy-1 predominance in neurological synapses, as well as its ability to mediate T cell mitogenesis inspired Norcross (40) to propose the synaptic model of T cell activation. The term “immunological synapse” was thus coined to describe junctions between T cells and APCs that are the immunological counterpart of synaptic junctions in the nervous system (41). The similarity between neuronal and immunological synapses may extend beyond nomenclature. Agran, an extracellular proteoglycan that functions in the clustering of receptors within neuromuscular junctions, is also expressed in lymphocytes, and is important in the creation of signaling domains in both immune and nervous systems through a common lipid raft pathway (42). Thy-1 is also recruited to the immunological synapses formed between primary resting T cells and surrogate APCs consisting of cell-sized microbeads coated with anti-CD3 and anti-CD28 mAbs (our unpublished data). Although it is not yet clear whether Thy-1 recruitment into the immunological synapse is an active process or a passive event simply reflecting the aggregation of lipid rafts, the presence and concentration of Thy-1 within the synapse is consistent with a role for Thy-1 in T cell activation.

Furthermore, Thy-1 deficiency has an unexpectedly subtle effect on cells of the T lineage. Although an initial report indicated that negative selection and self restriction to MHC class II occurs normally during T cell development in Thy-1$^{-/-}$ mice (43), a more careful analysis of mutant thymocytes revealed impaired maturation from the CD4$^+$CD8$^+$ double-positive to the CD4$^+$ or
CD8+ single-positive stage, as well as enhanced TCR signaling (44). In addition, peripheral T cells from Thy-1-/- mice show a modest decrease in their proliferative response to immobilized anti-CD3 mAb, although responses to Con A or PMA plus Ca2+ ionophore are normal (45). Cytokine synthesis is normal but cutaneous immune responses are moderately impaired in Thy-1-deficient animals. These findings are consistent with a role for Thy-1 in fine-tuning T cell activation. Nevertheless, Thy-1 deficiency does not generally compromise immunity (25), perhaps due to the extensive redundancy in signaling and adhesion molecules that exists within the immune system. In this regard, GPI-APs other than Thy-1 may compensate for Thy-1 deficiency. Moreover, at least some of the roles played by Thy-1 in rodents may be assumed by other membrane proteins in humans. One such protein is CD7, which is similar to murine Thy-1 in terms of gene sequence and cellular distribution (46). Interestingly, similar to Thy-1/CD3 coengagement in mouse T cells (47), mAb cross-linking of CD7 on human PBMCs up-regulates IL-2 production, IL-2R (CD25) expression, and cellular proliferation in response to suboptimal stimulation with immobilized anti-CD3 mAb (48).

**Signal transduction by Thy-1.** It is perplexing that GPI-anchored Thy-1 can signal T cell activation. Nevertheless, signaling through many, if not most, GPI-APs (Thy-1 included) induces qualitatively similar responses. The GPI anchor is believed to be an important signaling element because a genetically engineered transmembrane version of Qa-2 that lacks a GPI anchor is unable to signal (49). Conversely, Ab-mediated aggregation of MHC class I molecules engineered to contain a GPI anchor results in T cell activation. Both GPI anchor/lipid raft-dependent and -independent signaling pathways may be initiated following ligation of at least some GPI-APs (50). Furthermore, although lipid raft localization is regarded as a cardinal feature of GPI-APs, the signaling potency of these proteins may depend on additional factors. Although insertion of purified rat brain Thy-1 into the membrane of murine splenocytes results in rat Thy-1 surface expression, lateral mobility, and sensitivity to PI-PLC, T cell activation is achieved only with mAbs to endogenous mouse Thy-1 and not by mAb cross-linking of rat Thy-1 (51). Structural differences between rat brain Thy-1 and mouse Thy-1.2 in terms of posttranslational modifications, especially at the level of carbohydrate residues attached to the GPI anchor backbone, may account for this finding. Alternatively, murine lipid rafts may differ from their counterparts in the rat in terms of their microenvironment and/or kinase contents, and may therefore fail to provide a suitable platform for signaling through rat brain Thy-1.

Although signaling properties imparted by the GPI anchor may be a common characteristic of GPI-APs, distinct extracellular domains of these proteins ensure the specificity of interactions with their cognate ligands, and may also modulate consequent activation processes. However, because the physiological ligands for many GPI-APs, including T cell-expressed Thy-1, are yet to be identified, most information on signaling through these proteins has been obtained using cross-linking mAbs whose triggering action is presumed to mimic that of the putative ligands. Although this approach has several drawbacks, it is reassuring that, in cases in which the ligands for the GPI-APs in question are known, cognate ligation provides results that are similar to those obtained with cross-linking mAbs (52).

Mechanisms that have been proposed to account for signaling through Thy-1 include a functional association with the TCR/CD3 complex (53), an indirect effect on TCR signal transduction imposed by the abundant expression of Thy-1 (25), and generation of DAG and/or phosphatidic acid from the GPI anchor upon ligation of the protein moiety or its cleavage from the cell surface by endogenous phospholipases resulting in the production of bioactive second messengers (54). DAG, which is partially responsible for the membrane anchorage of Thy-1, has been proposed to play a role, via protein kinase C (PKC) activation, in Thy-1-mediated signaling (11). Another molecule that interacts with Thy-1 is p100, a transmembrane protein expressed by a subset of CD4+ T cells that coinmunoprecipitates with anti-Thy-1 mAb and is required by these cells for cytokine synthesis and cellular proliferation in response to Thy-1 triggering (55). However, p100 cannot account for Thy-1-triggered signaling in other cell types that do not express p100. CD45, fyn protein tyrosine kinase (PTK), and G proteins are also known to physically associate with Thy-1, and may, therefore, be involved in Thy-1 signal transduction (56–58). More recently, signaling triggered by GPI-APs has been linked to lipid raft aggregation that leads to the sequestration and juxtaposition of signaling entities enriched within these microdomains (59). Advocates of the raft hypothesis of GPI-AP-mediated signaling argue that cross-linking of these proteins causes lipid rafts to coalesce, thereby promoting clustering and activation of Src family PTKs that colocalize with GPI-APs within lipid rafts (60). Activated PTK could phosphorylate intracellular substrates that regulate signal transduction pathways. Alternatively, changes in distribution, conformation, or the localized microenvironment of PTKs may result in auto- or transphosphorylation of PTKs and the initiation of signal transduction. Cocapping of Src family PTKs with GPI-APs or the lipid raft marker ganglioside GM1 suggests that redistribution of the outer leaflet of the plasma membrane causes a concomitant redistribution of the inner leaflet membrane components.

**Thy-1 ligand(s)/counterreceptor(s).** Investigation of the role(s) of Thy-1 in T cell activation is hindered by the fact that a ligand or counterreceptor for T cell-associated Thy-1 has not yet been identified. Thy-1 is involved in cell-cell adhesion events and has even been reported to self-polymerize (26). Human, rat, and mouse Thy-1 share a conserved RGD-like sequence that functions as a binding motif for αvβ3, αvβ1, and αvβ2 integrins, and allows Thy-1 on EL-4 thymoma cells to bind a β3 integrin (most likely αvβ3) on astrocytes (61). The interaction between Thy-1 and astrocyte β3 integrin triggers tyrosine phosphorylation of astrocyte focal adhesion proteins, thereby promoting focal contact formation, as well as astrocyte attachment and spreading. Human polymorphonuclear leukocytes and monocytes, but not lymphocytes, express a putative Thy-1 ligand that is binding to polymorphonuclear leukocyte and monocyte binding to activated endothelial cells and fibroblasts that express Thy-1 (62). Thy-1 expressed by PMA-stimulated human dermal microvascular endothelial cells interacts with the leukocyte integrin αMβ2 to mediate leukocyte firm adhesion to activated endothelium and leukocyte transendothelial migration (63). Thy-1 αvβ3 interactions may also promote leukocyte extravasation during inflammation, because Thy-1 is expressed on endothelial cells in inflamed tissues such as the
sympathetic membrane of joints from patients with active rheumatoid arthritis (62). Malignant melanoma cells also express a Thy-1 ligand that binds human dermal microvascular endothelial cells induced to express Thy-1 by melanoma cell-derived soluble factors, suggesting that Thy-1 may be involved in melanoma metastasis (64). In view of the progress that has been made in identifying ligands/counterreceptors for human Thy-1, it is indeed remarkable that a ligand/counterreceptor for murine Thy-1 that functions in the context of T cell-mediated immune responses has not yet been identified.

Role of Thy-1 in murine T cell activation

Functions proposed for mouse T cell-associated Thy-1 range from an accessory role during T cell activation (47, 65) to negative regulation of T cell responses (25, 44). However, our current understanding of Thy-1 function in the context of T cell activation is mainly derived from studies using anti-Thy-1 Abs. This approach is far from perfect, because isotype, epitope specificity, and whether the Ab is monoclonal or polyclonal may all affect the outcome of Thy-1 ligation. Furthermore, some anti-Thy-1 mAbs must be immobilized on plastic or cross-linked by a secondary anti-Ig Ab or accessory cell FcRs to activate T cells (11, 50). In the latter case, accessory cells may contribute other membrane-bound molecules or produce soluble factors that complicate data interpretation. In addition, another stimulus such as the PKC activator PMA is sometimes required for robust Thy-1-induced T cell activation (7, 66). The apparent outcome(s) of Thy-1 cross-linking by mitogenic anti-Thy-1 Abs may vary depending on the experimental systems and readouts used to assess T cell responses. For instance, anti-Thy-1 mAb G7 induces peripheral mouse T cells to synthesize IL-2, up-regulate CD25, and proliferate (7, 66), whereas cross-linking of Thy-1 on thymocytes by the same mAb causes apoptosis (67).

We recently discovered that Thy-1 engagement by mAb G7 in the presence of syngeneic dendritic cells (DCs) induces vigorous T cell proliferation and IL-2 synthesis (68). DC-mediated enhancement of Thy-1-induced T cell activation is dependent on CD80/CD86 interactions with CD28, indicating that CD28 costimulates Thy-1-driven T cell activation. Indeed, stimulation of T cells with anti-Thy-1 and anti-CD28 mAbs cointernalized on microbeads resulted in a proliferative response that was comparable with that elicited by bead-immobilized anti-CD3 and anti-CD28 mAbs. These observations indicate that Thy-1 triggering can at least partially substitute for TCR signaling (signal 1) during T cell activation. Thy-1 engagement by a putative Thy-1 ligand on DCs may stabilize or fine-tune immunological synapses that form at the interface between naive T cells and DCs in the absence of cognate Ag (69), thereby ensuring continuous scanning of DC surface areas for foreign peptides and/or eliciting modest levels of T proliferation that may contribute to the maintenance of T cell homeostasis. Importantly, unlike signaling through the TCR, Thy-1 signaling is not sufficient to induce cytotoxic effector function (68), which ensures that Thy-1-stimulated T cells will not exhibit promiscuous cytotoxicity and therefore pose no threat to surrounding tissues. It is not clear whether the ability of Thy-1 to provide a signal 1 surrogate during T cell activation is shared by all T cell-associated GPI-APs, although the finding that human T cells proliferate in response to CD59 cross-linking with simultaneous engagement of CD28 lends support to this notion (70).

Signal 1 provided by the TCR is necessary but not sufficient for complete and productive T cell activation, which also requires a costimulatory signal (signal 2) that is delivered by other T cell surface molecules interacting with their ligands/receptors on APCs. Although interactions between CD28 on T cells and CD80/CD86 on APCs are arguably the principal source of signal 2, additional costimulatory molecules (e.g., CD2 and CD44) that are present within lipid rafts also participate in T cell activation (71). Thy-1 is also well situated to function as a costimulatory molecule due to its localization to T cell lipid rafts (10). Thus, Thy-1 signaling promotes Ag-induced effector function by cloned CTLs and enhances anti-CD3 mAb-induced activation of EL-4 thymoma or hybridoma T cell lines (47, 65). Moreover, T cells stimulated with microbeads coated with anti-CD3 mAb plus anti-Thy-1 mAb G7 exhibit a proliferative response that is comparable in magnitude with that of T cells stimulated through the TCR/CD3 complex and CD28, demonstrating that Thy-1 is similar to CD28 in its capacity to costimulate murine T cell activation (68). Therefore, Thy-1 is remarkable in its capacity to provide either signal 1 or signal 2, depending on the availability of concomitant signaling through the TCR or costimulatory molecules such as CD28.

Similarities and differences between Thy-1- and TCR-mediated signal transduction pathways. Mouse T cell activation via Thy-1 requires an intact and functional TCR/CD3 complex, because Thy-1 triggering does not activate TCR/CD3-deficient T cells (53). Thy-1 and the TCR have both been shown to physically associate with the membrane-based tyrosine phosphatase CD45, suggesting that a common interaction with CD45 accounts for the dependence of Thy-1 signaling on TCR coexpression (56). This is consistent with the finding that CD45– T cell clones fail to proliferate in response to anti-Thy-1 or anti-CD3 mAb (72). There is no evidence that Thy-1 and the TCR form a ternary complex with CD45; however, if such a complex exists, cross-linking of Thy-1 would be equivalent to cross-linking the TCR. Regardless, Thy-1- and TCR-mediated signaling events are in many ways strikingly similar (73), which suggests that Thy-1-associated signal transduction involves a functional association with at least some elements of the TCR/CD3 signal transduction pathway. However, the ability of Thy-1 to induce Ca2+ flux in TCR/CD3-deficient T cells argues that not all aspects of Thy-1 signaling are TCR dependent (53). The failure of Thy-1 triggering to induce cytolytic effector function indicates another fundamental difference between Thy-1 and TCR signaling pathways (68).

T cell mutants that lack GPI-APs (including Thy-1) show impaired tyrosine phosphorylation of TCR-ζ and ZAP-70, and reduced activation of TCR-associated Src kinases fyn and lck in response to TCR stimulation (74). However, it is difficult to reconcile these results with the finding that murine T cells pretreated with PI-PLC exhibit normal proliferation in response to anti-CD3 mAb (17). Moreover, T cell development, as well as activation in response to anti-CD3 mAb, occurs normally in mice that lack GPI-APs on thymocytes and peripheral T cells due to specific disruption of the phosphatidylinositol glycan-class A gene in their thymocytes (75). Studies on Thy-1–/– mice have also yielded contradictory findings. A negative regulatory function for Thy-1 was suggested by the hyperresponsiveness of Thy-1–/– thymocytes to TCR triggering, as evidenced by heightened activation of lck, phosphorylation of CD3-ζ and p23 TCR-ζ chains, Ca2+ flux and cell proliferation.
(44). Paradoxically, peripheral T cells from Thy-1−/− mice exhibit a diminished Ca2+ influx and reduced PTK activity following TCR engagement (45).

To clarify the often-conflicting data on the relationship between Thy-1 and the TCR signaling in T cells, we determined the effect of exposing mouse T cells to a panel of pharmacologic inhibitors of signaling intermediates before and during stimulation with either anti-Thy-1 G7 or anti-CD3 mAbs (73). The results indicate a similar requirement for Src family PTKs, PI3K, and calcineurin in both Thy-1- and TCR-induced T cell activation. However, Thy-1 signaling is more sensitive than TCR signaling to PKC inhibition, perhaps because Thy-1 triggering alone does not strongly induce PKC activation in T cells (66). During normal Ag-specific T cell activation in which Thy-1 may have an accessory role, optimal PKC activation is most likely provided through the TCR/CD3 complex and additional T cell surface molecules. The advent of new pharmacologic inhibitors of different PKC isoforms should allow further dissection of the Thy-1- and TCR-associated signaling pathways in terms of PKC isoform involvement, in particular whether Thy-1 stimulation causes PKCβ recruitment to the central region of the immunological synapse, as happens after TCR triggering (76). Thy-1-driven T cell proliferation is also more sensitive to MEK1 inhibition, suggesting suboptimal activation of ERK1 and ERK2 in response to Thy-1 signaling (73). There is also a preferential requirement for fyn activation during Thy-1 signaling because Thy-1-driven T cell activation, but not TCR-induced T cell activation, is impaired in fyn-deficient T cell clones (77). Interestingly, inhibition of p38 MAPK enhances T cell proliferation induced by soluble anti-Thy-1 mAb G7, whereas p38 inhibition prevents TCR-induced T cell proliferation (73). Ongoing studies seek to determine whether p38 inhibition has a similar effect on T cells stimulated with bead-immobilized anti-Thy-1 and anti-CD28 mAbs.

**Induction of T cell effector functions.** Several lines of evidence indicate that Thy-1 cross-linking on mouse T cells induces cytokine production. Ab-mediated ligation of Thy-1 leads to de novo synthesis of IL-2, whereas removal of GPI-APs by PI-PLC or mAb blockade of Thy-1 impairs signaling through the IL-2/IL-2R system via suppression of IL-2 production and/or assembly of the IL-2R (7, 11, 17, 65, 78). Different effects have sometimes been reported in different systems, most likely because of the mode in which anti-Thy-1 Abs were presented to T cells (i.e., soluble vs immobilized), the presence of FcR+ accessory cells in culture, and the capacity of anti-Thy-1 Abs to bind these FcRs. Moreover, whether these Abs generate a negative signal on their own, prevent interactions between Thy-1 and its putative ligand, or impede the mobility of Thy-1 or other molecules in the plane of the membrane may result in apparently conflicting outcomes. The origin, type, and activation state of responding T cell populations are also of prime importance in determining the amount(s) and type(s) of cytokines produced following Thy-1 cross-linking. Analysis of a panel of T cell hybridomas specific for the same Ag and expressing comparable levels of Thy-1 revealed remarkable heterogeneity in the magnitude of IL-2 responses elicited upon stimulation with the same anti-Thy-1 mAbs, even though these hybridomas produced similar levels of IL-2 in response to specific Ag or Con A (79).

The proliferation of primary CD8+ T cells and an IL-2-dependent T cell clone in response to plate-bound anti-TCR mAb is profoundly inhibited by coimmobilized nonstimulatory anti-Thy-1 mAb 30-H12 (78). IL-2 is secreted normally but is not used by the IL-2R to support T cell growth because of reduced assembly of high-affinity IL-2R and impaired phosphorylation of JAK1 and JAK3. CD25 is normally enriched within lipid rafts, whereas the other IL-2R chains, as well as JAK1 and JAK3, are found in soluble membrane fractions where trimerization of the IL-2R chains and subsequent IL-2-mediated signal transduction events are believed to occur (80). Immobilization of raft-associated Thy-1 by plate-bound mAb is believed to cause lipid rafts to sequester CD25, thereby preventing the assembly of the high-affinity IL-2R outside lipid rafts. In contrast, T cells stimulated with anti-CD3, anti-CD28, and anti-Thy-1 mAb 30-H12 coimmobilized on microbeads exhibited slightly enhanced proliferation in comparison with stimulation with only anti-CD3 and anti-CD28 mAb (our unpublished data). These conflicting results may be due to differences in the spatial orientation of the anti-Thy-1 Abs presented to T cells as Thy-1 ligand substitutes. Ab-coated microbeads may be superior to Abs immobilized on flat plastic surfaces due to the spherical shape of the beads, which better mimic the interactions between T cells and APCs. However, both systems ignore the physiological contributions of professional APCs such as DCs, which actively participate in synapse formation by rearranging their actin cytoskeleton (81).

Many studies have used blocking or activating anti-Thy-1 mAbs to explore the role of Thy-1 in CTL responses, although few have examined the role of Thy-1 during the induction phase of CTL responses. The lytic activity of allospecific CTLs generated in MLC is inhibited when blocking anti-Thy-1 mAb is present during the effector phase of cytolysis (82). Similarly, short-term pretreatment of some TCRαβ+ and TCRγδ+ viral Ag-reactive or alloreactive CTL clones with a nonstimulatory anti-Thy-1 mAb inhibits the subsequent lytic function of these clones (65). Ag-specific IFN-γ production by these cloned T cells is also inhibited by Thy-1 blockade, suggesting Thy-1 involvement in CTL activation. Studies with cloned CTLs and stimulatory anti-Thy-1 mAbs also support a role for Thy-1 in CTL responses. The cytolytic activity of several memory-like CTL hybridomas generated in vivo or in MLC is enhanced by pretreatment with anti-Thy-1 mAb G7 (83). The same anti-Thy-1 mAb also boosts the cytolytic function of OVA-specific CD4+ Th1 clones, as well as allosreactive CD8+ T cell clones in a redirected lysis assay (77, 84). In addition, coimmobilization of anti-Thy-1 mAb G7 with suboptimal doses of H-2-Kb alloantigen on a flat polystyrene surface causes serine esterase release from H-2-Kb-alloreactive CTLs, although Abs to other non-TCR surface molecules also elicit a similar response (85). However, this effect is seen only when mAbs are used in immobilized form, suggesting that coimmobilized mAbs may increase the overall avidity of interactions between CTLs and Ag-bearing surfaces, thereby leading to higher TCR occupancy with Ag at a given Ag density. Alternatively, mAb binding to the CTL surface molecules may generate a stimulatory signal that synergizes with TCR signaling.

Our findings using freshly explanted mouse T cells that were activated by Thy-1 cross-linking are in sharp contrast to these earlier results obtained with cloned CTLs. Purified T cells that
were stimulated with anti-Thy-1 mAb G7 in combination with potent costimulatory signals provided by DCs failed to develop cytotoxic effector function, even though these T cells expressed ample perforin (PFN), granzyme B (GzmB), and Fas ligand, and efficiently formed conjugates with target cells (68). The inability of Thy-1-stimulated T cells to trigger delivery of the lethal hit was attributed to defective polarization of cytotoxic granules to the T cell-target cell interface. Enhanced PFN and GzmB gene expression as a result of Thy-1 triggering conflicts with a previous report that Thy-1 cross-linking selectively activates Fas ligand but not granule-mediated cytotoxicity by CTL clones (86). Anti-Thy-1 mAb G7 also allowed for redirected lysis of FcR+ target cells by CTL clones (86), whereas, in our hands, the same mAb failed to function in a redirected lysis assay. These discrepant findings may reflect differences in activation requirements between resting T cells and CTL clones that have already been stimulated through the TCR by cognate Ag before Thy-1 ligation. Moreover, TCR-triggered Fas ligand induction takes place within hours of TCR ligation, whereas PFN and GzmB gene expression is a later event that is at least in part secondary to IL-2R signaling (87). Short-term exposure to stimulatory anti-Thy-1 mAb in a redirected lysis assay is therefore not sufficient to induce PFN and GzmB expression, whereas a longer exposure to anti-Thy-1 mAb in the context of strong costimulation provided by DCs will induce IL-2-dependent expression of GzmB and PFN. Importantly, Thy-1-stimulated T cells are capable of lysing FcR+ target cells in a redirected lysis assay when anti-CD3 mAb but not anti-Thy-1 mAb is present during the effector phase of cytolysis (68), suggesting a unique requirement for TCR triggering in the development of a fully functional cytotoxic phenotype.

Concluding remarks and future directions

Although Thy-1 has been studied for over four decades, several important questions regarding Thy-1 immunobiology remain unanswered. The abundance of Thy-1 expression, its signaling properties, and the stimulatory effect of Thy-1 cross-linking on mouse T cells indicate that Thy-1 is more than just a T cell marker. As shown in Fig. 1, Thy-1 is able to at least partially substitute for TCR-induced signal 1 during T cell activation, and also has the capacity to costimulate T cells. The dual signaling capacity of Thy-1 depends on the availability of signaling via other cell surface molecules. In the presence of strong costimulation and the absence of TCR ligation, Thy-1 provides a surrogate form of signal 1 that induces IL-2 production and T cell proliferation but not cytotoxic effector function. However, in the presence of a classical TCR-driven signal, Thy-1 acts as a costimulatory molecule to augment T cell responses. We hypothesize that Thy-1-driven T cell expansion without the induction of a fully functional lytic phenotype may constitute a mechanism for the maintenance of T cell homeostasis without the risk of developing cell-mediated autoimmunity. The signals that govern T cell homeostasis under normal or lymphoid-poor states are not well understood. Ongoing studies seek to determine the contribution of Thy-1 signaling to the maintenance of the peripheral T cell pool in normal adult mice, as well as to acute homeostatic T cell proliferation that occurs in response to a lymphopenic state.

A considerable body of evidence indicates that Thy-1 signaling requires a functional association with the TCR/CD3 complex. Nevertheless, fundamental differences exist between Thy-1- and TCR-associated signaling pathways. Although Thy-1 is enriched within lipid rafts, the extent to which signaling through Thy-1, and perhaps other GPI-APs, depends on lipid raft integrity and heterogeneity within the same cell or among different cell types is yet to be determined. In addition, lipid raft presence and content in Thy-1−/− mice remains to be assessed. Because lipid rafts have been implicated in TCR-mediated signal transduction, examination of possible recruitment of TCR/CD3 complex components (especially the ζ-chain) to lipid rafts as a result of Thy-1 cross-linking may clarify the nature of Thy-1 and TCR interactions. Given the abundance of Thy-1 in lipid rafts, a role for Thy-1 in the formation and/or fine-tuning of immunological synapses is more than plausible. Another area of inquiry that has received insufficient attention relates to glycoimmunobiology of Thy-1. Carbohydrate decoration of Thy-1 varies considerably among lymphoid cells at different stages of maturation, suggesting that Thy-1 glycosylation patterns may contribute to interactions with presumed ligand(s) in different lymphoid tissues.

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