CD45 and the Immune Response

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ABSTRACT

CD45 is a major transmembrane glycoprotein expressed on all nucleated hematopoietic cells. Eight isoforms of CD45 are distributed through the immune system according to cell type and degree of cellular differentiation. Heterogeneity among the isoforms is found entirely in the extracellular domain, arising from the differential splicing of up to four exons of a single gene. The control of isoform expression suggests that the extracellular domain may participate in protein-protein interactions with isoform-specific ligands. The intracellular domain of CD45 is large (~700 amino acids), identical for all isoforms, and highly conserved across species. Two nonidentical intracellular sequences of about 240 amino acids that are homologous with a tyrosine phosphatase consensus sequence have been identified. Studies with purified CD45 have shown that all isoforms possess enzymatic activity in vitro assays. In several T and B cell lines and in natural killer cells, it appears that CD45 is required for optimal signal transduction after stimulation through a number of surface receptors. Although an in vivo substrate has not been identified conclusively, one model suggests that CD45 functions to dephosphorylate a negative-regulatory tyrosine residue on one or more protein tyrosine kinases involved in receptor-mediated second messenger formation. In T cells, the src family kinases, lck and lyn, are candidates for this regulated kinase. In this review, some of the structural and functional aspects of CD45 and its role in signal transduction in the immune system are discussed.

Key Words: CD45, tyrosine phosphatase, T cells, signal transduction
that natural killer (NK) cells deficient in CD45 expression fail to signal via CD2 or to kill targets efficiently (27).

STRUCTURE/FUNCTION PROPERTIES OF CD45

The isoform heterogeneity found in CD45 is generated by the differential splicing of exons 4, 5, 6, and probably 7 of a single gene (1,2). These exons each encode approximately 50 amino acids inserted near the N terminus (1,2), which contains multiple sites for O-linked glycosylation (28,29) (Figure 1). Thus, both polypeptide structure and addition of carbohydrate contribute to differences in molecular weight. To date, eight isoforms of CD45 have been described in murine cells. However, there may be species-specific differences in exon splicing. Presently, there is evidence for five isoforms of CD45 in human cells (30,31). These appear in patterns of expression that are tightly regulated in lymphopoiesis, suggesting that the structural differences are involved in functional aspects of differentiation within the immune system. Initially, isoform-specific expression was thought to distinguish "naive" from "memory" T cells (1). However, recent data argue that isoform specificity may actually identify cells according to different requirements for activation (32). In addition, one recent study suggests that CD45 isoform expression relates to the lifespan of T cell subsets (33).

The ordered, cell-specific expression of isoforms suggests that specific ligands may bind to the various CD45 isoforms. Sufficient information is encoded in the large (400- to 500-amino-acid) extracellular domain for it potentially to function as a cell surface receptor. A number of investigators have attempted, without success, to demonstrate specific binding of soluble recombinant proteins consisting of the various CD45 isoform extracellular domains to known ligands or other cell surfaces (34). Recently, however, it was demonstrated that a B cell adhesion molecule, CD22, appears to interact with T cells (35). Initially, this was felt to be via a specific interaction with a particular CD45 isoform (CD45RO). Experiments supporting this result included studies performed with a soluble CD22-immunoglobulin Fc receptor chimeric protein, which bound to T cells in proportion to the surface expression of CD45RO (35). Further, a CD45RO-specific monoclonal antibody (mAb) prevented the binding of the CD22 chimera with T cells. However, subsequent studies with the chimeric CD22 molecule and coimmunoprecipitation experiments demonstrated that multiple isoforms of CD45 appear to interact with CD22 (36). The means by which CD22 binds to members of the CD45 family is not yet completely understood. It appears that the interaction is mediated via the ability of CD22 to bind carbohydrate moieties present on CD45. Further, it is suggested that CD22 may bind to a spectrum of lymphocyte ligands that share common glycosylation

Figure 1. Schematic structure of CD45. The extracellular region is generated by the differential splicing of four exons of a single gene. CD45 crosses the membrane once. Catalytic (PTP) activity resides in the two PTP-homology domains of the highly conserved cytoplasmic region. Conserved cysteine residues, which appear to be essential for enzymatic function, are indicated. aa, amino acid.
patterns. One such ligand is CD75, a B cell–specific antigen that does not share primary structure homology with CD45 (35). Although the physiologic relevance of the interaction between CD45 and CD22 remains unclear, an intriguing study demonstrated that cross-linking the TCR and soluble CD22 inhibited TCR-mediated signaling (36). This suggests that the binding of CD45 with ligand may modulate signal transduction. The mechanism of this effect is not known.

CD45 has also been shown to associate physically with other surface proteins expressed on T cells, including CD2 (37), CD4 (38), CD8 (38), Thy-1 (39), and the TCR (39). Such associations have been demonstrated by coimmunoprecipitation, fluorescent resonance energy transfer analysis, and cocapping techniques. An intriguing recent report provided evidence that the association between CD45 and several T cell surface antigens occurred in a CD45 isoform–specific manner (40), suggesting that these associations may represent ligand-receptor interactions specific to particular sequences found in the different isoforms. The physiologic significance of these protein-protein interactions, including their role in regulating enzymatic activity, is unclear. One recent study with a number of mAb directed against the extracellular domain of CD45 demonstrated that one of these mAbs was able to stimulate in vivo PTP activity in a murine T cell hybridoma (41), suggesting that endogenous ligands may exist that act through the extracellular domain to modify intracellular PTP activity.

The membrane-spanning domain of CD45 consists of 22 amino acids and crosses the membrane once (Figure 1). All members of the CD45 family have identical transmembrane domains (1). The functional contributions of this segment, if any, are presently unknown.

The large (~700-amino-acid) cytoplasmic domain of CD45 is the subject of intensive investigation. It is identical for all isoforms and is highly conserved across species (1). If conservative amino acid substitutions are considered, the cytoplasmic domain of CD45 is 95% homologous among human, mouse, and rat (42). The salient structural features of the intracellular segment are typical of transmembrane PTP (Figure 1). A membrane proximal segment (~80 amino acids) is followed by two catalytic domains of about 240 amino acids, which are separated by a short spacer (~55 amino acids) and which, in turn, are followed by the COOH-terminal segment (~80 amino acids). In vitro PTP activity of CD45 appears to be independent of the external domain, as shown in studies of recombinant CD45 cytoplasmic domain expressed alone in bacterial or baculovirus expression systems (20,43).

The cytoplasmic regions of the transmembrane PTP typically possess two domains, which exhibit homology with a PTP consensus sequence. (An exception is HPTP-B, a human placental transmembrane PTP possessing only one such region of cytoplasmic sequence homology [44].) Alternatively, the cytoplasmic PTP proteins described to date contain only a single enzymatic domain (2). The reason for this difference is unclear. Within the conserved regions of both receptor and nonreceptor PTP are sequences surrounding a cysteine that appear to be essential for enzymatic function (43,45). Extensive study of CD45 and other PTP has shown that mutagenesis of amino acids in the highly conserved region can alter or abolish PTP activity (19,20,43). Considerable effort has been made to understand the relative contribution of the two PTP domains of CD45 to its overall enzymatic function. One report showed that mutant CD45 molecules expressed in Escherichia coli possess no PTP activity if the cysteine of the first homology domain is altered, suggesting that the second PTP domain is inactive against in vitro substrates (19,43). In other experiments, however, it was shown that expression of the first domain alone resulted in a molecule with either much reduced or no enzymatic activity, implying that the presence of the second domain is necessary for the normal activity of the first. Experimental results may differ depending upon the choice of in vitro substrates, the sensitivity of the assay systems, or the differences in the expression systems used for the production of the mutant molecules. The most appropriate substrate reagents and assay conditions will depend upon the identification of in vivo substrate(s) for CD45. Until such are identified, the physiologic relevance of these in vitro studies remains speculative.

It remains unclear how the PTP activity associated with CD45 is regulated. Experiments have been reported suggesting that both the extracellular and cytoplasmic regions may play a role. One recent report showed that binding the extracellular domain with a mAb may alter in vivo PTP activity (41). A number of studies have investigated the effect of phosphorylation of the cytoplasmic domain on enzymatic activity. The cytoplasmic region of CD45 has been shown to be phosphorylated constitutively on serine residues (46). A concomitant decrease in the phosphorylation and CD45 enzymatic activity has been shown after the treatment of T lymphocytes with calcium ionophore (46). The relevant kinases and phosphates responsible for these events have not yet been determined. In addition, phosphorylation of CD45 has been shown to increase after the treatment of T cells with phorbol ester, presumably via protein kinase C (PKC) (47), and by treatment with interleukin-2 via a non-PKC serine kinase (48). Although CD45 isolated from T cells pretreated with phorbol ester was reported in one study to have less intrinsic PTP activity than CD45 from untreated cells (47), results from another study failed to show a
CD45 AND LYMPHOCYTE ACTIVATION

CD45 appears to play a significant role in the ability of immune cells to respond to activating stimuli. Initial experiments used mAb directed against CD45 in assays of lymphocyte activation. In a number of studies, the ligation of CD45 with mAb decreased T cell responses to antigen, whereas in other studies, TCR responses were augmented (50-52). Anti-CD45 mAb also appear to inhibit some B cell responses (53-56) and NK responses (57). Other experiments have reported looking at simultaneous ligation of the TCR and CD45 with mAb (58). These studies consistently demonstrated an inhibition of TCR-mediated stimulation by co-cross-linking CD45 with the TCR, suggesting that when CD45 is brought into close physical proximity to the TCR an inhibitory signal is delivered. Another explanation of these findings has been put forth in a recent review suggesting that the inhibition seen in these studies may be due to the prevention of TCR aggregation (which may be necessary for efficient signaling by TCR) and not due to a specific CD45 effect (59).

More recently, investigators have taken a genetic approach to understand the role of CD45 in lymphocyte activation. The identification of CD45-deficient mutants has contributed greatly to our current understanding of the role of CD45 in these responses. The first description of such cells by Pingel and Thomas (60) demonstrated that a CD45-negative mutant derived from a T cell clone failed to respond by proliferation to antigenic challenge. The defect in signaling was specific to the TCR because the CD45-negative clone was still able to proliferate in response to exogeneous interleukin-2 (IL-2). Revertants, which once again expressed surface CD45, responded to both antigen and IL-2 stimulation. Follow-up reports have also demonstrated that selection for a CD45-negative mutant of a cytolitic T cell clone gave rise to a cell that had impaired killing ability (61). Revertants expressing CD45 again were able to lyse target cells.

CD45 AND PROXIMAL SIGNAL TRANSDUCTION

Our laboratory has been interested in using a similar genetic approach to investigate the role of CD45 in the regulation of proximal signaling via the TCR. The first detectable biochemical event after TCR ligation is the activation of a protein tyrosine kinase (PTK) (62,63). Seconds after TCR ligation, proteins newly phosphorylated on tyrosine residues can be seen (64). The identity of this PTK has not yet been determined. In fact, several recent studies suggest that a cascade of tyrosine kinase activity involving multiple enzymes may be involved in TCR-mediated activation. Several candidates include the src family kinases lck (65,66) and jyn (67-69) and ZAP-70, a non-src PTK associated with the TCR-ζ subunit (70,71). An early substrate of TCR-associated PTK activity is phospholipase Cγ1 (PLCγ1) (72-74), the enzyme that initiates the production of phosphatidylinositol-4,5-bisphosphate into inositol-1,4,5-trisphosphate and diacylglycerol (75). Substantial evidence suggests that these two messengers are critical in initiating distal events in T cell activation such as cellular proliferation and lymphokine production. The phosphorylation of tyrosines on PLCγ1 is known in other systems to be required for the optimal production of P1-derived second messengers (76). This observation, together with experiments demonstrating that activation of PTK is necessary for TCR-mediated activation of the P1 pathway (64,77,78), links the PTK and P1 second messenger system in T cells.

As the importance of PTK activity associated with TCR activation became clear, our laboratory became interested in the potential role of CD45, the major membrane tyrosine phosphatase present on T cells, in this process. We have thus far obtained mutants of two human T cell leukemic lines that are deficient in CD45 expression. In both CD45-deficient variants, one obtained from the CD4+/CD8+ HPB-ALL line (21) and the other from the CD4+ Jurkat line (22), signal transduction via the TCR is defective. PTK activation and the appearance of P1-derived second messengers do not occur after ligation of the TCR. As expected from the proximal signaling results, distal markers of T cell activation (such as the production of IL-2 and the expression of activation antigens) are also defective in our CD45-deficient cells. Additionally, stimulation via CD2, another surface molecule that can deliver activation signals, is defective in our CD45-deficient Jurkat clone (22). We have shown, however, that our CD45-deficient cells do not have a global defect in signal transduction because signaling via the CD28 costimulus receptor remains intact (22). In addition, a heterologous receptor (the human muscarinic receptor type I) transfected into our CD45-deficient cells appears to couple normally to its sig-
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As required for proof of the role of CD45 in the signaling events via the TCR and CD2, reconstitution of CD45 expression by gene transfer rescues the signaling defect in both deficient cell lines (21,23). In similar studies with the HPB-ALL cell line, other laboratories have shown that the TCR fails to couple with its signaling machinery in the absence of surface expression of CD45 (24).

This signaling defect can be overcome by cross-linking the TCR with CD4 (79) (see below for a possible explanation of this finding) and by gene transfer of CD45 cDNA into the deficient clone (24).

A number of CD45-deficient T cell clones have now been described. Although it is clear that all CD45-deficient mutants described to date show differences in signaling when compared with wild-type cells, the mutant clones do not share identical signaling phenotypes. One CD45-deficient variant of Jurkat has been described in which TCR ligation resulted in small but measurable responses in PTK activity and increases in intracellular free calcium (80). However, TCR-mediated release of lymphokines, i.e., completion of activation, does not occur in these cells (80).

Analysis of another CD45-deficient cell line, derived from the YAC-1 line, also demonstrate abnormalities in TCR-mediated signaling (81). In this cell, ligation of the TCR results in a delayed, attenuated increase in intracellular free calcium. Immunoprecipitation of phosphotyrosine-containing proteins from these cells reveals an increase in constitutive phosphorylation of a number of proteins. The relationship between these proteins and the signaling differences between wild-type and CD45-deficient cells has not yet been elucidated. Thus, in some systems, it appears that a deficiency in CD45 results in a quantitative difference in signaling, whereas in others, the loss of CD45 completely uncouples TCR ligation from proximal signaling events. The reasons for this are unknown. Possible explanations may involve compensatory phosphatases expressed in some of the CD45 mutants or other PTK with regulatory requirements that vary according to cell type.

A similar genetic approach has been used to study the role of CD45 in proximal signaling events in other cells of the immune system. It has been shown that stimulation of the B cell antigen receptor slg fails to activate a PTK or generate PI-derived second messengers in a CD45-deficient variant of the J558 myeloma line (25). Transfer of CD45 cDNA resulted in the reconstitution of CD45 expression and in the rescue of signal transduction. The mechanism by which CD45 regulates slg signaling remains unclear. A recent study, however, has shown that CD45 appears to regulate the state of phosphorylation of two molecules, B29 and mb-1 (82), which associate with slg to form the complete antigen receptor complex (83). The functional relevance of the association between CD45 and these molecules is being investigated.

CD45 also appears to play a critical role in signal transduction in NK cells. Whereas wild-type NK cells respond with the production of PI-derived second messengers in response to ligation of the surface protein CD2, a CD45-deficient NK line does not exhibit this response (27). Further, these CD45-deficient cells are unable to lyse targets killed by the wild-type parent. Most recently, a preliminary report indicates that the high-affinity IgE receptor also signals only in cells that express CD45, at least when the IgE receptor is expressed in a T cell line (26). Thus, it appears that CD45 expression is required in order for an array of receptors on immune cells to couple with their proximal signal transduction machinery.

The structural features of CD45 that relate to its role in the regulation of signal transduction remain speculative. The observation that CD45 is required for signal transduction in diverse cells of the immune system, including T cells, B cells, and NK cells, despite differences in CD45 isoform expression, suggests that the cytoplasmic domain (with its PTP activity) is required for receptor-coupled signal transduction. The fact that the signal transduction defects in several CD45-negative variants have been rescued with cDNA encoding different CD45 isoforms likewise suggests an essential role for the conserved cytoplasmic domain in the signaling process. However, the observation that CD45 may interact with molecules present on the same cell in an isoform-specific manner also suggests a role for the extracellular domain in the regulation of CD45 function. This matter, as well as questions pertaining to the role of phosphorylation of CD45 in its ability to regulate lymphocyte signaling and the ability to modify PTP activity by ligation of the extracellular domain, remains unanswered. The availability of CD45-deficient mutants that can be transfected with strategically altered forms of CD45 cDNA will help to address these points.

A MODEL FOR THE ROLE OF CD45 IN TCR SIGNALING

It is reasonable to speculate that CD45 regulates transmembrane signaling by the dephosphorylation of crucial tyrosine residues on cytoplasmic protein substrate(s). This has led to a model for the role of CD45 in TCR-mediated signal transduction (Figure 2). The relationship between CD45 and the src family kinases, especially lck and fyn, is the subject of ongoing study. There is a growing body of evidence that lck is required for TCR-mediated signal transduction (65,66,84). Studies investigating mutants of the Jurkat T cell line demonstrated that, in the absence of lck, the TCR is uncoupled from its signaling pathways (65). Consistent with this finding, other studies have shown that if T cells are transfected...
with a constitutively active form of lck, enhanced signaling via the TCR is observed (66). As with all src family kinases, phosphorylation of lck on its COOH-terminal tyrosine (Y505) down-regulates its PTK activity (85). Although the mechanism for this down-regulation has not been proved, recent studies suggest that phosphorylation of COOH-terminal tyrosines on src family members induces a conformational change that prevents the effective association of the PTK with their substrates (86–88). CD45 has been shown to dephosphorylate lck at the Y505 residue in vitro (89,90). Additionally, in several wild-type murine T cell lymphoma lines and their corresponding CD45-deficient variants, the expression of CD45 corresponded to a decrease in the phosphorylation of Y505 and increased lck kinase activity (91–93). These findings suggest that the expression of CD45 is required for the dephosphorylation of lck on Y505 in vivo. This model thus suggests that, in the absence of CD45, lck Y505 is phosphorylated, rendering it unable to transduce an upstream signal from the TCR. CD45 would, therefore, play a permissive role in TCR signaling by dephosphorylating lck Y505, thereby allowing the kinase to transmit an activation signal. The possible relationship between CD45 and lck is more intriguing when one considers the recent data demonstrating a physical association between CD4 or CD8 and specific isoforms of CD45 (94). Elegant studies have shown clearly that both CD4 and CD8 associate tightly with lck (95). The apparent isoform-specific relationship between CD45 and CD4 or CD8 suggests the possibility that the ability of CD45 to regulate TCR signaling through lck Y505 phosphorylation may be modulated, at least in part, by the extracellular domain of CD45.

The most compelling, albeit indirect, evidence that CD45 and lck are functionally associated comes from studies in which CD45 was immunoprecipitated from detergent lysates of normal T cells or various T cell lines. In vitro kinase assays performed on these immunoprecipitates demonstrated the presence of a tyrosine kinase, and immunoblot analysis revealed the presence of lck (96–98). We have shown recently that the association between CD45 and lck does not require TCR stimulation, nor even the surface expression of the TCR (98). Further, we have shown that, at least in the Jurkat cell line, lck is the only PTK that appears to coprecipitate with CD45, even though these cells express other src family PTK (65,98). Experiments are currently underway to identify the regions of CD45 that are responsible for its physical interaction with lck.

Although considerable data suggest that there is a functional relationship between CD45 and lck and that the role of CD45 may be to regulate lck kinase activity by controlling phosphorylation of lck’s COOH-terminal tyrosine, several experimental results are not consistent with this model. In experiments where CD45 is co-cross-linked with the TCR, a decrease in signaling is seen (58). However, as noted above, an explanation for this finding is that, under these experimental conditions, TCR aggregation may be prevented (59). That is, the inhibition in TCR responses may not reflect a specific inhibitory effect of CD45. In other experiments, it was shown that, in a CD45-negative variant of the HPB-ALL cell line, co-cross-linking the TCR with surface CD4 overcame the signaling deficiency seen when the TCR alone was stimulated (79). A possible explanation for this finding is that when enough TCR and lck (via its association with CD4) are brought into sufficient proximity by co-cross-linking the two receptors, the
relative decrease in lck kinase activity due to its phosphorylation on Y505 can be overcome. Finally, an interesting report examining the relationship between CD45 and lck demonstrated that co-cross-linking CD4 and CD45 with mAb resulted in a decrease of lck kinase activity (90). The above model would predict an increase in lck activity due to enhanced dephosphorylation of Y505. This result and those showing increases in lck activity in cells expressing CD45 compared with lck recovered from CD45-deficient cells remain to be reconciled.

Although an increasing body of data suggests that lck plays an essential role in TCR activation, other PTK have also been shown to be important in this process. It is possible that CD45 plays a role in the regulation of these enzymes. For example, one recent study demonstrated an association between CD45 and fyn (99), another src family kinase expressed highly in T cells. fyn has been shown to interact directly with the TCR (67) and may be the most proximal PTK stimulated after TCR activation. The expression of this PTK has been shown to influence the signaling properties of T cells at various stages of development (68,69). The catalytic activity of fyn is also regulated by the state of phosphorylation of its COOH-terminal tyrosine. Evaluation of at least one CD45-deficient T cell clone has demonstrated decreased enzymatic activity present in fyn immunoprecipitates when compared with immunoprecipitates obtained from those cells after reconstitution of CD45 by gene transfer (24).

In addition to potential direct interactions between CD45 and a src family PTK, CD45 may also function to control the state of phosphorylation of substrates of the TCR-activated PTK. Whereas the identity of some of these substrates, e.g., PLCγ1, the ZAP-70 PTK, and the ζ chain of the TCR, are known, most remain unidentified. The role of CD45 in the regulation of the state of phosphorylation of these molecules remains to be determined.

CONCLUSION

CD45 isoforms are present on all nucleated hematopoietic cells. The heterogeneity of the extracellular region raises the possibility that CD45 may act as a receptor for soluble ligands, ligands expressed on the surface of other cells, or ligands present within the membrane of the same cell. Examples of possible ligands include the B cell antigen, CD22, and several molecules expressed on the surface of T cells. The intracellular domain of CD45 is large and highly conserved and contains two PTP domains. In several T and B cell lines and in NK cells, CD45 is required for optimal signal transduction mediated via a number of cell surface receptors. One model proposes that the cytoplasmic domain of CD45 acts by dephosphorylating critical tyrosine residue(s), thus up-regulating a PTK or more than one PTK, which in turn initiate a second messenger cascade, resulting in cellular activation. Candidates for such PTK include the src family kinases lck and fyn and the non-src PTK ZAP-70. The availability of CD45-deficient mutants should help to elucidate the structural features of CD45, which are important in its ability to regulate signal transduction, and to shed light on the physiologic relevance of the recently described interactions between CD45 and other molecules in lymphoid cells.

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