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CD45 regulates signal transduction and lymphocyte activation by specific association with receptor molecules on T or B cells

(cytoplasmic calcium mobilization/proliferation/leukocyte common antigen/protein tyrosine phosphatase)

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ABSTRACT **Evidence is presented that the leukocyte common antigen CD45 can regulate both signal transduction by lymphocyte receptor molecules and T- and B-cell proliferation in a manner dependent on specific interactions between these receptors on the cell surface. Formation of homoaggregates of CD3, CD2, or CD28 on the surface of T cells induced by crosslinking with monoclonal antibodies (mAbs) results in an increase in cytoplasmic free calcium concentration ($[Ca^{2+}]_i$). This increase in $[Ca^{2+}]_i$ was abolished when these receptors were crosslinked to CD45 on the cell surface. In contrast, the increase in $[Ca^{2+}]_i$ induced by formation of homoaggregates of CD4 was strongly amplified when CD4 was coupled to CD45. T-cell proliferation initiated by immobilized anti-CD3 was inhibited by anti-CD45 or anti-CD45R when immobilized on the same surface, but not when in solution. Similarly, proliferation after stimulation of the CD2 and CD28 receptors was inhibited when a CD45 mAb was crosslinked to either CD2 or CD28 mAbs, but not when a CD45-specific mAb was bound to the cell surface separately. In B cells, the increase in $[Ca^{2+}]_i$ and resulting proliferation induced by crosslinking either the CD19 or Bgp95 receptors was inhibited by coupling these molecules to CD45. Thus, CD45 appears to modify other cellular receptors functionally when brought into close physical association with them. The homology of the CD45 conserved cytoplasmic domains with a major human placental protein tyrosine phosphatase suggests that the effects of CD45 described here result from alterations in the phosphorylation state of tyrosyl residues in membrane-associated proteins.**

The CD45 leukocyte common antigen (L-CA, also known as T200 or Ly-5) is a family of major glycoproteins ranging from 180 to 220 kDa that is restricted to cells of hematopoietic lineages (1–5). Distinct isoforms of CD45 that arise from alternative mRNA splicing are differentially expressed on subpopulations of lymphocytes. Some monoclonal antibodies (mAbs) to human CD45 recognize epitopes shared by all CD45 isoforms of 220, 205, 190, and 180 kDa (6). However, other mAbs recognize only the 220-kDa isoform of CD45, designated CD45R, that is selectively expressed on B lymphocytes and a subpopulation of T cells (6–9). Another mAb, UCHL-1, selectively binds to the 180-kDa species, which is restricted to cortical thymocytes and a subset of activated or memory T cells (10).

Recently, the primary structures of rat (11, 12), mouse (13–16), and human (17, 18) CD45 (L-CA) have been deduced from cDNA nucleotide sequences. CD45 is an integral membrane protein with a large 705- to 707-amino acid cytoplasmic segment, a 22-amino acid transmembrane segment, and an extracellular domain ranging from 400 to 550 amino acids. The various isoforms of CD45 that are generated by alternative mRNA splicing of primary transcripts of a single gene have different extracellular domains but have the same transmembrane and cytoplasmic segments (12, 17, 18). The cytoplasmic segment has two homologous highly conserved domains of ≈300 residues.

Studies attempting to define the function of CD45 have yielded conflicting results (6). mAbs to the human or mouse antigen have been reported to inhibit T- and B-cell proliferation (19–21), antibody-forming cell production (22), and natural killer (NK) cell and cytotoxic T-cell activity (23–26). However, under certain conditions, anti-CD45 mAbs have also been reported to augment T-cell proliferation (6, 9, 27). Thomas *et al.* (11) have suggested that the cytoplasmic portion of the molecule with its two conserved homologous domains may play a critical role in CD45 function. Recently, these domains were found to be homologous to a major low molecular weight protein tyrosine phosphatase isolated from both the soluble and particulate fractions of human placenta (28–30). This suggests that CD45 is a membrane-bound protein tyrosine phosphatase that may function by interacting with other membrane-associated molecules. In this study, the influence of CD45 on signal transduction by T- and B-cell-associated receptors was investigated; the results show that CD45 is indeed a potent regulator of signal transduction and lymphocyte activation. These activities of CD45 depend on its proximity on the cell surface to other T- or B-cell signalling molecules. Thus, it is likely that CD45 regulates surface receptors by causing the dephosphorylation of tyrosine residues in membrane-associated proteins.

MATERIALS AND METHODS

Cell Preparations. Peripheral blood mononuclear cells, dense tonsillar E-rosette-negative lymphocytes, and nylon wool nonadherent lymphocytes from peripheral blood were prepared as described (31, 32).

mAbs and Reagents. The following mouse mAbs to human leukocyte markers were used: 9.4 (IgG2a) anti-CD45 (6); G1-15 (IgG1) and 3AC5 (IgG2a) anti-CD45R (9, 33); UCHL-1 (IgG1) anti-CD45 (180 form) kindly provided by P. Beverley (Imperial Cancer Research Fund Human Tumour Immunology Group, London) (10); G19-4 (IgG1) anti-CD3 (33); 9.6 (IgG2a) anti-CD2 (34); 9.3 (IgG2a) anti-CD28 (35); 1F5 (IgG2a) to CD20, a pan B-cell marker (31); G28-5 (IgG1) anti-CDw40 (36); G28-8 (IgG1) to a recently defined B-cell-associated receptor, Bgp95 (37); and HD37 (IgG1) anti-CD19 kindly provided by T. Pezzutto (University of Heidelberg) (38). The rat anti-mouse κ-specific mAb 187.1 (39) was used to crosslink mouse mAb.

Conjugation of mAbs with biotin utilized biotin-succinimide (Sigma) as described (40). Phorbol 12-myristate 13-

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Abbreviations: $[Ca^{2+}]_i$, intracellular free calcium concentration; mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate; rIL-2, recombinant interleukin 2.

acetate (PMA) and avidin were from Sigma. Recombinant interleukin 2 (rIL-2) was purchased from Genzyme.

Cell Cultures. Proliferation of blood T and tonsillar B cells was measured by incorporation of [^{3}H]thymidine (New England Nuclear; specific activity, 27 Ci/mmol; 1 Ci = 37 GBq) as described (9, 31) using 10^6 cells per ml in 200-μl microtiter wells.

Analysis of Cytoplasmic Free Calcium Concentration ($[Ca^{2+}]_i$). The method using Indo-1 (Molecular Probes) to measure $[Ca^{2+}]_i$ has been described in detail (41), again using 10^6 cells per ml.

Preparation of mAb Heteroconjugates. mAb heteroconjugates were prepared at a 1:1 molar ratio with the heterobifunctional crosslinker maleimidobutyryloxysuccinimide (GMBS; Calbiochem) and 5-iminothiolane hydrochloride (Pierce) as described for phycoerythrin coupling (40). This mode of coupling ensures that anti-CD3 can only be conjugated to anti-CD45.

RESULTS AND DISCUSSION

Triggering of the CD3/T-cell receptor with mAbs results in inositol phospholipid hydrolysis and production of the second messengers, inositol trisphosphate and diacylglycerol (42, 43). Measurement of the increase in $[Ca^{2+}]_i$ initiated by inositol trisphosphate formation provides a rapid and simple assay of this signal transduction pathway. Recent studies have indicated that the CD3/T-cell receptor can interact with other cell-surface antigens such as CD4 to augment inositol trisphosphate formation, calcium mobilization, and T-cell proliferative responses (44–46). We therefore investigated whether the activation of T cells could be influenced by a mAb-induced interaction between CD3 and CD45 on the cell surface.

To approach this question, we first measured proliferation of T cells after stimulation with the CD3 mAb G19-4 immobilized on the surface of microtiter wells with CD45 mAb 9.4 either in solution or also immobilized on the plastic surface. The results (Table 1) show that anti-CD45 immobilized together with anti-CD3 inhibited proliferation by >75%, whereas anti-CD45 in solution did not. The inhibitory effect of immobilized anti-CD45 was still apparent even in the presence of a suboptimal concentration of PMA or exogenous rIL-2. Inhibition was also evident when either 3AC5 or G1-15 CD45R mAbs were immobilized together with anti-CD3, but not when the CD45R mAbs were in solution (Table 2). Again, neither PMA nor rIL-2 could overcome this inhibition. A control IgG2a mAb, 96.5, against the melanoma antigen p97 did not inhibit proliferation in response to immobilized anti-CD3 when either present in solution or immobilized together with anti-CD3.

Table 1. CD45 regulates T-cell proliferation after stimulation of CD3

Activation	PMA (1 ng/ml)	Proliferation ([^{3}H]thymidine incorporation, cpm × 10^{-3}) Medium	Anti-CD45 in solution	Anti-CD45 immobilized
Medium	−	0.3	0.4	0.3
	+	0.5	0.5	0.4
Anti-CD3 (immobilized)	−	137.9	143.5	32.3
+ rIL-2 (100 units/ml)	+	204.3	198.3	93.2
	−	196.3	183.9	67.1

Proliferation of peripheral blood mononuclear cells was measured by uptake of [^{3}H]thymidine during the last 6 hr of a 3-day experiment. Cells were cultured in quadruplicate and means are shown. Standard errors were <11%. Activation with anti-CD3 used mAb G19-4 precoated to the wells of the microtiter plate at 10 μg/ml prior to the assay. Anti-CD45 mAb 9.4 in solution was 1 μg/ml and was immobilized on the microtiter plate at 10 μg/ml.

Table 2. CD45R regulates T-cell proliferation after stimulation of CD3

Activation	Proliferation ([^{3}H]thymidine incorporation, cpm × 10^{-3}) Medium	PMA (1 ng/ml)	rIL-2 (100 units/ml)
Medium	0.1	0.5	0.4
Anti-CD3 (immobilized) + medium	143.6	281.5	291.8
+ G1-15 anti-CD45R (soln)	137.5	261.5	309.4
+ 3AC5 anti-CD45 R (soln)	177.8	266.2	294.6
+ 96.5 (soln)	147.9	262.1	312.4
Anti-CD3 (immobilized) + G1-15 anti-CD45R (immobilized)	0.1	0.7	0.6
+ 3AC5 anti-CD45R (immobilized)	10.1	68.2	95.5
+ 96.5 (immobilized)	154.4	257.1	280.4

Proliferation was measured as described in Table 1. Cells were cultured in quadruplicate and means are shown. Standard errors were <11%. Anti-CD45R mAbs G1-15 and 3AC5 were used at 1 μg/ml in solution and immobilized at 10 μg/ml. mAb 96.5 is an IgG2a against melanoma antigen p97 and was used here as a nonreacting control at 1 μg/ml in solution (soln) and 10 μg/ml immobilized.

To ensure that the inhibition seen with immobilized CD45 mAb 9.4 was not due simply to a displacement of the CD3 mAb G19-4 from the plastic surface, microtiter wells were coated with anti-CD3 alone, anti-CD3 plus anti-CD45, or anti-CD3 plus control mAb 96.5. Fig. 1 shows that (*i*) as more anti-CD3 was used for coating the wells, there was more proliferation, consistent with previous reports (47); (*ii*) the CD45 mAb 9.4 caused substantial inhibition at all concentrations, but only when immobilized; and (*iii*) there was essentially no inhibition caused by the simultaneous coating with the isotype control 96.5 mAb, indicating that the inhibition by anti-CD45 was not the result of a displacement of anti-CD3. Addition of PMA (1 ng/ml) to the cultures reversed the inhibition by immobilized anti-CD45 only when the mAb was coated at 4 μg/ml or less, but not at higher concentrations (Fig. 1*B*). This suggests that a critical concentration of anti-CD45 may need to be in close proximity to anti-CD3 on the plastic surface to maintain inhibition in the presence of PMA.

T-cell activation resulting in proliferation can proceed by stimulation of CD28 and CD2 using mAbs 9.3 and 9.6 in solution, respectively, with subsequent crosslinking in a second step using anti-κ mAb 187.1 (48, 49). To investigate the effects of CD45 ligation in this system, anti-CD45 was added to anti-CD28 or to anti-CD2 in solution with or without the crosslinking mAb 187.1. It was apparent that addition of anti-CD45 alone could promote cell proliferation or increase that induced by either CD2 or CD28 mAbs without requiring exogenous rIL-2 (Table 3). In all instances, addition of rIL-2 further augmented cell proliferation even in the absence of other stimulants. These phenomena may be due to an effect of CD45 ligation on expression of high-affinity receptors for IL-2 (9). By contrast, crosslinking CD45 to CD2 or CD28 by addition of the 187.1 anti-κ mAb reversed the stimulatory effect of CD45 alone and reduced the stimulation by CD2 or CD28 (Table 3) in either the presence or absence of rIL-2. This was not due to a nonspecific inhibitory activity of 187.1 since CD28 and CD2 stimulation were both enhanced by 187.1 when the CD45 mAb 9.4 was not included.

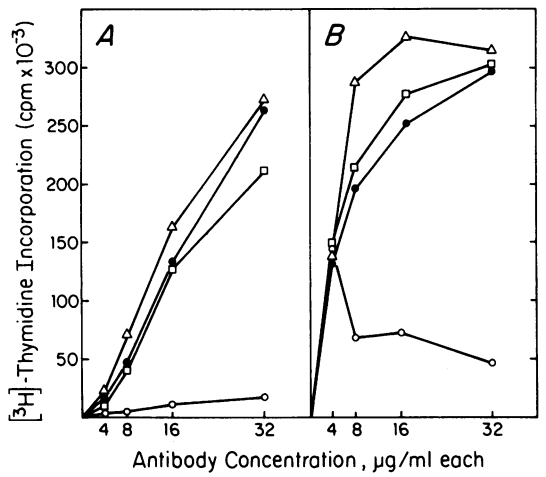

FIG. 1. CD45 inhibition of proliferation induced by anti-CD3 requires a CD45–CD3 interaction. Peripheral blood mononuclear cells were stimulated with anti-CD3 mAb G19-4 immobilized to the wells of the microtiter plate by incubation at room temperature in phosphate-buffered saline for 2 hr at the indicated concentrations either alone (●) or together with CD45 mAb 9.4 (○) or anti-p97 mAb 96.5 (□) at the same concentration followed by the addition of bovine serum albumin to prevent further protein attachment. Proliferation with CD45 mAb 9.4 in solution (△) is also shown for comparison. Proliferation in the absence (*A*) or presence (*B*) of PMA (1 ng/ml) was measured. Cells were cultured in quadruplicate for 3 days and pulsed with [^{3}H]thymidine during the last 6 hr. Means are shown, and standard errors were <15% of the mean at each point.

CD3, CD2, and CD28 are coupled to signal transduction pathways that result in an increase in $[Ca^{2+}]_i$ when these receptors are ligated on the cell surface (49). We therefore measured $[Ca^{2+}]_i$ after crosslinking these receptors in the presence or absence of CD45 mAb 9.4 (Fig. 2). In this experiment, biotin-conjugated mAbs were bound to peripheral blood T cells and then crosslinked on the cell surface by the addition of avidin. It was apparent that the increase in $[Ca^{2+}]_i$ seen after CD3, CD2, and CD28 crosslinking was inhibited by the presence of biotin-conjugated mAb 9.4. When mAb 9.4 was not biotinylated, and thus not crosslinked by avidin, a small decrease in the extent of anti-CD3-induced calcium mobilization was still observed (Fig. 2*A*). Complete inhibition, however, appeared to require that CD45 and CD3 be brought into close proximity. Furthermore, 50 μg of a heteroconjugate of anti-CD45 and anti-CD3 per ml was unable to directly increase $[Ca^{2+}]_i$ (Fig. 2*B*) even though it could bind to both receptors on the cell surface (data not shown) and 25 μg of anti-CD3 per ml could elicit a strong calcium response. CD45R mAbs such as 3AC5 were able to partially inhibit $[Ca^{2+}]_i$ responses when ligated to mAbs reactive with the cell receptors, such as illustrated for CD2 (Fig. 2*C*). The partial inhibition afforded by CD45R mAb may

Table 3. CD45 regulates T-cell proliferation after CD2 and CD28 stimulation

Stimulus	rIL-2 (100 units/ml)	Proliferation ([^{3}H]thymidine incorporation, cpm × 10^{-3}) Medium	mAb 187.1	Anti-CD45	Anti-CD45 + mAb 187.1
Medium	−	0.2	0.3	1.6	0.1
	+	6.8	4.2	37.6	2.5
Anti-CD28	−	0.2	4.3	47.4	0.4
	+	12.6	36.1	72.5	9.6
Anti-CD2	−	0.5	0.2	12.7	0.2
	+	4.4	7.0	37.3	4.0

Proliferation was measured by uptake of [^{3}H]thymidine during the last 6 hr of a 3-day culture using peripheral blood mononuclear cells. Cells were cultured in quadruplicate and means are shown. Standard errors were <12% of the mean at each point. Activation was with CD28 mAb 9.3 and CD2 mAb 9.6 at 1 μg/ml each. Anti-CD45 mAb 9.4 was used at 1 μg/ml and anti-κ mAb 187.1 was used at a 4:1 final ratio of mAb 187.1 to mouse mAb.

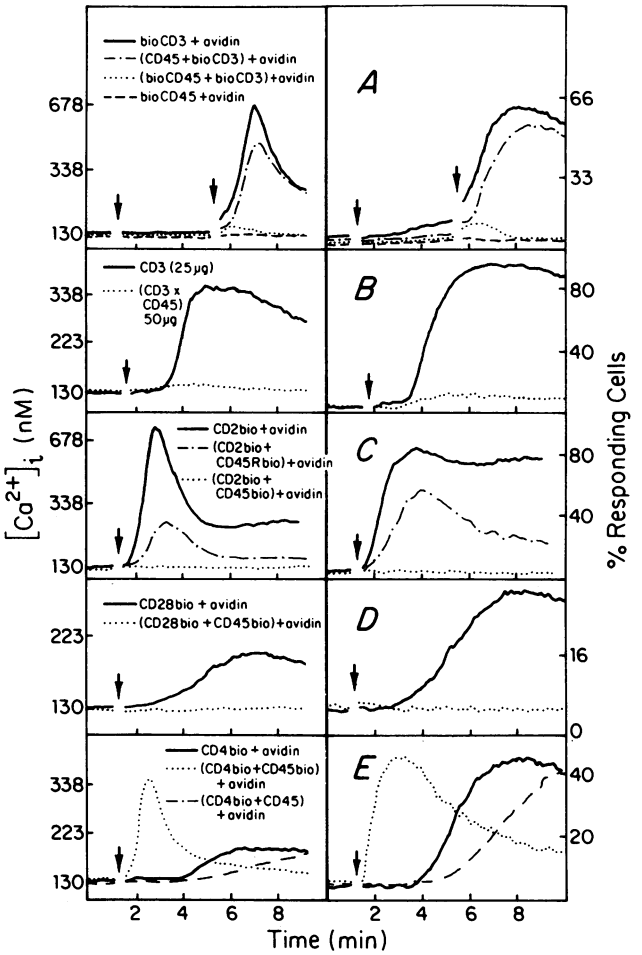

FIG. 2. CD45 regulates signal transduction in T cells by steric association with receptor molecules. Increases in $[Ca^{2+}]_i$ in peripheral blood mononuclear cells loaded with Indo-1 were measured after crosslinking receptors on T cells either alone or together with CD45 mAb 9.4 or CD45R mAb 3AC5 as indicated. Mean $[Ca^{2+}]_i$ (*Left*) and percentage of responding cells (*Right*) are shown for each experiment. (*A*) CD3 was crosslinked with biotin-conjugated mAb G19-4 (2 μg/ml) at 1.5 min followed by avidin (8 μg/ml) at 5.5 min (—). This was compared with the same crosslinking in the presence of 10 μg of CD45 mAb 9.4 per ml (–·–) or to 10 μg of biotin-conjugated 9.4 per ml followed by 48 μg of avidin (···). An additional control of biotin-conjugated 9.4 (10 μg/ml) followed by avidin (40 μg/ml) is also shown (---). (*B*) Comparison of 25 μg of CD3 mAb G19-4 per ml (—) to 50 μg of a CD3/CD45 heteroconjugate of mAbs G19-4 and 9.4 per ml (···). (*C*) Crosslinking of CD2 with 10 μg of biotin-conjugated 9.6 mAb per ml added at $t = -3$ min followed by addition of 40 μg of avidin per ml at $t = 1.5$ min (—). (*D*) Crosslinking of CD28 with 10 μg of biotin-conjugated anti-CD28 mAb 9.3 per ml added at $t = -3$ min followed by addition of 40 μg of avidin per ml at $t = 3$ min (—) compared with simultaneous crosslinking of CD28 and CD45 using 10 μg of 9.3 per ml and 10 μg of 9.4 per ml added at $t = -3$ min followed by addition of 80 μg of avidin per ml at $t = 1.5$ min (···). (*E*) Crosslinking of CD4 by addition of 10 μg of biotin-conjugated anti-CD4 mAb G17-2 per ml at $t = -3$ min followed by 40 μg of avidin per ml at $t = 1.5$ min (—). This was compared with simultaneous crosslinking of CD4 and CD45 by addition of 10 μg of biotin-conjugated G17-2 anti-CD4 per ml and 10 μg of biotin-conjugated 9.4 anti-CD45 per ml at $t = -3$ min followed by addition of 80 μg of avidin per ml at $t = 1.5$ min (···). The comparison of biotin-conjugated anti-CD4 (10 μg/ml) plus anti-CD45 (10 μg/ml) (not conjugated) followed by 40 μg of avidin per ml at $t = 1.5$ min is also shown (–·–).

reflect the expression of CD45R isoform on some but not all T cells (9). In contrast to the inhibitory effects on CD3, CD2, and CD28, ligation of CD45 to CD4 gave a strong and reproducible augmentation of signalling as compared to when CD4 is crosslinked to itself (Fig. 2*E*). This occurred without an increase in the number of responding cells, showing that the same CD4-positive cells were more responsive after ligation of CD45 and CD4. Thus, CD45 appears to either up- or down-regulate transmembrane signalling depending on the receptor with which it is interacting.

Because CD45 is expressed on all leukocytes, we also examined its function on B cells. Anti-immunoglobulin alone stimulates a strong increase in $[Ca^{2+}]_i$ in B cells without requiring a second antibody for crosslinking, making it difficult to assess the effects of CD45 on immunoglobulin signalling. However, two other B-cell receptors, CD19 (38) and Bgp95 (37), induce rapid increases in $[Ca^{2+}]_i$ after more extensive crosslinking on the cell surface and thus were investigated. In addition to increases in $[Ca^{2+}]_i$, crosslinking the CD19 or Bgp95 molecules causes cell proliferation in the presence of PMA (ref. 37; unpublished data). The increase in $[Ca^{2+}]_i$ was abolished by ligation with CD45 mAb 9.4 and partially inhibited by ligation with CD45R mAb 3AC5 (Fig. 3). Similarly, the proliferation of B cells induced by CD19 or Bgp95 mAb in the presence of PMA was inhibited by coupling to CD45 (Table 4). A CDw40 mAb, G28-5, which acts synergistically with PMA to drive B-cell proliferation (36), was also inhibited by crosslinking to CD45. This might suggest that CD45 could alter the activity of surface receptors that do not initiate phosphatidylinositol turnover and calcium mobilization, since the activation signal delivered by G28-5 binding to CDw40 does not increase $[Ca^{2+}]_i$ in B cells (unpublished data). However, some direct measurement of phosphatidylinositol turnover would be required to confirm this.

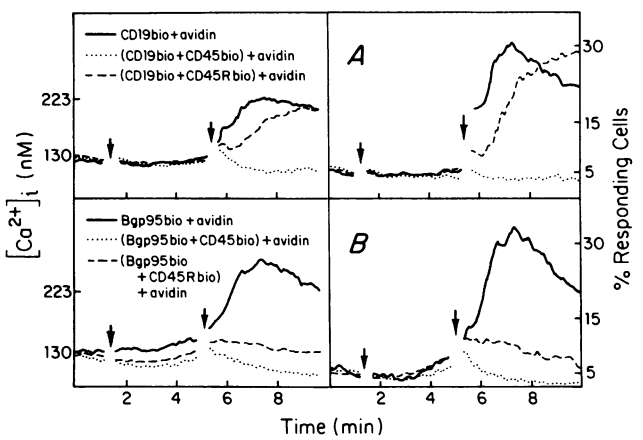

FIG. 3. CD45 regulates signal transduction in B cells by steric association with receptor molecules. Increases in $[Ca^{2+}]_i$ in dense E-rosette-negative tonsillar B cells were measured after crosslinking receptors alone or together with CD45 mAb 9.4 or CD45R mAb 3AC5. Mean $[Ca^{2+}]_i$ (*Left*) and percentage of responding cells (*Right*) are shown. (*A*) CD19 crosslinking with 10 μg of biotin-conjugated HD37 per ml added at the first arrow followed by 40 μg of avidin per ml added at the second arrow (—). This was compared with simultaneous addition of 10 μg of biotin-conjugated anti-CD19 per ml and 10 μg of biotin-conjugated anti-CD45 per ml (···) or 10 μg of biotin-conjugated anti-CD19 per ml and 10 μg of biotin-conjugated 3AC5 anti-CD45R per ml (–·–) at the first arrow and 80 μg of avidin per ml at the second arrow. (*B*) Bgp95 crosslinking with 10 μg of biotin-conjugated G28-8 per ml added at the first arrow followed by 40 μg of avidin per ml added at the second arrow (—). This was compared with simultaneous addition of 10 μg of biotin-conjugated anti-Bgp95 per ml and 10 μg of biotin-conjugated anti-CD45 per ml (···) or 10 μg of biotin-conjugated anti-Bgp95 per ml and 10 μg of biotin-conjugated 3AC5 anti-CD45R per ml (–·–) at the first arrow followed by 80 μg of avidin per ml added at the second arrow.

Table 4. CD45 regulated B-cell proliferation after stimulation with CD19 or Bgp95

		Proliferation ([^{3}H]thymidine incorporation, cpm × 10^{-3})			
	PMA	Medium	Avidin	b-CD45 mAb only	b-CD45 mAb + avidin
Medium	−	0.3	0.5	0.3	0.2
	+	32.0	28.8	37.4	29.0
b-CD19 mAb	−	0.3	0.5	0.3	0.1
	+	70.1	84.6	67.7	42.6
b-Bgp95 mAb	−	3.6	2.5	4.3	0.1
	+	125.8	133.6	97.3	45.9
b-CDw40 mAb	−	1.9	2.0	3.7	0.2
	+	165.7	120.2	166.7	69.3

Proliferation was measured by uptake of [^{3}H]thymidine during the last 18 hr of a 3-day culture using dense E-rosette-negative tonsillar B cells (31). Means of quadruplicate cultures are shown in which standard errors were <5%. All mAbs were used at 5 μg/ml final concentration; PMA was used at 2 ng/ml; avidin was added at a 4:1 ratio of avidin/biotinylated mAb 15 min after addition of mAb.

Previous studies of CD45 suggest that it may function to either up- or down-regulate lymphocyte activity (9, 19–27). Soluble mAbs to CD45 or CD45R are costimulatory with phytohemagglutinin or anti-CD3 attached to beads in increasing IL-2 production, IL-2 receptor expression, and T-cell proliferation (9, 27). Soluble anti-CD45 can act cooperatively with CD2 or CD28 mAb (Table 3). These costimulatory effects are restricted to $CD4^+$ cells and are not seen with $CD8^+$ cells, which do not express CD4 (27). This may be due in part to the fact that CD45 has a characteristic effect on CD4 distinct from its action on other cell-surface antigens such as CD3 or CD2: when CD45 is brought into close association with CD4, it acts to accelerate and increase the calcium signal transmitted via CD4, while inhibiting signal transduction when coupled to CD3 and CD2 (Fig. 2).

Anti-CD45 mAbs have also been reported to inhibit lymphocyte proliferation or effector activity (19–26). Here we report that the inhibitory effects of anti-CD45 are best demonstrated under conditions that place CD45 into close contact with signal transducing elements such as the T-cell receptors CD2, CD3, or CD28 or the B-cell antigens CD19 or Bgp95. It can act very early in lymphocyte activation, inhibiting the mobilization of intracellular free calcium normally detectable within 30–60 sec.

Why do these dramatic effects appear to require a close interaction between CD45 and a signalling receptor? The recently described homology (30) between the cytoplasmic domains of CD45 and the major protein tyrosine phosphatase of human placenta (28, 29) suggests that the former is in fact a membrane-bound protein tyrosine phosphatase. CD45 may function to modify signal transduction in leukocytes by dephosphorylating key tyrosyl residues on other membrane-associated molecules such as on the ζ chain of CD3 (50). It could also modify protein tyrosine kinase activity associated with CD4 that would normally function to regulate antigen-driven signal transduction (51). This model would predict that phosphorylation of key tyrosyl residues precedes and is necessary for the mobilization of $[Ca^{2+}]_i$. The cytoplasm of lymphocytes has an abundance of protein tyrosine phosphatase activity (unpublished observation); thus, an integral membrane form of the enzyme that functions only in close proximity with other membrane-associated proteins would serve to localize protein tyrosine kinase/phosphatase regulation to specific cell-surface molecules.

How might CD45 normally come into close contact with T- and B-cell-associated receptors when it is normally quite immobile in the membrane? CD45 is phosphorylated after treatment of T cells with PMA (52) and phorbol diesters enhance the capping of certain lymphocyte surface glycoproteins including CD45 (53). Thus, after cell activation and subsequent phosphorylation, the mobility of CD45 may change. Murine CD45 (T200) forms a direct connection with the cytoskeletal protein fodrin (54); modulating this association may in some way facilitate intracellular interactions between CD45 and other molecules. CD45 has several alternative extracellular domains, the ligands for which have yet to be identified. One possibility is that these distinct extracellular domains may function to promote the interaction of different sets of surface molecules with the putative protein tyrosine phosphatase domains. This could be accomplished either by specific intercellular interactions between CD45 and ligands on characteristic accessory cells or by preferential intracellular interactions of the various CD45 forms with T- or B-cell signalling molecules.

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