The major tyrosine phosphatase activity against angiotensin detected in membranes of the antigen-specific T cell hybridoma 2B4 is contained in the cytoplasmic tail of the CD45 molecule. When these cells are stimulated with either an antibody directed against the T cell antigen receptor or an activating anti-Thy-1 antibody, there is a rapid redistribution of CD45 in the cells. The redistribution can be observed in two ways: morphological and subcellular fractionation. Morphological examination of resting cells reveals intense CD45 staining of the Golgi as well as surface staining. Upon activation the Golgi is rapidly cleared of CD45. This redistribution is specific for CD45 and is not observed for an intrinsic Golgi protein, mannosidase II, or a protein traversing the secretory pathway, the T cell receptor. In activated cells, in contrast to resting cells, approximately 30% of the total cellular CD45 is precipitated either at 280 × g or at 200,000 × g through a 2.2 m sucrose cushion after cell homogenization. This fraction is not accessible to cell surface labeling. CD45 redistribution does not require hydrolysis of phosphatidylinositol lipids and cannot be reproduced by the addition of phorbol ester and calcium ionophore. It does require the presence of an intact functional T cell receptor on the cell surface. These studies suggest that the residence time of CD45 within an intracellular organelle can be acutely regulated by a signal mediated via the T cell receptor. This regulation may control access of this phosphatase to critical substrates.

The regulation of protein phosphorylation on tyrosine residues is believed to play a crucial role in intracellular signal transduction pathways that regulate cellular activation and differentiation (Hunter and Cooper, 1985, 1986). Although the identification and biochemical characterization of tyrosine kinases have advanced rapidly over the past several years, relatively less is known about tyrosine phosphatases. Considerable effort is currently being directed toward the purification and characterization of cytosolic and membrane protein tyrosine phosphatases (PTPases).1 The major tyrosine phosphatase activity (Pingel and Thomas, 1989; Koretzky et al., 1990). Recently, a major advance in our understanding of tyrosine phosphatases and their potential role in transmembrane signaling has been reported. Tonks et al. (1988a, 1988b) have isolated and sequenced a molecular clone encoding a tyrosine phosphatase from human placenta. When the sequence was compared with known protein sequences a striking similarity to the cytoplasmic domain of the common leukocyte surface antigen CD45 was noted (Charbonneau et al., 1988). This was followed by the demonstration of PTPase activity of CD45 (Tonks et al., 1988c, 1990). These findings raise the possibility of a direct role of tyrosine phosphatases in surface activation events.

CD45 is a so-called leukocyte common antigen and is expressed in cells of the hematopoietic lineage including lymphoid and myeloid cells (Trowbridge, 1978; Thomas, 1989). One of its striking characteristics is the lineage-specific expression of various isoforms. The cell type-specific expression of CD45 variants demonstrates an exquisite level of differentiation (Thomas, 1989). Thus B cells express a different form than T cells, and different subsets of T cells express different CD45 forms. These varieties arise as a result of alternative splicing such that various combinations of three exons are found in the mRNA encoding this protein (Saga et al., 1987). This alternative splicing results in the expression of isoform-specific epitopes and altered biochemical characteristics. The latter is manifested by apparent molecular masses, as determined by SDS-polyacrylamide gel electrophoresis, that vary between 180 and 200 kDa. Much of this heterogeneity is the result of isoform-specific glycosylation differences although there are also differences in the core polypeptide size.

Recent studies have demonstrated the efficacy of anti-CD45 antibodies in modulating T cell activation (Ledbetter et al., 1988). These studies emphasized the potential importance of the structural proximity of CD45 to the T cell antigen receptor for the observed effects on cellular activation. The recent reports pointing to a possible requirement for CD45 for T cell activation emphasized further the importance of tyrosine phosphatase activity (Pingel and Thomas, 1989; Koretzky et al., 1990). As with kinases, establishing the mechanisms underlying the regulation of tyrosine phosphatase function poses a major challenge. Phosphatases can be envisioned to be regulated by altering their intrinsic activity, by inhibitors and/or activators, or by determining their access to substrates. The final control could be accomplished by altering the subcellular localization of the phosphatase. In this paper we report that CD45, which is the major membrane tyrosine phosphatase in a T cell hybridoma, undergoes a novel and rapid intracellular redistribution in response to cell activation.

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1 The abbreviations used are: PTPase(s), protein tyrosine phosphatase(s); SDS, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; PBS, phosphate-buffered saline; ER, endoplasmic reticulum; TCR, T cell antigen receptor; 1987; Shrinser and Brautigan, 1984; Tonks et al., 1988a, 1988b; Jones et al., 1989). Recently, a major advance in our understanding of tyrosine phosphatases and their potential role in transmembrane signaling has been reported. Tonks et al. (1988a, 1988b) have isolated and sequenced a molecular clone encoding a tyrosine phosphatase from human placenta. When the sequence was compared with known protein sequences a striking similarity to the cytoplasmic domain of the common leukocyte surface antigen CD45 was noted (Charbonneau et al., 1988). This was followed by the demonstration of PTPase activity of CD45 (Tonks et al., 1988c, 1990). These findings raise the possibility of a direct role of tyrosine phosphatases in surface activation events.

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CD45 Redistribution on T Cell Activation

EXPERIMENTAL PROCEDURES

Materials—[Val]-angiotensin II peptides and activated charcoal were purchased from Sigma. Bio-Gel P-2 and P-81 phosphocellulose paper were purchased from Bio-Rad and Whatman, respectively. The biotin labeling kit was from Clontech Laboratories, Inc. Rhodamine-avidin and horseradish peroxidase-streptavidin were from Enzo Biochem, Inc. (New York).

Cells and Antibodies—The T cell hybridoma 2B4 cells and LSTRA cells were maintained continuously under the conditions described previously (Samelson et al., 1985a; Herskovic et al., 1979). The β-deficient T cell line 21.2.2, β-deficient T cell line MA5.8, and γ-deficient T cell line EV3 were derived by repetitive subcloning of 2B4 cells (Susman et al., 1988) and were kindly provided by Dr. J. Ashwell (NCI). The cell line 2A7 was obtained by transfection of MA5.5 cells with the γ chain gene. The trans-act4833-transfected 2B4 cell line was obtained by retroviral transfection. A2B4-2 is a monoclonal mouse IgG2a that reacts with a clonotypic determinant of the 2B4-α chain (Samelson et al., 1983). 145-2C11 (2C11) is a hamster monoclonal antibody that binds the murine CD3-α chain (Leo et al., 1987). G7 (rat IgG2c) is an activatable monoclonal antibody that binds a nonpolymorphic determinant on mouse Thy-1 (Gunter et al., 1984). Either of two nonactivating antibodies was used as a control, SH11 an anti-Thy-1 antibody or 10.2.16, an antibody to Aα+Aβ. Anti-CD45 monoclonal antibodies used were M1/3.4 (M1/9) (Springer et al., 1978) and 55.10.1. Rabbit anti-mouse mannosidase II antibody was a generous gift from Dr. K. Keough. Fluorescein-conjugated goat anti-rabbit IgG and fluorescein-conjugated affinity-purified goat anti-hamster IgG were from Cappel Laboratories (Malvern, PA) and Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD), respectively.

Preparation of [32P]-Labeled [Val]-angiotensin II Peptides—For steady-state metabolic labeling, the 2B4 cells (106/ml) were grown on 12-mm glass coverslips overnight and incubated for 8 h with [32P]orthophosphate (200 Ci/mmol) in 50 mM Hepes, pH 7.4, 150 mM NaCl, containing 0.1% Triton X-100 and washed once for 5 min at room temperature with PBS. Cells were then incubated with biotin-labeled M1/9 and anti-mannosidase antibody for 1 h at room temperature. After washing twice with 4 mM PBS, cells were incubated with biotin-labeled 2C11 and irrelevant ascites (1:50 dilution) as a control. Cells were then washed with PBS and fixed with 2% formaldehyde in PBS for 15 min at room temperature. After permeabilization with 0.1% saponin, 1% bovine serum albumin in PBS, cells were incubated with a biotin-labeled antibody against rabbit IgG and fluorescein-conjugated affinity-purified goat anti-rabbit IgG. Cells were then washed with PBS and fixed with 2% formaldehyde in PBS for 15 min at room temperature. After permeabilization with 0.1% saponin, 1% bovine serum albumin in PBS, cells were incubated for 1 h at room temperature with rhodamine-avidin and fluorescein-conjugated goat anti-rabbit IgG. After washing twice with 4 × SSC for 2 min, coverslips were mounted on glass slides using Fluorescent T (Southern Biotechnology Associates, Birmingham, AL) and examined with an inverted microscope (ICM 405, Cal Zeiss, Inc., Thornwood, NY).

Electron Microscopy—The ultrastructural localization of CD45 and CD45-δ was studied in 2B4 cells by immunoperoxidase electron microscopy using biotin-labeled antibodies, M1/9 and 500A2. Cells were grown on 12-mm glass coverslips overnight and incubated for 8 h with [32P]orthophosphate. Cells were then washed with PBS and fixed with 2% formaldehyde in PBS for 15 min at 37°C either with anti-Thy-1, G7 ascites (1:50 dilution), or with irrelevant ascites (1:50 dilution) as a control. Alternatively, cells were incubated for 30 min at 37°C on a glass coverslip that had been coated with either 2C11 or an irrelevant antibody. Cells were then washed with PBS and fixed with 2% formaldehyde in PBS for 15 min at room temperature. After permeabilization with 0.1% saponin, 1% bovine serum albumin in PBS, cells were incubated for 1 h at room temperature with rhodamine-avidin and fluorescein-conjugated goat anti-rabbit IgG. After washing twice with 4 × SSC for 2 min, coverslips were mounted on glass slides using Fluorescent T and examined with a Zeiss EM 109 (Jena, Germany).

RESULTS

CD45 Is the Major Membrane Tyrosine Phosphatase in T Cell Membranes—T cell tyrosine phosphatase (PTPase) activity was detected using tyrosine-phosphorylated [Val]-angiotensin II as a substrate. The results obtained using the activated charcoal method were similar to those obtained using the P-81 phosphocellulose paper binding method (Table 2).
Tyrosine phosphatase activity assays: Activated charcoal method versus p-81 phosphocellulose paper binding method

Postnuclear supernatants were prepared from B4 cells. Each assay was performed with a volume of supernatant containing either 100 or 75 μg of protein. Protein tyrosine phosphatase assays were performed as described under "Experimental Procedures" using 1 μM phosphotyrosyl [Val15]-angiotensin I1 as the substrate.

| 2B4 cell lysate protein | Time (s) | 32P released |
|-------------------------|----------|--------------|
| µg                      |          | Charcoal     | Phosphocellulose |
| 100                     | 0        | 0            | 0               |
|                         | 3        | 7,311        | 9,650           |
|                         | 6        | 12,851       | 14,038          |
|                         | 10       | 17,524       | 20,972          |
|                         | 15       | 22,641       | 21,520          |
| 75                      | 0        | 0            | 0               |
|                         | 3        | 1,442        | 899             |
|                         | 6        | 3,407        | 3,550           |
|                         | 10       | 4,669        |                 |
|                         | 15       | 8,427        | 12,008          |

TABLE I

CD45 Redistribution on T Cell Activation

- Postnuclear supernatants were prepared from 2B4 cells. Each assay was performed with a volume of supernatant containing either 100 or 75 μg of protein. Protein tyrosine phosphatase assays were performed as described under "Experimental Procedures" using 1 μM phosphotyrosyl [Val15]-angiotensin I1 as the substrate.

- Tyrosine phosphatase activity assays were performed using 1 μM phosphotyrosyl [Val15]-angiotensin I1 as a substrate. The amount of phosphate released is calculated relative to the amount of total solubilized cell protein used for immunoprecipitation.

- Immunoprecipitation of CD45 and the CD45 tyrosine phosphatase activity measured on anti-CD45 antibody-conjugated beads. A, 2B4 cells were surface iodinated, solubilized, and immunoprecipitations were carried out with either anti-CD45 antibody (M1/9.9.3.4) or irrelevant antibody. B, 2B4 cells were solubilized, immunoprecipitated either with anti-CD45 antibody (M1/9.3.4)-conjugated beads (C) or with control antibody-conjugated beads (B). Tyrosine phosphatase assays were performed using 4 μM phosphotyrosyl [Val15]-angiotensin I1 as a substrate. The amount of phosphate released is calculated relative to the amount of total solubilized cell protein used for immunoprecipitation.

- Klaunser et al., 1987; Baniyash et al., 1988; Hsi et al., 1989. Because activation results in the rapid change of tyrosine phosphate content of a variety of proteins, we were interested in determining whether activation resulted in any alteration in CD45 phosphatase activity. T cells were activated with an anti-Thy-1 monoclonal antibody termed G7 (Gunter et al., 1984). This antibody has been demonstrated previously to activate 2B4 T cells (Klaunser et al., 1987) and does not require presenting cells to achieve activation. T cells were treated with either G7 or a variety of monoclonal antibodies which fail to activate the cells. Membranes were prepared from a 280 × g supernatant of Dounce homogenized cells and assayed for angiotensin tyrosine phosphatase activity. We noted a 20% drop in the specific activity of phosphate release only after activation with G7 (Fig. 2B). When antibody was added to isolated 2B4 membranes no change in phosphatase activity was observed (data not shown). If cells were instead lysed directly in Triton X-100 and assayed, G7 stimulation resulted in no change in phosphatase activity (Fig. 2A). The missing activity in the membrane fraction could be accounted for by examining the 280 × g pellet obtained after Dounce homogenization of the cells. When this fraction was assayed after solubilization in Triton X-100 a significant increase in phosphatase activity was observed after G7 activation. This effect of cell activation on the PTPase activity could be observed more clearly after centrifugation through 2.0 or 2.2 M sucrose at 200,000 × g (Fig. 2C). The 200,000 × g sucrose pellet (200 suc-pellet) showed virtually no phosphatase activity in unactivated cells whereas after activation, up to 20% of total cellular angiotensin phosphatase activity was found in this fraction. These changes were complete within minutes of the addition of G7.

- We next asked whether the redistributed PTPase activity included CD45. When membranes were assayed for PTPase activity, membranes from activated cells contained about 20–25% less total activity (Fig. 3A). After depletion with M1/9 antibody beads, the PTPase activity from control and activated membranes was nearly identical (Fig. 3A). This demonstrated that the vast majority of the redistributed PTPase activity was CD45. To demonstrate this directly the pelleted...
PTPase activity was subjected to immunodepletion with the M1/9 beads. This resulted in 100% depletion of PTPase activity (Fig. 3B). Thus, all of the redistributed PTPase activity was attributable to CD45.

**Activation Results in Rapid Depletion of CD45 from the Golgi Region—** We next examined the intracellular distribution of CD45 by immunofluorescence microscopy in 2B4 cells. These cells displayed diffuse surface staining under conditions in which they were not permeabilized. When permeabilized with saponin an intracellular staining pattern was observed. This intracellular CD45 was found in a structure that colocalized with the region of the cell containing the Golgi apparatus (Fig. 4). The latter was identified with a polyclonal antibody against the Golgi enzyme mannosidase II. Little if any CD45 was found in the ER and nuclear envelope, and its distribution did not overlap that of antibodies directed against resident proteins of the ER (data not shown). When the T cells were activated either with G7 or with 2C11-coated coverslips, the morphology of the cell changed rapidly as the cell rounded up and became swollen. The Golgi apparatus was now found over the nucleus in the center of the cell and remained heavily stained by the mannosidase II antibodies. However, the strong Golgi staining region of CD45 seen in control cells became more diffuse in the area of the Golgi. These morphological changes were not observed in cells treated with 3H11, a non-stimulatable anti-Thy-1 antibody (data not shown).

The intracellular distribution of CD45 was examined at the ultrastructural level using immunoperoxidase staining. As shown in Figs. 5A and 6A all cisternae of the Golgi apparatus were labeled by anti-CD45 with no detectable labeling of the ER or nuclear envelope. This pattern can be contrasted to the intracellular localization of the TCR which showed a more common pattern of proteins in transit through the secretory pathway. Thus, staining with either an anti-α or an anti-CD3-ε monoclonal antibody revealed ER, partial Golgi staining, and peripheral vesicle staining. In addition, significant lysosomal staining was observed (Fig. 5B). When the cells were examined by electron microscopy after G7 activation, there was a dramatic loss of the Golgi staining pattern (Fig. 6B). In contrast to the rapid loss of Golgi staining of CD45 upon activation, no activation-induced change in the intracellular staining pattern of the TCR-ε chain was seen in the same cells (Fig. 6D).

Metabolic pulse-chase studies failed to demonstrate any differences in the rate of synthesis or carbohydrate processing of CD45 between control and activated cells (data not shown). The latter was measured by the rate of acquisition of resistance to endoglycosidase H and of susceptibility to neuraminidase as assessed by two-dimensional gel electrophoresis. The disappearance of the heavy pan-Golgi staining by CD45 after activation was not a result of inhibition of CD45 biosynthesis with subsequent clearing of the secretory pathway. We can achieve clearing of the Golgi staining pattern by inhibition of new protein synthesis but only after several hours of treatment with cycloheximide. Concomitant with this, we observed a loss of the activation-induced redistribution of CD45 to the 200 suc-pellet. The half-time for the loss of redistribution (measured by phosphatase activity) was approximately 2 h. Less than 5% of total cellular mannosidase II was detected in the 200 suc-pellet both before and after cell activation (see...
CD45 Redistribution on T Cell Activation

Fig. 3. Redistribution of the CD45 tyrosine phosphatase upon T cell activation. A, 2B4 cells were treated with G7 for 30 min at 37 °C. After activation, membrane lysates were immunodepleted either with anti-CD45 antibody-conjugated beads (●) or with control antibody-conjugated beads (□) as a mock depletion. Membrane lysates from an equal number of 2B4 cells, treated with irrelevant antibody for 30 min at 37 °C, were immunodepleted either with anti-CD45 antibody-conjugated beads (●) or with control antibody-conjugated beads (□). The supernatants were subjected to phosphatase assays using 4.8 μM phosphotyrosyl [Val5]-angiotensin I1 as a substrate. B, 2B4 cells were treated with G7 for 30 min at 37 °C. After activation lysates of the 200 suc-pellets were prepared as described under “Experimental Procedures” and were immunodepleted either with anti-CD45-conjugated beads (●) or with control antibody-conjugated beads (□) as a mock depletion. Lysates of the 200 suc-pellets from an equal number of 2B4 cells, treated with irrelevant antibody for 30 min at 37 °C, were immunodepleted either with anti-CD45 antibody-conjugated beads (●) or with control antibody-conjugated beads (□). Phosphatase assays were performed using 1 μM phosphotyrosyl [Val5]-angiotensin II as a substrate.

Fig. 4. Intracellular distribution of CD45 by immunofluorescence microscopy in the absence or presence of stimulation. 2B4 cells were incubated either with irrelevant antibody (A and B) or with G7 (C and D), or on 2C11-coated coverslips (E and F) for 30 min at 37 °C. Cells were fixed with formaldehyde, permeabilized with saponin, and incubated with anti-mannosidase II antibody (B, D, and F) and with biotin-conjugated M1/9.3.4 (A, C, and E) followed by fluorescein-conjugated goat anti-rabbit IgG and rhodamine-conjugated avidin. Note the loss of CD45 staining in Golgi cisternae after activation although there is no change in the intensity of Golgi staining by the anti-mannosidase II antibody.

Fig. 5. Ultrastructural distribution of CD45 and CD3-ε in 2B4 cells. 2B4 cells were fixed, permeabilized with saponin, and labeled either with biotin-conjugated M1/9.3.4 (A) or with biotin-conjugated 500A2 (B) followed by horseradish peroxidase-conjugated streptavidin. After reaction with diaminobenzidine hydrochloride/H2O2, the cells were prepared for electron microscopy. A, overview of the cytoplasm of 2B4 cells stained with biotin-conjugated anti-CD45 antibody (M1/9.3.4) showing the distribution of the horseradish peroxidase reaction product in pan-Golgi cisternae and its vesicles. Note the relative absence of horseradish peroxidase staining in ER cisternae. B, overview of the cytoplasm of 2B4 cells stained with biotin-conjugated anti-ε antibody (500A2) showing the distribution of the horseradish peroxidase reaction product in ER cisternae, in lysosomes. Note the low levels of horseradish peroxidase staining in Golgi cisternae (compared with Fig. 5A). G, Golgi; er, endoplasmic reticulum; nu, nucleus; ly, lysosome; arrow/head, plasma membrane.
cells stained with biotin-conjugated anti-CD45 antibody (M1/9.3.4) and horseradish peroxidase-conjugated streptavidin. After reaction with diaminobenzidine hydrochloride/H2O2, the cells were prepared stabilized with saponin, and labeled either with biotin-conjugated M1/9.3.4 antibody showing the similar extent of Golgi staining in the absence or presence of T cell activation. 2B4 cells followed by activation with biotin-conjugated anti-CD45 antibody (M1/9.3.4) showing the significant loss of heavy pan-Golgi staining after activation for electron microscopy. This was demonstrated by surface iodination of resting 2B4 cells followed by activation with G7 and fractionation of the 200 suc-pellet (Fig. 7A). The 200 suc-pellet had virtually no radioactivity as determined by γ counting and no labeled immunoprecipitable CD45. If cells were first activated and then subjected to surface iodination, again no labeled CD45 was detected in the 200 suc-pellet. In contrast, when these cells were labeled overnight with [35S]methionine, we could readily detect CD45 in the 200 suc-pellet after G7 activation (Fig. 7B). Although a small amount of CD45 could be found in this fraction from unactivated 2B4 cells, much greater amounts could be recovered after activation. Approximately 30% of the total labeled CD45 redistributed to the 200 suc-pellet after activation. This fit well with the percentage found in this fraction when assessed by phosphatase activity. Thus, no change in specific activity of the phosphatase was observed during this redistribution. These findings suggested that the activation-induced redistributed CD45 was derived from a pool of the phosphatase which was inaccessible to external labeling. This differed from observations on neutrophils in which an increase in cell surface expression of CD45 was observed during activation (Lacal et al., 1988).

Role of the T Cell Antigen Receptor in CD45 Redistribution—

We addressed the question of the role of the TCR in CD45 redistribution in this hybridoma by examining variants of 2B4 that lack surface TCR molecules because of defined genetic defects. 21.2.2 cells are 2B4 variants that lack expression of TCR β chains in which cell surface TCR expression can be reconstituted by the introduction of the β chain gene. When these cells were fractionated to look for CD45 redistribution, two interesting differences from parental 2B4 cells emerged: 1) G7 induced no CD45 redistribution; and 2) the unstimulated cells were indistinguishable, in terms of CD45 distribution, from the activated, receptor-positive parental cell (Fig. 8). To confirm this observation we examined another 2B4 variant, MA5.8, which expresses no γ chain of the TCR. This cell possesses about 3-5% of the number of cell surface receptors present on 2B4, but these are abnormal in terms of both structure (they lack γ) and function. As in the 21.2.2 cells these cells demonstrated no redistribution of CD45 in response to G7, and whether stimulated or not they have a...
judged by fluorescence-activated cell sorting. When we correct phosphatidylinositides in response to activating signals.

Multiple signaling pathways emanating from the TCR have been defined. These include the breakdown of phosphoinositides and the activation of one or more tyrosine kinases. Activation of protein kinase C and a rise in intracellular calcium (at least in part) result from the former pathway. The consequences of this pathway can be mimicked partially (in terms of sub-
strate phosphorylation) by transfecting 2B4 cells with the cDNA encoding v-src (O'Shea et al., 1991). Transfection of 2B4 cells with v-src did not result in the redistribution of CD45. Incubation of 2B4 cells with 0.5 mM dibutyryl cAMP, an analog of cAMP, another common intracellular second messenger, also failed to stimulate the redistribution of CD45. In addition, none of these maneuvers abrogated the structural response to G7 stimulation. Recently we have identified variants of 2B4 which are deficient in the γ chain of the TCR (Mercep et al., 1988). These cells break down little if any phosphatidylinositides in response to activating signals.

When one of these lines, EV3, was tested for its ability to redistribute CD45 we found this response to be intact (see Fig. 8). Thus, although EV3 had less total CD45 PTPase activity than 2B4, a marked change in the distribution of CD45 was observed after treatment with G7. Thus, the γ chain of the TCR does not appear to be required for this phenomenon.

DISCUSSION

Early Cellular Events of T Cell Activation—The studies reported here provide a new and unusual addition to our identification of the early events of T cell activation. A variety of phenomena have been described over the past several years which take place within seconds to minutes of the stimulation of surface molecules on T cells (Samelson et al., 1985a, 1987; Oetgen et al., 1985; Gardner et al., 1989). These include the breakdown of phosphatidylinositides, external calcium influx, release of internal calcium stores into the cytosol, the activation of one or more tyrosine kinases, and the phosphorylation of a variety of cellular substrates by both serine and tyrosine kinases. It is still unclear exactly how these multiple signals are translated into the complex pattern of gene expression and altered proliferative status which characterizes the activated T cell. In addition to these early biochemical events, the stimulated T cell undergoes a marked and rapid morphological change that includes swelling, microvillus formation, and reorientation of the Golgi apparatus as Kuper and Den
ett (1984) observed in cloned cytotoxic T lymphocytes. Recently, Lee and co-workers (1988) reported that a per-Golgi accumulation of spectrin rapidly reorganizes beneath the plasma membrane upon activation of a T cell hybridoma. It is not yet apparent how the currently described biochemical pathways relate to these structural changes. This list of structural changes we can add the redistribution of at least a portion of the cellular population of CD45. Direct stimulation of the T cell receptor was effective in inducing the change. The ability to change the intracellular distribution of CD45 acutely in response to the stimulatory anti-Thy-1 antibody clearly depends upon the expression of functional T cell receptors on the cell surface. This was demonstrated by studying a variety of mutants (or variants) of the 2B4 hybridoma. In cells that failed to express parental levels of full receptor complexes because of the absence of γ, α, β, or δ, no effect of the antibody was seen. When the receptor complex was reconstituted in these cells by cDNA or gene transfection, the CD45 redistribution phenomenon was restored. Interestingly, in cells that lack the newly described γ chain, CD45 redistribution did not take place. These cells failed to break down phosphatidylinositides or to activate protein kinase C in response to G7 stimulation (Mercep et al., 1988). Thus, the redistribution of CD45 in γ-negative cells is consistent with our failure to mimic CD45 redistribution with added phorbol esters and calcium ionophores. A surprising aspect of the phenotype of the T cells lacking surface TCR was that CD45 appeared constitutively redistributed, as assessed by subcellular fractionation. This observation is reminiscent of the recent findings concerning spectrin redistribution in which a TCR-negative variant of the T cell hybridoma demonstrated constitutive redistribution to the plasma membrane, yielding a phenotype indistinguishable from the activated parental TCR-positive cells (Lee et al., 1988). These observations suggest the intriguing possibility that at least in T cell hybridomas, the presence of a functional surface TCR complex may exert a tonic signal that affects the structure of the cell.

Regulating the Residence Time of a Protein within an Organelle—Perhaps the most surprising finding of these studies
The determinants of the residence time of membrane proteins within the organelles of the secretory pathway are the object of intense interest in cell biology. Much of this interest has been focused on the mechanisms for the retention of proteins within specific organelles. A variety of mechanisms can be responsible for retention including binding to retention proteins, transport incompetence, and sorting (Klausner, 1989). Progress is beginning to be made in defining possible signals within proteins responsible for their retention in the ER (Nilsson et al., 1989). Residence questions have been raised largely to address the problems of establishing and maintaining the identity of distinct organelles within the secretory pathway. However, the role of retention in the ER in preventing the expression of incomplete oligomeric complexes on the cell surface represents a use of organelle resistance in regulating cellular functions. The rapid loss of Golgi residence of CD45 in response to receptor-mediated signaling now suggests that the residence of a molecule in organelles along the secretory pathway can be regulated acutely by surface-generated signals.

Speculations on the Functional Implication of the Redistribution of CD45—After many years of intense interest in the role of protein tyrosine kinases it is now becoming clear that PTPases play important regulatory roles in cell signaling (Hunter, 1989; Tonks and Charbonneau, 1989). Interest in CD45 as one of the major membrane PTPases of T cells has been driven by at least two types of observations. First CD45 “looks” like a receptor and thus raises the possibility of ligand-regulated membrane PTPase activity. Second are a variety of observations on the potential functional roles of CD45 in T cell activation. These include the finding that CD45 is redistributed on T cell activation (Ledbetter et al., 1988) and the finding that T cell variants that lack surface CD45 are incapable of TCR-mediated signal transduction (Pingeil and Thomas, 1989). Furthermore, Koretzky et al. (1990) demonstrated recently the essential role of CD45 for coupling T cell antigen receptor to the phosphatidylinositol pathway. We do not know what the basal level of PTPase activity of CD45 is within T cells. However, the extremely high activity of this (and other) phosphatases in vitro suggests strongly that its activity must be regulated to allow tyrosine phosphorylation. Two ways to regulate this PTPase are by controlling its enzymatic activity and by controlling its spatial access to substrates. The rapid loss of Golgi residence of CD45 in T cells has been suggested by observing changes in the mobility of CD45 in the membrane upon treatment of cells with phorbol esters and the finding that CD45 interacts with fodrin in a manner that changes with activation (Bourguignon et al., 1985). The receptor-mediated redistribution of the internal pool of CD45 observed in cultured T cell hybridoma can be thought of in two ways. First, the cell may move this population of CD45 into a compartment in which it now functions in important dephosphorylations. Alternatively, this may function tonically in the Golgi, and the removal from this organelle may function then to allow tyrosine phosphorylations of structures near or associated with the Golgi. These two possibilities are not mutually exclusive. The latter possibility is intriguing because it might explain the unusually long residence time of newly synthesized CD45 in the Golgi. This residence, coupled with the half-life of the protein in these cells, resulted in about 30% of total CD45 (and about 20% of total membrane angiotensin PTPase activity) being present in or around the Golgi and correlation of changes with CD45 redistribution would point to the possible functional effects of the phenomenon reported in this study.

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CD45 Redistribution on T Cell Activation

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