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Immunology

A phosphorylated, disulfide-linked membrane protein in murine cytotoxic T lymphocytes

(interleukin 2/fetal thymocyte/cAMP-dependent protein kinase/T-cell "γ gene")

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Contributed by Herman N. Eisen, June 17, 1986

ABSTRACT **The previously determined sequence of the murine T-cell γ gene and its transcription in cloned T lymphocytes suggests that the polypeptide encoded by this gene is generally present in cytotoxic T cells as a 33-kDa monomer in a disulfide-bonded dimer. The γ chain is also expected to be phosphorylated because a sequence in its cytoplasmic domain is homologous to an active site for serine phosphorylation in the regulatory subunit of cAMP-dependent protein kinase. We describe here a cytotoxic-T-cell-associated phosphorylated protein, many of whose properties suggest that it may be the product of the T-cell γ gene. Its phosphorylation is greatly enhanced by interleukin 2 stimulation.**

Some T cells that express genes encoding the α and β polypeptide chains of the heterodimeric antigen-specific T-cell αβ receptor have been found to transcribe a third immunoglobulin-like gene, called γ. Like the α- and β-chain genes, γ is formed from rearranged variable (V) and joining (J) gene segments and has coding sequences that are similar to immunoglobulin V and constant (C) domains (1, 2). Sequence diversity is more limited in γ than in α and β cDNA but it is, nonetheless, clearly apparent at the junction of the γ gene's V and J gene segments (3). Recently, additional gene segments encoding V_{γ} and C_{γ} regions have been described, increasing initial estimates of murine γ diversity (4–6). The rearranged γ gene is transcribed in all murine cytotoxic T (T_c) cells so far examined, but not in most helper T (T_h) cells (3, 7). γ transcripts have also been found at relatively high levels in immature thymocytes, raising the possibility that the γ gene might play a key role in the process by which developing T cells learn to distinguish between self and nonself major histocompatibility complex (MHC) molecules (8). To help evaluate these and other possible functions of the γ gene, we have been interested in identifying and characterizing the γ gene's protein product. We describe in this report a T_c-cell-associated protein many of whose properties are consistent with those expected of the γ chain. Although our findings are not conclusive, we present them now in view of the intense current interest in the γ gene and its product.

The strategy we adopted to search for the γ chain was based on the amino acid sequence deduced from the γ cDNA clones (pHDS4/203) isolated from a cloned murine T_c-cell line (clone 2C) (2, 3). The sequence predicts that (*i*) the γ chain is an integral membrane protein disulfide-linked to another polypeptide chain, (*ii*) the polypeptide molecular mass of the γ-chain monomer is 33 kDa, and (*iii*) the chain lacks asparagine-linked oligosaccharides. A fourth and crucial prediction emerged from a search for amino acid sequence homology between the cytoplasmic domain of γ and the approximately 2700 protein sequences listed in the Protein Identification Resource Protein Sequence Data Base (August 1984, Division of Research Resources of the National Institutes of Health, National Biomedical Research Foundation). The most striking homology was with a sequence (9) in the regulatory subunit of cAMP-dependent protein kinase (Table 1). Phosphorylation of the serine in the regulatory subunit sequence activates the enzyme's kinase activity, and Arg-Arg-Xaa-Ser/Thr-Yaa (where Xaa is any amino acid and Yaa is a hydrophobic one) is now recognized as an important consensus sequence for serine phosphorylation by cAMP-dependent protein kinase (10). Since it seemed possible that the corresponding serine of the γ chain might also be phosphorylated, we searched in ^{32}P-labeled T_c cells for a disulfide-linked dimer having a phosphorylated monomer of 33 kDa. To facilitate the search, we used a form of two-dimensional gel electrophoresis (the first dimension carried out under nonreducing conditions and the second under reducing conditions) that displays disulfide-linked multimeric proteins as "off-diagonal" spots (11).

MATERIALS AND METHODS

Cell Lines. Murine alloreactive cloned T_c-cell lines 2C and G4 (12); 2.1.1 (3); 3H-2, 3C-11, and 4K-3 (13); and clone 3 (14) have been described. T_c-cell clones 4K-4 and 4K-11 resemble 4K-3 and were generously provided by E. B. Reilly. Murine alloreactive cloned T_h-cell line 18 (7), as well as clones 5.5 and D10 (15, 16), has been described, and clones SV-G11-B3 and SV-G11-C7 directed against Sendai virus were developed and generously provided by H. Ertl (17). T_h line TDH-1, specific for trinitrophenylated H-2^d cells, was produced and generously provided by G. B. Sigal. Two other cell lines examined were the B-cell lymphoma A20 and the mastocytoma line P815 (H-2^d).

^{32}P-Labeling. Cloned T cells were labeled 3 days after stimulation with antigen-bearing cells and recombinant interleukin 2 (rIL-2). Cells ($\approx 10^7$) were incubated for 3 hr at 37°C with 0.5 mCi (1 Ci = 37 GBq) of carrier-free [^{32}P]orthophosphate in 2 ml of phosphate-free medium, in the presence (200 units/ml) or absence of rIL-2 (Biogen, Boston). The cells were then washed three times with Dulbecco's modified Eagle's medium without phosphate and lysed with a previously described "extraction buffer" (18). Some cells were also ^{125}I-labeled by use of lactoperoxidase (19). "Double-negative" thymocytes were obtained by isolating thymocytes from 3-week-old mice and treating them with anti-L3T4 (GK1.5) and anti-Lyt-2 [AD4(15) and 3.168.8] plus complement (8). Day 16 fetal thymocytes were harvested from timed pregnancies (day 0 corresponds to day of detection of the

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Abbreviations: T_c cell, cytotoxic T cell; T_h cell, helper T cell; Endo F, endo-β-*N*-acetylglucosaminidase F; IL-2, interleukin 2; rIL-2, recombinant IL-2; V, variable; J, joining; C, constant.

Table 1. Amino acid sequence homology between the predicted cytoplasmic domain of the T-cell γ chain and cAMP-dependent protein kinase

	Sequence
γ cDNA	-Arg-Arg-Thr-Ser-Val-Cys-Gly-Asn-Glu-Lys-Lys-Ser-OH
cAMP-dependent protein kinase	-Arg-Arg-Leu-Ser*-Val-Cys-

Sequences based on γ cDNA clone pHDS4/203 (amino acids 275–286; refs. 1 and 2) and the regulatory subunit of cAMP-dependent protein kinase; asterisk marks the serine that is phosphorylated in the kinase (9).

vaginal plug). Double-negative and fetal thymocytes were ^{32}P-labeled immediately after harvest, with or without rIL-2 (500 units/ml) or 10 nM phorbol 12-myristate 13-acetate.

Membrane Preparations. Approximately 10^7 washed ^{32}P-labeled cells were suspended in 200 μl of phosphate-buffered saline (pH 7, without added divalent cations) with 100 μM sodium metavanadate and subjected to two freeze–thaw cycles. The lysed cells were then centrifuged at 100,000 × *g* for 20 min and the pelleted membrane was extracted with "extraction buffer" (18).

Two-Dimensional ("Diagonal") NaDodSO$_4$/Polyacrylamide Gel Electrophoresis. Extracts of labeled cells (1.5 × 10^6 cell equivalents) and of membrane preparations (10^7 cell equivalents) were electrophoresed under nonreducing conditions in a cylindrical 7.5% polyacrylamide gel containing NaDodSO$_4$. The "tube gels" were then incubated for 2 hr in 5% 2-mercaptoethanol and subjected to electrophoresis in the second dimension (10% polyacrylamide slab gels containing NaDodSO$_4$) (11).

RNA Blot Analysis. Total RNA or oligo(dT)-purified [i.e., poly(A)$^+$-enriched] RNA were electrophoresed in 1% agarose gels, blotted onto nitrocellulose filters, and hybridized at 42°C in 50% formamide/5× standard saline citrate (SSC: 1× is 0.15 M NaCl/0.015 M sodium citrate, pH 7), and the filters were washed at 65°C in 0.2× SSC. Probes corresponding to the α, β, and γ genes were derived from a cDNA library of T$_c$ clone 2C (pHDS58, pHDS11, and pHDS4, respectively; see refs. 1 and 2).

Endoglycosidase F Treatment. The ^{32}P-labeled 34-kDa "off-diagonal" protein (pp34) from clone 2C was electroeluted from a two-dimensional gel and digested with endoglycosidase F (Endo F) as described (20).

Phospho Amino Acid Analysis. Lysozyme (40 μg) was added as carrier protein to electroeluted ^{32}P-labeled pp34 and the mixture was precipitated with 50% (wt/vol) trichloroacetic acid. After successive washes three times each with 25% trichloroacetic acid, 5% perchloric acid, and acetone, samples were dried, hydrolyzed for 90 min at 100°C in 6 M HCl, dried again, washed with double-distilled H_2O, and subjected to two-dimensional thin-layer chromatography/high-voltage electrophoresis as described (21).

CNBr Digestion. ^{32}P-labeled pp34 was electroeluted from a two-dimensional gel and completely reduced and alkylated in 4.8 M guanidine HCl with 10 mM dithiothreitol followed by 22 mM iodoacetamide. After dialysis overnight against 1 M acetic acid, samples were dried, dissolved in 75 μl of 70% formic acid containing 185 μg of CNBr, and incubated at room temperature overnight.

Production and Purification of Peptide-Specific Antisera. Peptides corresponding to residues 54–65, 196–206, and 275–286 of the predicted sequence of the γ-gene protein product were synthesized and generously contributed by G. Matsueda (Massachusetts General Hospital). A cysteine residue was added to the amino terminus of each peptide to facilitate conjugation to keyhole limpet hemocyanin via maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) (22). The internal cysteine at residue 280 was carboxymethylated to prevent coupling via this thiol. Rabbits were immunized subcutaneously with 100 μg of conjugated peptide in complete Freund's adjuvant. After 21 days, the animals were given booster injections and, 7 days later, were bled. A 40% saturated ammonium sulfate fraction of each antiserum was affinity-purified on a peptide-Sepharose column prepared by coupling cysteinyl-peptide to aminohexyl-Sepharose activated with either maleimidobenzoyl-*N*-hydroxysuccinimide ester or iodoacetic anhydride. Bound antibody was eluted with 0.15 M acetic acid.

Immunoprecipitation. ^{32}P-labeled membrane extracts, in 150 μl of extraction buffer (18), were "precleared" twice by incubating them with 10 μl of nonimmune rabbit serum on ice for 30 min followed by continuous shaking at 0°C in the presence of 40 μl (packed volume) of protein A-Sepharose beads (Sigma). After removal of the sedimented beads (at 8000 × *g*), affinity-purified rabbit antibodies to the γ peptides (see above) were added (to 10 μg/ml) to the cleared supernatants. After 30 min on ice, 40 μl of packed protein A-Sepharose was added and the samples were shaken overnight at 0°C. The Sepharose pellets were washed until the Cerenkov cpm in successive washes differed by less than 10%, and the antibodies and peptides were then eluted by heating at about 100°C for 7 min in NaDodSO$_4$ sample buffer without mercaptoethanol.

RESULTS AND DISCUSSION

pp34: A 34-kDa Phosphorylated Protein. The predominant off-diagonal ^{32}P-labeled protein in lysates of the T$_c$-cell clone 2C has an apparent molecular mass of 34 kDa (Fig. 1). This protein, which we refer to as pp34, appears to be a disulfide-linked component of a molecule that in nonreduced form has a molecular mass of 52 ± 5 kDa. The size difference between reduced (34 kDa) and nonreduced (52 kDa) forms suggests that the component linked to pp34 has a molecular mass in the vicinity of 18 kDa. The nonreduced form is unlikely to be a homodimer, since electroeluted pp34 spontaneously oxidizes during electroelution to form a (presumed) homodimer of 68

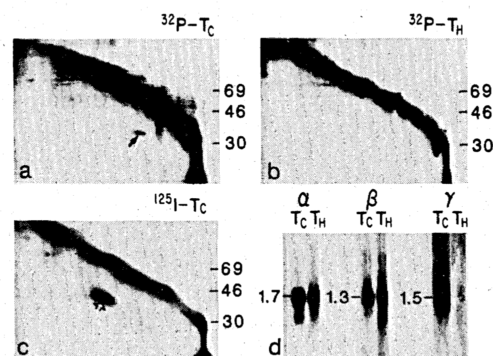

FIG. 1. (*a–c*) Expression of a phosphorylated disulfide-linked protein in T$_c$ lymphocytes, detected by two-dimensional (nonreducing/reducing) "diagonal" gel analyses of lysates from ^{32}P-labeled T$_c$ clone 2C (*a*, arrow points to pp34), ^{32}P-labeled T$_h$ clone 18 (*b*), and ^{125}I-labeled T$_c$ clone 2C (*c*, arrow points to α and β subunits of the T-cell receptor). Positions of standards (kDa) are indicated at right of each autoradiogram. (*d*) γ-gene transcription in T$_c$ clone 2C and T$_h$ clone 18 were compared by RNA blot analyses using α-, β-, and γ-specific probes. Estimated lengths of hybridizing RNAs are given in kilobases.

kDa. Since we did not detect a phosphorylated spot at 18 kDa, the partner chain is probably not phosphorylated.

Phosphorylation of pp34 Depends upon IL-2. To evaluate the effects of IL-2 and antigenic stimulation on phosphorylation of pp34, clone 2C cells were harvested, maintained for 15 hr in the presence or absence of rIL-2 or P815 cells (which have the L^d class I molecule recognized by 2C cells), and then labeled with [^{32}P]orthophosphate over a 3-hr period in the presence or absence of rIL-2. Diagonal electrophoresis showed that pp34 was most conspicuous in cells that had been exposed to rIL-2 both prior to (15 hr) and during (3 hr) the labeling with ^{32}P (Fig. 2*a*). When, in addition, P815 cells were also present, the intensity of the pp34 spot was not significantly enhanced. With rIL-2 absent and P815 cells present, pp34 was evident but much less conspicuous (Fig. 2*b*). In the absence of both rIL-2 and P815, the phosphorylated off-diagonal protein was barely detectable (Fig. 2*c*). The cells that had been continuously exposed to rIL-2 also had more intensely phosphorylated on-diagonal components, suggesting that the increased ^{32}P-labeling of pp34 in the presence of rIL-2 was associated with a general increase in protein kinase activity in IL-2-stimulated cells.

In lysates of ^{125}I-labeled 2C cells, the α and β subunits of the antigen-specific receptor migrate as expected, as an intensely labeled off-diagonal spot at 40–45 kDa (Fig. 1*c*). However, at the position of pp34 there was only a faint ^{125}I-labeled spot, and it was not clearly resolved from another ill-defined spot (or spots) that probably correspond to the Lyt-2 glycoprotein complex. (The ^{32}P-labeled spot, however, was not diminished by preabsorption with anti-Lyt-2 monoclonal antibody.) Conversely, the T-cell $\alpha\beta$ receptor was not phosphorylated.

pp34 Is a Membrane-Associated Protein. Although pp34 was not clearly labeled when surface proteins of intact 2C cells were labeled with ^{125}I, it seemed to be highly enriched in membrane preparations of ^{32}P-labeled 2C cells (Fig. 3 *a* and *b*). Moreover, when intact 2C cells were treated for 1 hr with Pronase at 0°C, the pp34 signal disappeared (Fig. 3 *c* and *d*). Taken together, these findings suggest that pp34 is a membrane protein present (at least in cloned 2C cells) at a much lower level than the T-cell antigen-specific ($\alpha\beta$) receptor.

Some Chemical Characteristics of pp34. When pp34 was isolated by electroelution from the off-diagonal spot of a two-dimensional gel and subjected to NaDodSO$_4$/PAGE electrophoresis in one dimension, it could be resolved into two closely spaced phosphorylated bands. Since their electrophoretic mobility was too similar to allow their separation, both members of the doublet were analyzed together. Phospho amino acid analysis revealed them to have only phosphoserine, but other phospho amino acids at one-tenth the level would not have been detected (data not shown).

Treatment of isolated pp34 with Endo F resulted in no change in its apparent size (Fig. 4*a*). Therefore, pp34 is not N-glycosylated, in accord with the absence of sequences for N-glycosylation (Asn-Xaa-Ser/Thr) in the sequenced γ cDNA clone (1, 2). Sequences corresponding to N-glycosylation sites are also absent from the two other previously described V_γ gene segments (3) but are present in another recently found V_γ and C_γ gene segment (4, 5).

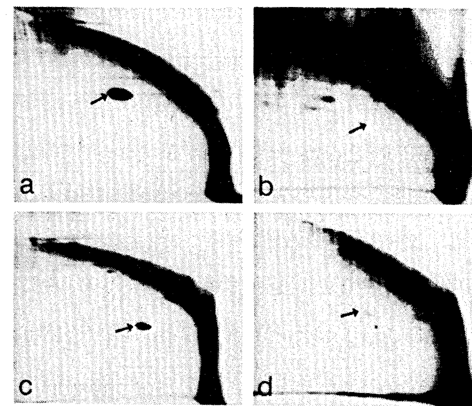

FIG. 3. pp34 as an integral membrane protein. Diagonal gel of membrane fraction of ^{32}P-labeled 2C cells (*a*) compared with diagonal gel of equal cell equivalents of cytosolic fraction (*b*). Pronase digestion at 0°C of ^{32}P-labeled 2C cells selectively reduces pp34 signal (*d*) when compared with a sample of the same preparation of labeled 2C cells, but untreated with enzyme. (*c*). (Arrow in all figures points to pp34, or its expected position, as verified by molecular mass markers, which are not shown.)

Isolated pp34 (from 2C cells) was digested with CNBr to compare the resulting fragments with those predicted for the product of the previously described rearranged γ gene in 2C cells. This product is expected to have 286 amino acid residues, with methionine at positions 98, 189, and 201 (1, 2). Hence, if the phosphorylated serine residue(s) of γ is confined to the cytoplasmic domain (positions 275–286) and if, as is likely, CNBr cleavage is relatively inefficient (because of methionine oxidation under the nonreducing conditions of electroelution), then CNBr digestion of ^{32}P-labeled γ chain should yield the undigested chain (33 kDa) and three fragments (21.3, 11.0, and 9.6 kDa). CNBr digestion of isolated pp34 (Fig. 4*b*) yielded a considerable amount of undigested chain (36 ± 4 kDa) and three labeled fragments, of 22.5 ± 1.5, 13 ± 4, and 8.6 ± 1 kDa, all of which agreed reasonably well with the sizes of the fragments expected from the sequenced γ-gene transcript in 2C cells. In addition, however, there was an ill-defined fourth fragment (≈28 kDa) whose origin is unclear: it could be due to cleavage at a tryptophan residue or at an acid-labile residue or due to a contaminant in the electroeluted sample.

Frequency of pp34 in T_c and T_h Cells. Since a γ-gene probe (pHDS4/203, refs. 1 and 2) had been found to hybridize with RNA transcripts from all T_c clones tested but from none of four T_h clones, we examined several additional cloned T-cell lines for the presence of pp34. Every one of nine alloreactive T_c-cell lines (2C, 2.1.1, G4, 4K4, 3H2, 4K11, 3C11, 4K3, and clone 3) had a phosphorylated spot that corresponded in

FIG. 2. Effect of IL-2 and antigen stimulation on ^{32}P-labeled pp34. 2C cells were incubated for 14 hr with rIL-2 plus P815 target cells (see Fig. 1*a*) or with rIL-2 alone (*a*), irradiated P815 cells alone (*b*) or neither rIL-2 nor P815 (*c*) prior to labeling for 3 hr with [^{32}P]phosphate. Lysates (of equivalent numbers of cells) were then analyzed by two-dimensional (nonreducing/reducing) gel electrophoresis. Positions of standards are indicated (molecular mass values at right, in kDa).

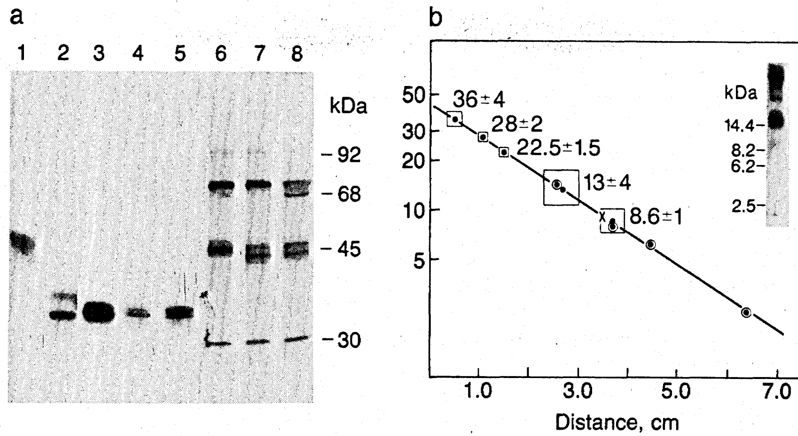

FIG. 4. Effect of Endo F and CNBr cleavage on electroeluted pp34. (*a*) Eluted ^{125}I-labeled β chain of the T-cell receptor of 2C cells (lane 1) after treatment with Endo F shows the expected decrease in size due to removal of its N-linked oligosaccharides (20) (lane 2). Eluted pp34 (lane 3) treated with Endo F (lane 4) shows no change in size, consistent with expected lack of N-glycosylation sites. Loss of intensity of pp34 signal with Endo F treatment is partly restored by performing Endo F incubation in the presence of phosphatase inhibitors contained in extraction buffer (18) (lane 5), suggesting the presence of phosphatase in Endo F preparation. Standards (lane 6), including glycosylated ovalbumin (45 kDa), show that effect of Endo F (lane 7) is not blocked by the presence of phosphatase inhibitors (lane 8). (*b*) CNBr cleavage of electroeluted pp34 analyzed by one-dimensional NaDodSO$_4$/15% PAGE (*Inset* shows autoradiograph of a gel) (23). CNBr digest of myoglobin provided external molecular mass standards (circled) and λ2 immunoglobulin light chain major CNBr fragment (X) of predicted size 8.9 kDa (based on its amino acid sequence) served as an internal standard (24).

position to clone 2C's pp34 on diagonal gels. However, this spot was not detected in five of six T_h-cell lines [D10, clone 18 (Fig. 1*b*), TDH-1, SV-G11-B3, and SV-G11-C7]. Nor was it found in a B-cell lymphoma (A20) or in the mastocytoma cell line P815. Low levels of pp34 (<10% of that found in T_c cells) in the negative cell lines would not have been visible above background.

The exceptional T_h-cell line was clone 5.5 (generously provided by C. Janeway). Like many cloned T_h-cell lines, clone 5.5 has cytolytic activity: it has the "helper" phenotype (L3T4$^+$, Lyt-2$^-$) and its specificity for ovalbumin is restricted by H-2^b class II glycoprotein. It is of special interest that poly(A)$^+$ RNA from the 5.5 cell line hybridized with the previously described γ-gene probe (pHDS 4/203, refs. 1 and 2) when examined after electrophoresis and transfer to nitrocellulose (data not shown). The finding of both pp34 and γ transcript in the T_h-cell line 5.5 is consistent with the possibility that pp34 is encoded by the γ gene.

Since high levels of γ-positive RNA transcripts have been found in fetal thymocytes (detectable on day 14 and peaking on day 15) and in the immature adult "double-negative" (Lyt-2$^-$, L3T4$^-$) thymocytes (8), we metabolically ^{32}P-labeled day-16 fetal thymocytes and adult double-negative thymocytes. In day-16 fetal thymocytes stimulated with either rIL-2 or phorbol myristate acetate and in adult double-negative cells stimulated with rIL-2, an off-diagonal phosphoprotein was present (Fig. 5). It shared with pp34 the fine structure of a closely spaced phosphorylated doublet, but it had a somewhat lower molecular mass (31 ± 1 kDa), and therefore we term it pp31. ^{32}P-labeled pp31 was not detectable in unstimulated day-16 fetal thymocytes.

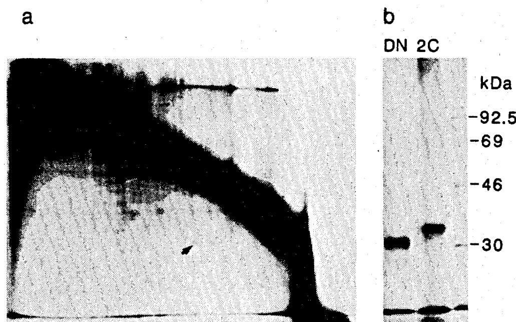

FIG. 5. Analysis of double-negative (Lyt-2$^-$, L3T4$^-$) thymocytes for phosphorylated disulfide-linked dimer. (*a*) Diagonal gel analysis of lysate of ^{32}P-labeled double-negative thymocytes (arrow points to pp31). (*b*) Comparison of electroeluted pp31 from double-negative (DN) thymocytes with pp34 from T_c clone 2C.

Immunoprecipitation with Anti-Peptide Antibodies. To determine whether pp34 and the γ-gene transcript have sequences in common, we tested ^{32}P-labeled 2C cell extracts with antisera raised in rabbits to three synthetic peptides corresponding to the deduced amino acid sequence of the 2C γ gene in positions 54–65 in the V region, 196–206 in the C region, and 275–286 in the cytoplasmic domain. The latter contains the sequence homologous to the serine phosphorylation site of cAMP-dependent protein kinase. None of the antisera reacted positively in immunoblots with pp34 (data not shown). However, antiserum to the cytoplasmic domain peptide immunoprecipitated pp34 from ^{32}P-labeled membrane extracts of cloned 2C cells but not from the T_h clone D10 (Fig. 6).

Immunoprecipitation of ^{32}P-labeled fetal thymocyte ex-

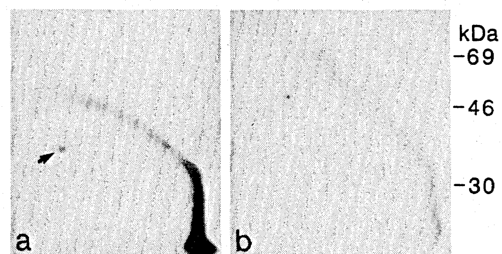

FIG. 6. Immunoprecipitation of ^{32}P-labeled 2C membrane preparation with rabbit antiserum raised against γ peptides. (*a*) Immunoprecipitation with antiserum raised against γ cytoplasmic domain peptide. The second precleared pellet contained no detectable pp34 (data not shown). pp34 was not detectable above background in immunoprecipitates analyzed by one-dimensional gel electrophoresis but was enriched >100-fold by precipitation with antipeptide antiserum. Some of the precipitated on-diagonal phosphoproteins could also be expected to have been phosphorylated by cAMP-dependent protein kinase and to contain the peptide consensus sequence. (*b*) pp34 is not immunoprecipitated with antiserum raised against the peptide corresponding to residues 54–65 of the predicted γ gene product.

tracts with antiserum to the cytoplasmic domain peptide enhanced the signal of pp31 but, compared with the background, did not allow us to distinguish decisively between nonspecific binding and specific immunoprecipitation.

CONCLUDING REMARKS

The properties described here for pp34 agree to a considerable extent with those predicted for the T-cell protein product of the γ gene. Like the predicted protein, pp34 is a $\approx$34-kDa component of a membrane-associated disulfide-linked dimer that is phosphorylated on serine and is not glycosylated on asparagine. The CNBr digest of pp34 is also in reasonable agreement with the location of methionine residues predicted by a sequenced γ cDNA, and pp34 is immunoprecipitated by an antiserum to a 12 amino acid peptide encoded in the γ-gene cytoplasmic domain. The fact that antisera to two other γ peptides did not react with pp34 is not inconsistent, since anti-peptide antibodies often react weakly with the corresponding intact proteins.

However, the identification of pp34 as the T-cell γ product runs into some difficulties in light of several other findings. One problem is its absence in day-16 fetal thymocytes and in double-negative thymocytes. However, these cells contain an off-diagonal ^{32}P-labeled protein of 31 kDa, not 34 kDa. We do not know whether "pp31" in these immature thymocytes is related to pp34; possibly it represents an alternative splicing of the γ transcript or posttranslational modification of the γ product.

Perhaps the most troubling difficulty arises from the recently determined sequences of γ cDNA clones from T_c-cell clones 4K-3, 3H-2, and 3C-11 (13). In all of these cell lines, γ cDNA sequences at the V–J joining region are out-of-phase, suggesting premature termination of the translated protein. Nevertheless, the levels of pp34 in 4K3, 3H-2, and 3C-11 are comparable to that found in clone 2C, which contains a γ gene whose V–J join is in-frame (1, 2). If pp34 is the γ product, this result suggests that there is a greater diversity of murine γ genes than was initially found or suspected, and that there are as yet undescribed members of the γ gene family that do not hybridize with the 2C γ probe pHDS4.

The key to visualizing pp34 in T_c cells was its IL-2-enhanced phosphorylation. This suggests that pp34 may function as a receptor that transduces membrane signals and that its functional activity may depend upon its phosphorylation state. Since pp34 is immunoprecipitated by antiserum raised against a short peptide containing the consensus sequence for cAMP-dependent serine phosphorylation, it is probable that its phosphorylation is mediated by cAMP-dependent protein kinase. However, 4 out of 12 amino acids in the cytoplasmic peptide are basic, and it is possible (although less likely) that the phosphorylation is mediated by protein kinase C (25). Protein kinase C activity may well be enhanced in T_h and T_c cells following binding to the $\alpha\beta$ T-cell receptor–T3 complex (26) and following IL-2 stimulation (27). The fact that pp34 phosphorylation is more dependent on IL-2 than antigenic stimulation suggests that the various end points of T-cell activation may involve multiple regulatory and phosphorylating enzymes.

Note Added in Proof. In two recent reports a protein that appears to correspond to the γ chain has been identified in human peripheral blood T cells from immunodeficient patients (28) and in immature human thymocytes (29). The putative human γ chain differs from pp34 in several respects: e.g., it is not disulfide-linked to another chain, it is glycosylated, and it is larger (44–55 kDa). These differences are in accord with the different amino acid sequences predicted from the human and most murine γ-gene segments.

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