mains of the type II TGF-β receptor was constructed by a polymerase chain reaction (PCR)–based strategy. The extracellular domain of the type II activin receptor was amplified with primers to introduce a Bam HI site at the 5′ end and a Spe I site at the 3′ end. We generated the fragment encoding the transmembrane and cytoplasmic domains of the type II TGF-β receptor as a Spe I–Xba I fragment using a PCR reaction. Both fragments together were subcloned as a Bam HI–Xba I fragment into the vector pRK7 (*21*). The sequences of the primers used were as follows: 5′ activin receptor primer, 5′-GCTAGAATTCGGGAAAATGGGAGCTGCTGCA-3′; 3′ activin receptor primer, 5′-GGCGGACTAGTAACAAGGGTGGCTTCGGTGTAACAGG-3′; 5′ TGF-β receptor primer, 5′-TTGTTACTAGTCATATTTCAAGTG-3′; 3′ TGF-β receptor primer, 5′-GGCTCTAGAGCTATTTGGTAGTGTTTAG-3′.

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18. Recombinant human activin and human TGF-β1 were prepared and purified to homogeneity from transfected CHO overproducer cell lines and ^{125}I-labeled by the use of a modified chloramine T method [C. A. Frolik *et al.*, *J. Biol. Chem.* **259**, 10995 (1984)].
19. To insert the HA–epitope tag (Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ser-Leu) at the COOH-terminus of the full-length Tsk 7L, a Hind III–Bam HI fragment corresponding to nucleotides −7 through 1522 of the tsk 7L cDNA, was generated by PCR and was ligated to the 5′ of a double-stranded synthetic adaptor encoding the HA epitope tag. The resulting ligation product was inserted into the Hind III and Eco RI cloning sites of the pRK5 (*21*) expression vector.
20. Immunoprecipitations were carried out as follows: After cross-linking and lysis in the presence of protease inhibitors, cell extracts were solubilized in Triton X-100, clarified by centrifugation, and were diluted tenfold in 0.5× phosphate-buffered saline with 0.4% gelatin (Bio-Rad) and 0.2% of both Tween 20 and Thimerosal (Serva). After incubating with 5 to 15 μg of specific antibody for 6 to 12 hours at 4°C, 10 mg of protein A–Sepharose CL-4B beads (Pharmacia), preswollen in the same buffer, were added and incubation continued for another 4 to 8 hours. Beads were harvested by centrifugation, washed extensively in the immunoprecipitation buffer, and radiolabeled complexes were then dissociated from the beads by boiling in electrophoresis sample buffer.
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Interaction of Shc with the ζ Chain of the T Cell Receptor upon T Cell Activation

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The *shc* oncogene product is tyrosine-phosphorylated by Src family kinases and after its phosphorylation interacts with the adapter protein Grb2 (growth factor receptor–bound protein 2). In turn, Grb2 interacts with the guanine nucleotide exchange factor for Ras, mSOS. Because several Src family kinases participate in T cell activation and Shc functions upstream of Ras, the role of Shc in T cell signaling was examined. Shc was phosphorylated on tyrosine after activation through the T cell receptor (TCR), and subsequently interacted with Grb2 and mSOS. The Src homology region 2 (SH2) domain of Shc directly interacted with the tyrosine-phosphorylated ζ chain of the TCR. Thus, Shc may couple TCR activation to the Ras signaling pathway.

The activation of Ras proteins appears to be a crucial early event in the intracellular signaling pathways initiated by a number of receptor tyrosine kinases (RTKs) (*1*). Activation of RTKs increases the amount of Ras in the active guanine triphosphate (GTP)–bound state, and mutational inactivation of *ras* genes or dominant negative inhibitors of Ras proteins block the effects of several RTKs (*2*, *3*).

The intermediary steps between RTK activation and the conversion of Ras to its active GTP-bound state have been delineated (*4*). Grb2 is an adapter protein that lacks a catalytic domain and is composed of one SH2 domain and two SH3 domains (*5*). The SH2 domains bind to specific phosphopeptide sequences, whereas SH3 domains bind to proline-rich sequences (*6*, *7*). The protein Grb2 binds to the autophosphorylated RTK through its SH2 domain, and it simultaneously associates through its SH3 domains with mSOS, a guanine nucleotide exchange protein that activates Ras by inducing exchange of guanine diphosphate for GTP on Ras (*4*, *8*). Thus, in RTK signaling, Ras (which is normally membrane-bound) is activated when Grb2 shuttles mSOS to the membrane.

Receptors that are not tyrosine kinases but signal through activation of associated tyrosine kinases also induce Ras activation. Another protein that interacts with Grb2 is the *shc* oncogene product. Shc is also an adapter protein that is widely expressed in all tissues and contains an SH2 domain and a collagen-like domain but no obvious catalytic domain (*9*). This protein is phosphorylated on tyrosine in cells transformed by nonreceptor tyrosine kinases such as v-Src and v-Fps (*9*). Through its SH2 domain Grb2 associates with tyrosine-phosphorylated Shc (*10*). Like Grb2, Shc appears to function upstream of Ras because the differentiation of PC12 pheochromocytoma cells induced by overexpression of Shc is blocked by the dominant inhibitory N17Ras mutant (*10*). Because several nonreceptor Src family tyrosine kinases (namely Lck, Fyn, and ZAP-70) participate in T cell signaling, and TCR stimulation leads to Ras activation (*11*, *12*), we speculated that Shc might couple the TCR-CD3 complex to the Ras activation pathway.

To evaluate the role of Shc in T cell activation, we determined whether Shc is tyrosine-phosphorylated during T cell activation. A murine T cell hybridoma By155.16 (By) (*13*), expressing a Vβ8$^+$ TCR and CD4, was activated by antibody-mediated cross-linking of the TCR, either alone or with the CD4 coreceptor. Shc was immunoprecipitated with antibodies to Shc (anti-Shc) from activated T cell lysates and immunoblotted for phosphotyrosine. Marked tyrosine phosphorylation of both the 48- and 52-kD isoforms of the Shc protein was detected after the cross-linking of TCR and CD4 (TCR×CD4) and was only weakly detected after cross-linking TCR alone or CD4 alone (Fig. 1A). Our analysis of the time course of phosphorylation indicated that Shc phosphorylation occurred early, as it could be detected within 15 s (Fig. 1B). The Shc phosphorylation peaked at about 5 min and its phosphorylation started to diminish by 15 min. Similarly, immunoprecipitation of proteins from By cell lysates after TCR activation with antibody to phosphotyrosine (anti-phosphotyrosine) and immunoblotting for Shc showed activation-dependent tyrosine phosphorylation of Shc (Fig. 1, C and D). Anti-Shc also immunoprecipitated an unidentified 140-kD tyrosine-phosphorylated protein.

We assessed whether Shc would interact

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with Grb2 after T cell activation. Immunoblotting of anti-Shc immunoprecipitates with antibody to Grb2 (anti-Grb2) revealed the coprecipitation of Grb2 only after T cell activation (Fig. 2A). Consistent with the amount of Shc tyrosine phosphorylation, greater amounts of Grb2 were precipitated after TCR×CD4 cross-linking than after TCR cross-linking alone. We also found Grb2 in anti-phosphotyrosine immunoprecipitates after T cell activation (*14*). Because Grb2 was not detectably tyrosine-phosphorylated, it was probably precipitated through its association with another tyrosine-phosphorylated protein, most likely Shc.

The SH2 domain of Grb2 (Grb2SH2) interacts with tyrosine-phosphorylated Shc (*10*). To determine whether the coprecipitation of Grb2 with Shc after TCR activation occurs through this interaction, we incubated Grb2SH2 [expressed as a glutathione-S-transferase (GST) fusion protein and immobilized on glutathione beads] with lysates from activated or unactivated T cells. The proteins that bound to the beads were immunoblotted with anti-Shc (Fig. 2B). The Grb2SH2 precipitated Shc only from activated lysates (strongly detected after TCR×CD4 cross-linking and barely detectable after TCR cross-linking alone), and precipitates contained mainly the 52-kD isoform. In contrast, GST alone did not precipitate Shc from activated lysates. Immobilized GST fusion proteins containing SH2 domains from other proteins (p85 subunit of PI-3 kinase, PLC-γ1, or Shc itself) did not precipitate Shc from activated or unactivated lysates (*14*).

Through its SH3 domains, Grb2 binds to the guanine nucleotide exchange factor, mSOS (*4*). We examined whether mSOS would be part of the complex with Shc and Grb2 during T cell activation. Immunoblotting of anti-Shc immunoprecipitates with antibody to mSOS (anti-mSOS) showed activation-dependent coprecipitation of mSOS with Shc (Fig. 2C). Again, greater amounts of mSOS were detected after TCR×CD4 cross-linking than after TCR cross-linking alone.

These data suggested that, after T cell activation, phosphorylation of Shc is an intermediary event through which the activation of Ras may occur. Because Shc is a cytoplasmic protein and activation of Ras occurs at the plasma membrane, we speculated that Shc, by means of its SH2 domain (ShcSH2), might interact with one of the membrane-associated proteins that becomes tyrosine-phosphorylated during T cell activation. This would provide a mechanism to shuttle Grb2 and mSOS to the membrane. Lysates from activated and unactivated T cells were incubated with an immobilized GST-ShcSH2 fusion protein. The proteins that bound to ShcSH2 were identified by immunoblotting with anti-phosphotyrosine (Fig. 3). The ShcSH2 domain specifically precipitated a doublet of 21 to 23 kD and also a weak band of 70 kD. GST alone did not precipitate these phosphoproteins. The mobility of the 21- to 23-kD doublet closely matched that of the doublet seen in immunoprecipitates with antibodies to TCR ζ chain (anti-ζ) (*14*). Because ζ chain is tyrosine-phosphorylated after T cell activation, we assessed whether Shc, through its SH2 domain, interacts with the tyrosine-phosphorylated ζ chain.

Biotinylated peptides corresponding to the third tyrosine-based activation motif (TAM) (*15*) present in TCR ζ chain were synthesized with both tyrosines either phos-

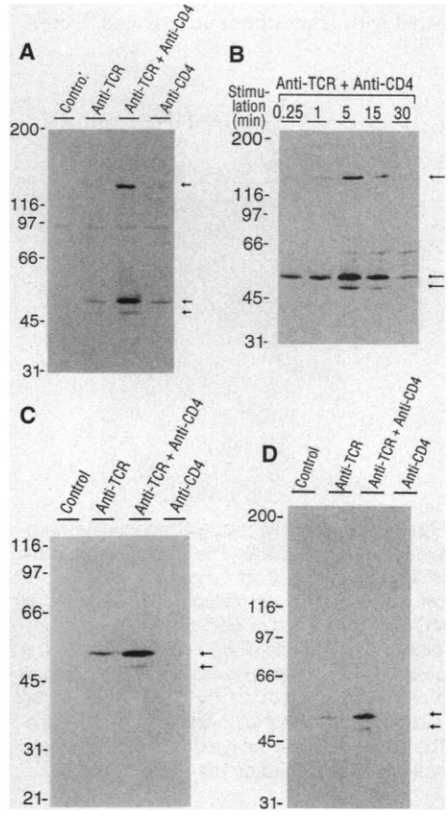

Fig. 1. Tyrosine phosphorylation of Shc after T cell activation. (**A**) Anti-phosphotyrosine blotting of Shc immunoprecipitates. By cells were stimulated with antibody to CD3 (anti-CD3) (designated as anti-TCR) or antibody to CD4 (anti-CD4), or both, and cross-linked with rabbit anti-mouse IgG, then incubated for 2 min at 37°C (*22*). After cell lysis in 1% NP-40, the lysates were immunoprecipitated with anti-Shc. The proteins were resolved by 6 to 12% SDS-PAGE, immunoblotted with anti-phosphotyrosine, and developed by ECL. The molecular size standards in (A) through (D) are indicated on the left of each in kilodaltons. The 48- and 52-kD isoforms of Shc and the unidentified 140-kD protein are indicated by arrows. Rabbit anti-mouse IgG was used for the control. (**B**) Time course of tyrosine phosphorylation of Shc. The By hybridoma cells were stimulated with anti-CD3 and anti-CD4 for the indicated times at 37°C. Shc immunoprecipitation and anti-phosphotyrosine blotting were performed as in Fig. 1A. (**C**) Immunoblotting of Shc in phosphotyrosine immunoprecipitates. By cells were stimulated for 2 min and immunoprecipitated with anti-phosphotyrosine (4G10) and rabbit anti-mouse IgG bound to protein A–Sepharose beads. After they were washed, the tyrosine-phosphorylated proteins bound to the beads were eluted by incubation for 30 min on ice with 10 mM *p*-nitrophenylphosphate (pNPP) in the washing buffer. The eluted proteins from 8×10^6 cell equivalents were separated by SDS-PAGE and immunoblotted with anti-Shc. The arrows indicate the 48- and 52-kD isoforms of Shc. (**D**) Immunoprecipitation of Shc from 4G10 immunoprecipitates. The By cells (2×10^7) were stimulated and immunoprecipitated with 4G10 as above. Shc was immunoprecipitated from proteins eluted with pNPP, resolved by SDS-PAGE, and immunoblotted with 4G10.

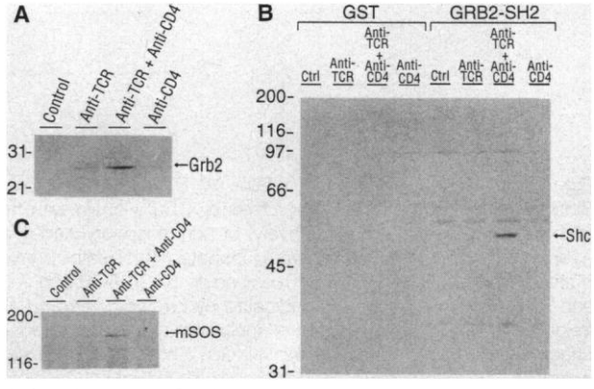

Fig. 2. Association of Shc with Grb2 and mSOS after T cell activation. (**A**) Immunoblotting for Grb2 in anti-Shc immunoprecipitates. By cells were stimulated for 10 min, immunoprecipitated with anti-Shc as in Fig. 1A, and immunoblotted with monoclonal anti-Grb2 (Transduction Laboratories). (**B**) Association of Shc with Grb2SH2. Lysates from activated and unactivated T cells (1×10^7) were incubated for 2 hours at 4°C with GST alone or GST-Grb2SH2 (2 to 4 μg) bound to glutathione agarose beads for 2 hours at 4°C. After they were washed, the proteins bound to the beads were resolved by SDS-PAGE (8%) and immunoblotted with monoclonal anti-Shc (Transduction Laboratories). Ctrl, control. (**C**) Immunoblotting of mSOS in anti-Shc immunoprecipitates. By cells were stimulated for 10 min, immunoprecipitated with anti-Shc, and immunoblotted with affinity-purified polyclonal anti-mSOS (AMRAD Corporation, Victoria, Australia). Data shown in (A) and (C) are from the same experiment.

phorylated (ζ-P) or unphosphorylated (ζ) (*16*). TAMs in ζ chain are tyrosine-phosphorylated on T cell activation. The sequences in this region contain the motif that is preferentially bound by the SH2 domain of Shc (*17*). As controls, tyrosine-phosphorylated or -unphosphorylated peptides corresponding to a similar motif present in the CD3 ε chain were also synthesized (ε-P and ε, respectively). The peptides were immobilized on streptavidin-agarose beads and incubated with lysates from unactivated T cells.

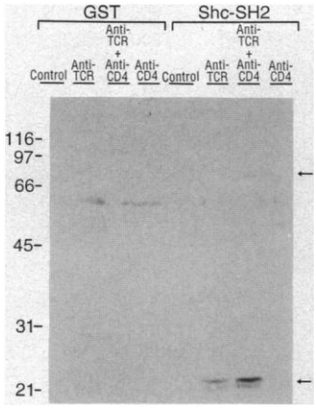

Fig. 3. Analysis of tyrosine-phosphorylated proteins bound to ShcSH2. The By cells were stimulated as described above and lysates (1×10^7 cell equivalents) were incubated for 2 hours at 4°C with GST alone or GST-ShcSH2 fusion proteins bound to glutathione agarose beads. The beads were washed extensively, and the bound proteins were resolved by SDS-PAGE (10%) and immunoblotted with anti-phosphotyrosine. The arrows indicate the tyrosine-phosphorylated proteins precipitated by the ShcSH2 domain.

The proteins bound to the beads were resolved by SDS–polyacrylamide gel electrophoresis (PAGE), and the presence of Shc was assessed by immunoblotting. Shc associated only with the ζ-P peptide, not with the nonphosphorylated ζ peptide or with the ε peptides (Fig. 4A). The Shc-ζ peptide interaction appeared to be specific, because the ζ-P did not precipitate other proteins containing SH2 domains such as Grb2, p120RasGAP, or SHPTP1 (*14*).

We next determined whether ζ-P could competitively inhibit the 21- to 23-kD proteins from binding to ShcSH2. Lysates from activated or unactivated T cells were incubated with the GST-ShcSH2. After they were washed, the beads were incubated with the phosphorylated and unphosphorylated ζ and ε peptides. The proteins that remained bound to the beads were resolved by SDS-PAGE and immunoblotted with anti-phosphotyrosine. Incubation with ζ-P specifically displaced the proteins bound to the ShcSH2 domain, whereas the other peptides had no effect (Fig. 4B).

Cross-linking of chimeric molecules containing the cytoplasmic domain of the ζ chain fused to the extracellular domains of either CD4, CD8, interleukin-2 receptor, or CD16 molecules can initiate tyrosine kinase activation resulting in tyrosine phosphorylation of the ζ chain (*18*, *19*). We therefore expressed a CD16-ζ chimeric molecule in the human Jurkat T cell line, and immunoblotting revealed that Shc associated with the cytoplasmic domain of ζ after cross-linking CD16 (Fig. 4C). When the CD16 immunoprecipitates were probed with anti-ζ, equal amounts of CD16-ζ were detected in Jurkat cells expressing CD16-ζ, whether or not the cells had been activated with antibody to CD16 (anti-CD16) (*14*). Thus, Shc appears to interact directly with the phosphorylated cytoplasmic domain of the ζ chain.

These data suggest that upon T cell activation Shc binds to the phosphorylated ζ chain through its SH2 domain. Shc may then be tyrosine-phosphorylated by any one of the Src family kinases that participates in T cell activation. The enhanced phosphorylation of Shc seen after TCR×CD4 cross-linking and the low but detectable phosphorylation of Shc by CD4 cross-linking alone suggest that Lck may be involved in Shc phosphorylation. Phosphorylation of Shc might help to shuttle Grb2 and mSOS to the membrane. So far, we have not been able to detect the direct interaction of Grb2 with the TCR using Grb2SH2, ζ and ε phosphopeptides, or CD16-ζ (*14*). Thus, Shc, by interacting with the phosphorylated ζ chain on the one hand and with Grb2 and mSOS on the other, may couple T cell activation to the Ras signaling pathway. The proto-oncogene *vav* can also act as a guanine nucleotide exchange factor for Ras after T cell activation (*20*). It remains to be determined if more than one pathway operates in T cells to activate Ras or whether alternate means of Ras activation may occur under different conditions of T cell stimulation.

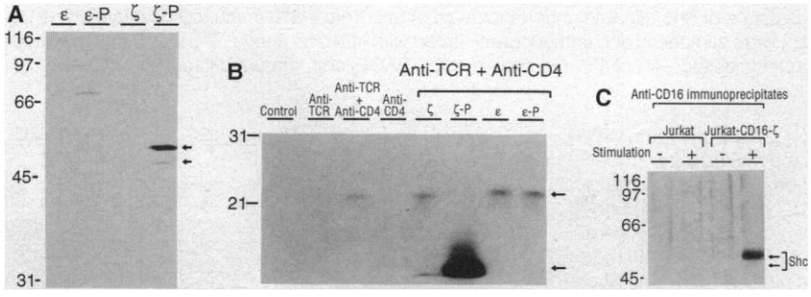

Fig. 4. Interaction of Shc with ζ chain. (**A**) Binding of Shc to tyrosine-phosphorylated ζ peptide. Biotinylated peptides from TCR ζ chain or CD3 ε chain with the tyrosines either phosphorylated (denoted as ζ-P and ε-P, respectively) or nonphosphorylated (ζ and ε) were bound to streptavidin-agarose beads (*23*). The beads were incubated with lysates from unactivated T cells. After the bound proteins were washed, they were resolved by SDS-PAGE and immunoblotted with anti-Shc. The 48- and 52-kD isoforms of Shc are indicated by arrows. The band at 70 kD was seen in all lanes after longer exposure. (**B**) Competitive inhibition by ζ phosphopeptide. Lysates from activated or unactivated T cells were incubated with GST-ShcSH2 fusion proteins as described in Fig. 3. After the beads were washed, they were incubated with 500 μM of each of the four peptides for 90 min on ice. After they were washed, the proteins still bound to the beads were analyzed by immunoblotting with anti-phosphotyrosine. The upper arrow indicates the 23-kD band. The ζ phosphopeptide that was bound to the ShcSH2 beads ran as a diffuse band at the bottom of the gel (lower arrow). (**C**) Association of Shc with CD16-ζ chimeric molecule after activation. Control Jurkat cells or Jurkat cells expressing CD16-ζ were stimulated with anti-CD16 for 2 min (*24*). After cell lysis, the proteins were immunoprecipitated with anti-CD16, resolved by SDS-PAGE, and immunoblotted with anti-Shc.

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21. Abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
22. By cells (3×10^7/ml) were incubated with or without anti-CD3 (2C11, 1 μg/ml) or anti-CD4 (Leu3a, 1 μg/ml), or both, for 10 min on ice. Rabbit antibody to mouse immunoglobulin G (anti-mouse IgG) (10 μg/ml) was added for cross-linking, and the cells were incubated for a further 10 min on ice and then incubated at 37°C for 2 min. The cells were pelleted by a pulse spin, washed once with phosphate-buffered saline, and lysed [lysis buffer contained 1% NP-40, 50 mM tris (pH 7.6), 150 mM NaCl, 1 mM Na_3VO_4, 10 mM NaF, leupeptin and aprotinin (10 μg/ml of each), and 2 mM phenylmethylsulfonyl fluoride]. After the nuclei were sedimented, the lysates were immunoprecipitated with anti-Shc (2 μg per 10^7 cell equivalents, Transduction Laboratories, KY) and 40 μl of 50% protein A agarose solution for 2 hours at 4°C. The beads were washed four times [0.1% NP-40, 20 mM Hepes (pH 7.4), 150 mM NaCl, 1 mM Na_3VO_4, 5 mM NaF, and leupeptin and aprotinin (10 μg/ml of each)], analyzed by 6 to 12% SDS-PAGE, transferred to nitrocellulose and blotted with the anti-phosphotyrosine 4G10 (Upstate Biotechnology Inc., Lake Placid, NY), and developed by enhanced chemiluminescence (ECL) (Amersham). Similar results have been obtained with the antibody to TCR, F23.1
23. Biotinylated peptides (10 μg) from ζ chain (amino acid sequence GKGHDGLYQGLSTATKDTYDALH) (*21*) or CD3 ε chain (NPDYEPIRKGQRDLYSG) were synthesized as described (*16*) with the tyrosines either phosphorylated (denoted as ζ-P and ε-P, respectively) or nonphosphorylated (ζ and ε) and were incubated with 50 μl of streptavidin-agarose beads (Oncogene Sciences) for 1 hour on ice. The beads were incubated with 4% bovine serum albumin for 15 min, washed extensively, and incubated with lysates (1×10^7 cell equivalents) from unactivated T cells for 2 hours at 4°C. After the beads were washed, the bound peptides were resolved by SDS-PAGE and immunoblotted with anti-Shc.
24. Jurkat T cells were infected with vaccinia virus containing complementary DNA (cDNA) encoding CD16-ζ chimeric molecule (CD16 extracellular, CD7 transmembrane, and ζ cytoplasmic tail) for 6 hours at 37°C (*18*). Fluorescence-activated cell sorter (FACS) analysis showed that 50% of the cells had surface expression of CD16-ζ at 6 hours. Control Jurkat cells or CD16-ζ–expressing Jurkat cells (1.3×10^7 per sample) were stimulated as in Fig. 1A with or without anti-CD16 for 2 min at 37°C. Cells were lysed and the proteins were immunoprecipitated with anti-CD16 and protein G–agarose (Oncogene Sciences). The proteins were resolved by SDS-PAGE (10%) and immunoblotted with anti-Shc.
25. Supported by NIH grant AI-17258 (S.J.B.) and a grant from F. Hoffmann–La Roche Inc. We thank T. Pawson for the constructs encoding the GST-Grb2SH2 and GST-ShcSH2, B. Seed for CD16-ζ chain vaccinia vector, and T. Vorherr for ζ and ε peptides. K.S.R. thanks U. Lorenz for helpful discussions and dedicates this work to his father.

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Identification of an Alternative CTLA-4 Ligand Costimulatory for T Cell Activation

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Stimulation of T cell proliferation generally requires two signals: The first signal is provided by the T cell receptor binding to antigen, and the second signal or costimulus is provided by a different receptor-ligand interaction. In mouse and human, the CD28-B7 interaction has been identified as a source of costimulatory signals. We have identified a cell surface molecule (GL1) that is distinct from B7 and abundantly expressed on activated B cells. On activated B cells GL1, rather than B7, is the predominant ligand for the T cell–activation molecule CTLA-4. GL1 provides a critical signal for T cell–dependent responses in vitro and in vivo.

The CD28-B7 interaction is regarded as a critical costimulus for T cell activation (*1–3*). Consistent with this model, a soluble fusion protein of CTLA-4 (CTLA4Ig), a T cell surface molecule with a high affinity for B7 (*4*), inhibits T cell–dependent responses in vivo and in vitro (*5–9*). To identify additional cell surface molecules that provide costimulatory signals to T cells, we screened monoclonal antibodies (mAbs) from rats immunized with activated mouse B cells for the ability to inhibit T cell activation and to identify ligands for CTLA-4.

The GL1 mAb identified a determinant expressed minimally on unstimulated B cells but at high density on B cells activated by lipopolysaccharide (LPS) (Fig. 1), interleukin-5 (IL-5) (*10*), or antibody to immunoglobulin D (IgD) (anti-IgD) (*10*). The B7 mAb (*11*) minimally stained activated B cells but stained B7-transfected Chinese hamster ovary (CHO) cells brightly, whereas CTLA4Ig bound strongly to both activated B cells and B7-transfected CHO cells. GL1 mAb did not react with B7-transfected cells but reacted with activated B cells from B7-deficient mice (*12*), demonstrating that the GL1 product is not encoded by the B7 gene. The GL1 mAb also brightly stained dendritic cells (*13*) but only minimally stained activated T cells (*10*).

The cell surface molecule identified by GL1 mAb was immunoprecipitated from surface-iodinated, LPS-activated B cells and analyzed by SDS–polyacrylamide gel electrophoresis (PAGE). A broad band, 65 to 100 kD, was precipitated under both reducing (Fig. 2) and nonreducing condi-

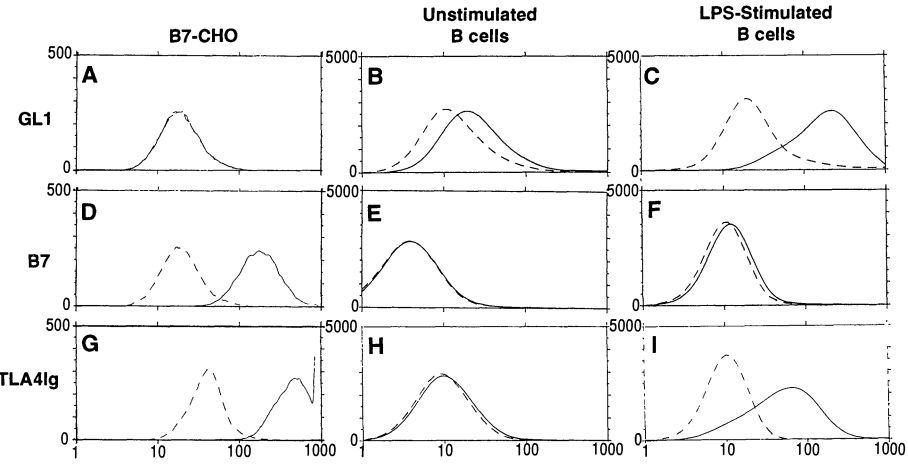

Fig. 1. Reactivity of GL1 mAb and B7 mAb with activated B cells. B7-transfected CHO cells (*14*) (**A, D**, and **G**) or T cell–depleted spleen cells that were either unstimulated (**B, E**, and **H**) or LPS-stimulated (**C, F**, and **I**) were stained with the GL1 mAb (A through C), hamster B7 mAb (D through F), (*11*), or CTLA4Ig (G through I) (*4*). The rat IgG2a hybridoma GL1 was produced by immunization with LPS-activated murine B cells, fusion, and selection as described (*15*). The DBA/2 spleen cells were T cell–depleted and cultured for 60 hours in medium alone or with LPS (15 μg/ml). Cells were stained with GL1 mAb (solid line) or control rat IgG2a (dashed line) (A through C), B7 mAb (solid line) or normal hamster Ig (dashed line) (D through F), or human CTLA4Ig (solid line) or CD7Ig (dashed line) (G through I). Cells were counterstained with antibody to B220. GL1 mAb reactivity with B cells was analyzed by electronic gating on $B220^+$ cells (*16*).