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25. The full length and truncated Ikaros proteins were expressed in the *E. coli* BL21 strain from the pet3a vector. One microliter of a 1:4 and of a 1:40 dilution of bacterial lysate (approximately 4 and 0.4 μg of total protein) that expressed Ikaros and Ikaros ZF-D2 were used respectively in a gel retardation assay with 100,000 and 10,000 cpm of kinased double-stranded oligonucleotide encoding the δA element as described previously (*10*). A 1:4 dilution of bacterial lysate programmed with wild-type pet3a vector was used as a control. A 50- and 200-fold molar excess of unlabeled double stranded oligonucleotides encoding δA wild-type and mutant variants were used as competitors.
26. Embryos were harvested from timed, pregnant CD1 mice (Charles River) and were fixed in 4% paraformaldehyde for 2 hours to 2 days depending on size. A series of dehydration steps was performed in alcohols followed by xylenes before paraplast embedding (*27*). Sections were prepared and treated according to published protocols (*27*). Sense and antisense ^{32}P-UTP (uridine 5′-diphosphate)–labeled RNA probes 320 bp in size were made from the 3′ of the Ikaros cDNA that contains 100 bp of coding and 220 bp of untranslated sequence (1230 to 1550 bp of Ikaros cDNA) and were used to hybridize to selected slides at 48°C overnight. After high-stringency washing, slides, were dehydrated, dipped in diluted photographic emulsion (NBT2), and exposed for 3 weeks. Dipped slides were developed, stained with Giemsa, and analysed by bright- and dark-field illumination on an Olympus dissecting microscope.
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29. We thank D. Dietch and R. Bruns for advice and help with in situ studies; D. Galson, J. Hensold, and M. Gurish for MEL, RBL, and J774 RNA, respectively; S. Grabee for preparing T-depleted splenic B cells; and B. Morgan and P. Goetinck for critical discussions and comments. This work was supported by a Shisheido grant to the Cutaneous Biology Research Center and from the Leukemia Society of America (to K.G.).
30. The Ikaros cDNA sequence has been submitted to GenBank. The accession number is L03547.

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A GDP Dissociation Inhibitor That Serves as a GTPase Inhibitor for the Ras-Like Protein CDC42Hs

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Members of the family of Ras-related guanosine triphosphate (GTP) binding proteins appear to take part in the regulation of a number of biological processes, including cell growth and differentiation. Three different classes of proteins that regulate the GTP binding and GTP hydrolytic activities of the Ras family members have been identified. These different regulatory proteins inhibit guanosine diphosphate (GDP) dissociation (designated as GDIs), stimulate GDP dissociation and GDP-GTP exchange (designated as GDSs), or stimulate GTP hydrolysis (designated as GAPs). In the case of the Ras-like protein CDC42Hs, which is the human homolog of a *Saccharomyces cerevisiae* cell division cycle protein, the GDI protein also inhibited both the intrinsic and GAP-stimulated hydrolysis of GTP. These findings establish an additional role for the GDI protein—namely, as a guanosine triphosphatase (GTPase) inhibitory protein for a Ras-like GTP binding protein.

The GTP binding and GTP hydrolytic (GTPase) activities of the Ras-like GTP binding proteins are essential for their normal regulation and function. The exchange of bound GDP for GTP is influenced by GDS proteins, which stimulate GDP dissociation, and by GDI proteins, which inhibit GDP dissociation. GDIs also influence the cellular localization of the Ras-like proteins by stimulating the release of GTP binding proteins from membranes (*1–3*). Both GDS and GDI activities have been identified for the Ras-like protein CDC42Hs (*3, 4*). The *dbl* oncogene product (*5, 6*) serves as a CDC42Hs-GDS (*4*), whereas a 28-kD protein, purified from bovine brain, serves as the GDI protein (*3*). The latter protein appears to be structurally very similar, if not identical, to a protein that attenuates the dissociation of GDP from the Rho (*2, 7*) and Rac (*8*) GTP binding proteins.

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The regulatory effects of GDI on the Rho protein may result from a specific interaction between the GDI protein and the GDP-bound form of Rho (*2*). However, the GDI protein elicits a weak but measurable (~10%) inhibition of the dissociation of guanosine-5′-O-(3-thiotriphosphate) (GTP-γ-S) from CDC42Hs (*3*) and causes the release of the GTP-γ-S–bound CDC42Hs (as well as the GDP-bound CDC42Hs) from membranes (*3*). This raised the question of whether the GDI protein might alter functional properties of the GTP-bound CDC42Hs species. The results presented below demonstrate an additional regulatory activity for the GDI protein—specifically, the inhibition of the GTP hydrolytic activity of CDC42Hs.

Figure 1A shows that in the presence of GDI (~1 μg), the rate of the intrinsic GTPase activity of the platelet CDC42Hs (~0.2 μg) was significantly inhibited. After 12 min at room temperature, less than 40% of the bound [γ-^{32}P]GTP was hydrolyzed by CDC42Hs in the presence of the GDI protein, whereas greater than 70% of the GTP was hydrolyzed by CDC42Hs in the absence of the GDI protein. The GDI protein also inhibited the rate of the GTPase activity of CDC42Hs that had been stimulated by the human platelet CDC42Hs–GTPase activating protein (GAP) (*9*). When an identical set of experiments was performed with [α-^{32}P]GTP, there was no difference in the amount of labeled GTP that remained associated with the platelet CDC42Hs in the presence or absence of the GDI protein or the platelet CDC42Hs-GAP (Table 1). These results indicated that the effects of the GDI and CDC42Hs-GAP proteins on the association of labeled GTP were in fact specific for the hydrolysis of GTP [and the release of inorganic phosphate ($^{32}P_i$)] and did not reflect changes in the rate of dissociation of GTP. We find a similar inhibition of the GTPase activity of CDC42Hs by the GDI protein (*10*) when the CDC42Hs was denatured with 10% trichloroacetic acid before quantitation of the release of $^{32}P_i$ by extraction with ammonium molybdate and isobutanol plus benzene (*11*). This indicates that the GDI protein inhibits the GTP hydrolytic event and not the release of $^{32}P_i$ from CDC42Hs.

The inhibition of the GTPase activity by the GDI protein was assayed in parallel with the inhibition of [^{3}H]GDP dissociation throughout the purification of the GDI protein from bovine brain cytosol. At all stages of the purification, the inhibition of the intrinsic GTPase activity of CDC42Hs

directly overlapped with the inhibition of [^{3}H]GDP dissociation. This purification yielded a single protein with an apparent molecular size of 28 kD (Fig. 1A, inset).

We have expressed the GDI protein as a glutathione-S-transferase (GST) fusion protein in *Escherichia coli* (*3*), and we find that the recombinant GST-GDI protein acts as a GTPase inhibitor in a manner identical to the purified brain GDI (*10*). Essentially identical results also were obtained when the CDC42Hs expressed in *Spodoptera frugiperda* cells was substituted for the CDC42Hs protein from human platelets (*10*). However, this was not the case when the *E. coli*–expressed CDC42Hs was used. Neither the intrinsic GTPase activity of the *E. coli*–expressed CDC42Hs nor the CDC42Hs-GAP–stimulated GTPase activity was inhibited by the GDI protein (Fig. 1B). The inability of the GDI protein to influence the GTPase activities of the *E. coli*–expressed CDC42Hs is consistent with earlier findings that demonstrated that the GDI protein was ineffective in the attenuation of GDP dissociation from the *E. coli*–CDC42Hs protein (*3*). These results suggest that a posttranslational modification (such as geranylgeranylation) of CDC42Hs, which cannot occur in *E. coli*, may be essential for this GTP binding protein to bind the GDI protein. Further evidence that the same binding interaction between the GDI and the CDC42Hs proteins was responsible for the inhibition of GDP dissociation and GTP hydrolysis was obtained from direct comparisons of the dose-response profiles for these GDI-mediated effects on the human platelet CDC42Hs protein (Fig. 2).

The effects of the GDI protein on the GTPase activity of CDC42Hs were examined in the presence of varying amounts of the platelet CDC42Hs-GAP. The platelet GAP (0.25 to 1.25 ng) stimulated GTP hydrolysis in a dose-dependent manner (Fig. 3A). When the same amounts of the platelet GAP were assayed in the presence of GDI (0.2 μg and 0.5 μg), the extents of the stimulation of GTP hydrolysis by CDC42Hs-GAP were significantly reduced. However, by further increasing the amount of the platelet CDC42Hs-GAP (from 2.5 to 12.5 ng), we overcame the GDI-mediated inhibition of GTP hydrolysis (Fig. 3B). These results suggest that the platelet CDC42Hs-GAP and the GDI protein may compete for a common binding domain on CDC42Hs.

The platelet CDC42Hs-GAP has biochemical properties (*9*) that are similar to those of the spleen Rho-GAP (*12*) and appears to be a member of a subgroup of GAPs (*13*, *14*) that includes the Bcr protein, a cytoskeleton-associated protein of 160 kD [which as an outcome of chromosomal translocation is fused to the Abl tyrosine kinase (*15*)], chimerin, an ~34-kD

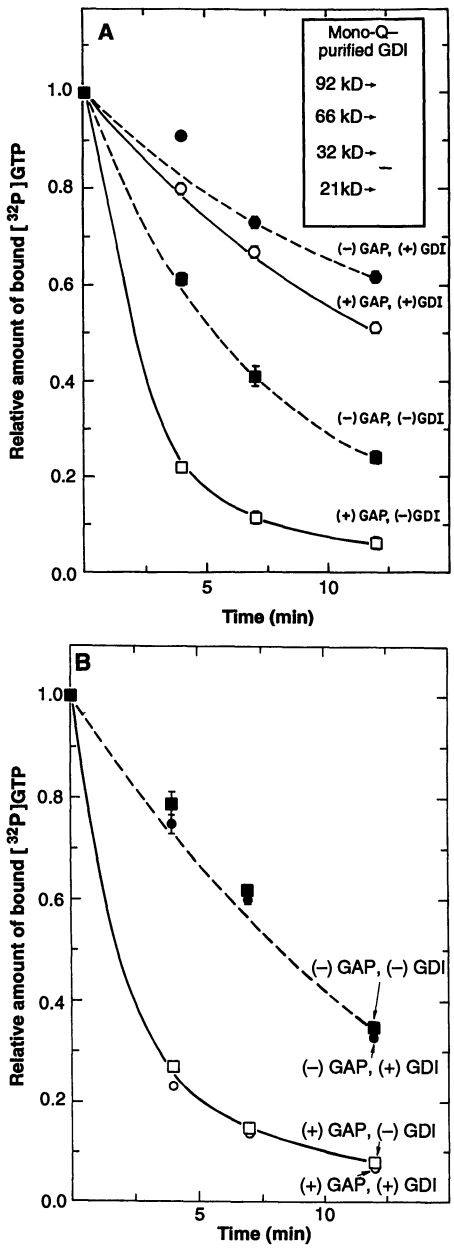

Fig. 1. Time courses for the intrinsic and GAP-stimulated GTP hydrolytic activity for the CDC42Hs protein in the presence and absence of the GDI protein. (**A**) Measurements of the GTP hydrolytic activity of the human platelet CDC42Hs. The CDC42Hs protein and the CDC42Hs-GAP were purified from human platelets as described (*9*). The GDI protein was isolated from bovine brain cytosol (*3*), and its GTPase inhibitory activity was used as an assay for purification (*29*). The platelet CDC42Hs (6.5 μg) was incubated with 6 μM [γ-^{32}P]GTP (41.6 Ci/mmol) in a final volume of 115 μl of a solution that also contained 15 mM tris-HCl (pH 7.5), 0.3 mM 5′-adenylylimidodiphosphate (AMP-PNP), 5 mM $MgCl_2$, 0.75 mM DTT, 0.75 mM EDTA, 75 mM potassium phosphate, and 0.35% CHAPS for 5 min at room temperature. Portions (4 μl) from this binding incubation were then assayed for GTP hydrolytic activity for the indicated periods of time in the presence (open squares) and absence (closed squares) of the CDC42Hs-GAP protein (~1 ng) or in the presence of the GDI protein (1 μg) alone (closed circles) or with the CDC42Hs-GAP (open circles). All assay solutions that lacked the CDC42Hs-GAP contained an equivalent amount of the GAP storage buffer [20 mM tris HCl (pH 7.5), 1 mM EDTA, 1 mM DTT, and 0.1% CHAPS], and all assays that lacked the GDI protein contained the GDI storage buffer [20 mM Na-MES (pH 6.1), 1 mM EDTA, 1 mM DTT, 100 mM NaCl, and 0.1% CHAPS]. The GTP hydrolytic activity is represented as the loss of bound radioactivity ($^{32}P_i$ released as a consequence of GTP hydrolysis) from CDC42Hs. (**Inset**) SDS–polyacrylamide gel (10%) electrophoresis of the purified GDI (1.5 μg) used in these experiments. Molecular size standards are shown on the left. (**B**) Measurements of the GTP hydrolytic activity of CDC42Hs expressed in *E. coli*. The CDC42Hs protein was expressed from the pET-3A expression vector under the control of the T7 promoter and then purified (*9*). The CDC42Hs was incubated with [γ-^{32}P]GTP, as described in (A), and portions from this binding incubation were assayed for GTP hydrolytic activity in the presence (open squares) and absence (closed squares) of the CDC42Hs-GAP (~1 ng), in the presence of GDI protein (1 μg) alone (closed circles), or in the presence of both GDI and CDC42Hs-GAP (open circles). In both (A) and (B), the data points represent the average of duplicates (the range of the determinations is indicated by error bars; the lack of error bars indicates that the duplicates fell within the range covered by the symbols).

Table 1. Comparisons of the effects of GDI on the [γ-^{32}P]GTP-bound and [α-^{32}P]GTP-bound CDC42Hs. The platelet CDC42Hs and the platelet CDC42Hs-GAP were purified as outlined in Hart *et al.* (*9*). The brain GDI was purified as described (Fig. 1A). The platelet CDC42Hs (6.5 μg) was preloaded with [γ-^{32}P]GTP or [α-^{32}P]GTP (Fig. 1A), and then the amount of the radiolabeled GTP that remained bound to the CDC42Hs, after a 5-min incubation at room temperature in the absence or in the presence of the CDC42Hs-GAP or GDI, was determined (*9*). The relative amount of [γ-^{32}P]GTP or [α-^{32}P]GTP still bound to the platelet CDC42Hs in the absence of the GAP or GDI proteins was set at 1.00. All data represent the average determinations from two assays (the ranges are indicated), and all values are expressed relative to the average amount of radiolabeled GTP bound to CDC42Hs alone.

Sample	Bound [γ-^{32}P]GTP	Bound [α-^{32}P]GTP
CDC42Hs	1.00 ± 0.001	1.00 ± 0.020
CDC42Hs + GDI	1.63 ± 0.040	1.07 ± 0.035
CDC42Hs + GAP	0.26 ± 0.070	1.03 ± 0.005
CDC42Hs + GDI + GAP	1.32 ± 0.010	1.03 ± 0.005

protein from human brain (*16*), and the Ras-GAP binding protein p190 (*17*). The *E. coli*–expressed, COOH-terminal portion of the Bcr protein, which includes a region of similarity to the Rho-GAP, functions as a GAP for the Rac1 GTP binding protein (*13*). We found that the full-length Bcr protein, when overexpressed in *S. frugiperda* cells, acted as a GAP for CDC42Hs (Table 2). Therefore, we determined whether the GDI protein could influence the Bcr-stimulated GTPase activity of CDC42Hs. As with the platelet CDC42Hs-GAP, the Bcr-stimulated GTPase activity of CDC42Hs was inhibited by the GDI protein (Table 2).

The GTPase activity of Ras and other members of the Ras family is an essential regulatory event, as evidenced by the findings that GTPase-defective forms of Ras are tumorigenic (*18*). Although a number of GAPs have been identified and characterized for Ras and other members of this family (*14*), much less is known regarding regulatory proteins that prolong the GTP-bound state of the Ras family members by the inhibition of GTP hydrolysis. A Ras-GTPase inhibitory activity has been characterized in extracts from mouse brain (*19*), and a protein kinase C–sensitive Ras-GAP inhibitory factor has been postulated (*20*); however, these regulatory factors have yet to be purified. The demonstration that a GDI protein also serves as a GTPase inhibitor or a GIP for CDC42Hs has implications for a number of biological processes. It now seems likely that one or more of the Rho subgroup GTP binding proteins are involved in some aspect of cell growth, because the *dbl* oncogene product serves as a GDS for CDC42Hs and a second oncogene product (that is, Vav) (*21*) contains a *dbl*-like domain (*22*). In addition, different members of the Rho-GAP family (such as Bcr and p190) are suspected to be involved in cell growth pathways, and recently the Rho GTP binding protein has been implicated in growth factor–stimulated formation of actin stress fibers (*23*), whereas the Rac1 protein has been implicated in growth factor–stimulated membrane ruffling (*24*). It seems possible that GDI-GIP serves as a shuttle molecule that directs the movement of CDC42Hs or a related Rho subgroup protein between a site at a membrane or cytoskeletal location (where guanine nucleotide exchange can occur) and a GAP target site (where GTP hydrolysis occurs). The multiple regulatory activities of GDI-

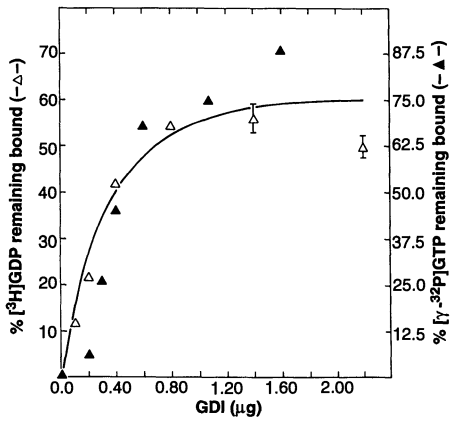

Fig. 2. Dose-response profiles for the effects of the GDI protein on the dissociation of [^{3}H]GDP and the hydrolysis of [γ-^{32}P]GTP. Purified CDC42Hs from platelets (3.4 μg) was loaded with 8.0 μM [^{3}H]GDP (12.4 Ci/mmol) or with 6 μM [γ-^{32}P]GTP (41.6 Ci/mmol) in a total volume of 65 μl of a solution that contained 14 mM tris-HCl (pH 7.5), 0.3 mM AMP-PNP, 5 mM $MgCl_2$, 0.70 mM DTT, 0.70 mM EDTA, 70 mM potassium phosphate, and 0.35% CHAPS for 10 min at room temperature. Portions (4 μl) from these binding incubations were then assayed for [^{3}H]GDP binding in assay solutions (22-μl total volume) that lacked $MgCl_2$ and contained the indicated amounts of brain GDI (open triangles) or were assayed for [γ-^{32}P]GTP hydrolytic activity (closed triangles) as described (Fig. 1A). The effect of GDI is plotted as the percentage of [^{3}H]GDP that remained bound to CDC42Hs or the percentage of [γ-^{32}P]GTP that remained bound (as an outcome of the inhibited release of $^{32}P_i$) as a function of the amount of GDI protein added to the mixture. All data points represent the average of duplicates with the range of the determinations indicated by the error bars. The lack of error bars indicates that the duplicates fell within the range covered by the symbols.

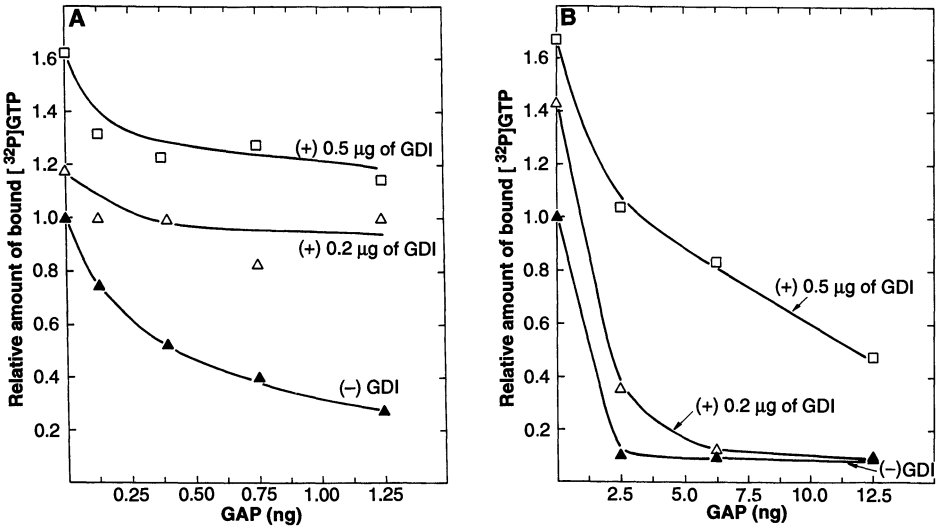

Fig. 3. Dose-response profiles for the GAP-stimulated GTP hydrolytic activity in the presence and absence of the GDI protein. (**A**) The GAP-stimulated GTPase activities of the platelet CDC42Hs were measured (after 7 min) over a range of 0.13 to 1.25 ng of the CDC42Hs-GAP from platelets. CDC42Hs was incubated with [γ-^{32}P]GTP, and the GTP hydrolytic activities were assayed as described (Fig. 1A). Closed triangles, GAP-stimulated activities measured in the absence of purified brain GDI; open triangles, GTPase activities assayed in the presence of purified GDI (0.2 μg); open squares, activities measured in the presence of purified GDI (0.5 μg). (**B**) The GAP-stimulated GTPase activities of the platelet CDC42Hs measured over a range of 2.5 to 12.5 ng of the platelet CDC42Hs-GAP. Symbols are as in (A).

Table 2. Effects of the GDI protein on the Bcr-stimulated GTPase activity of CDC42Hs. The platelet CDC42Hs was purified from human platelets (*9*), and GDI was purified from bovine brain (Fig. 1A). The platelet CDC42Hs (4 μg) was incubated with [γ-^{32}P]GTP, and then the amount of labeled GTP that remained bound to CDC42Hs after the indicated times (at room temperature) was measured in the absence or in the presence of *S. frugiperda* (Sf9) cell lysates that expressed the cDNA for full-length *bcr* or in the absence or presence of GDI. The expression of the Bcr protein in Sf9 cells and the preparation of cell lysates were done as described (*28*). We assayed GTPase activity by adding an aliquot (4 μl) from the initial [γ-^{32}P]GTP-CDC42Hs binding incubation to an assay solution (21 μl) that included 1.5 μl of the insect cell lysates that expressed Bcr (that is, ~45 ng of Bcr protein estimated by comparison of protein staining of insect cell lysates expressing Bcr with that of known amounts of protein standards). The indicated ranges were obtained from two GTPase assays. All data are expressed relative to the amount of [γ-^{32}P]GTP bound to the platelet CDC42Hs before it was added to the GTPase assay (that is, at time = 0 min).

Sample	Time (min)	Relative amount of bound [γ-^{32}P]GTP
CDC42Hs	0	1.0
CDC42Hs	4	0.49 ± 0.015
CDC42Hs + Bcr	4	0.08 ± 0.003
CDC42Hs + Bcr + GDI	4	0.74 ± 0.011
CDC42Hs	12	0.21 ± 0.005
CDC42Hs + Bcr	12	0.07 ± 0.016
CDC42Hs + Bcr + GDI	12	0.41 ± 0.003

GIP would be well suited for such a function. For example, the ability of GDI-GIP to stimulate the release of CDC42Hs from membranes could serve to initiate the cycling of this GTP binding protein between different cellular compartments, and its ability to inhibit GDP dissociation as well as GTP hydrolysis would insure that CDC42Hs remains in the GDP- or GTP-bound state while in transit to these locations. Recently, the GDI protein has been shown (*25*) to be part of a cytosolic complex with the Rac GTP binding protein involved in superoxide production in neutrophils (*25–27*). This raises the question of whether the GDI protein functions both in the assembly of this complex and in the maintenance of the activation of the reduced nicotinamide adenine dinucleotide oxidase by preserving the GTP-bound state of Rac.

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29. GTPase inhibitory protein (GIP) activity was first detected in DEAE fractions enriched in GDI. Therefore, the first two purification steps were performed to obtain partially purified GDI. These fractions were obtained by homogenization of bovine cerebral tissue (70 g) in 250 ml of TED [25 mM tris-HCl (pH 8.0), 1 mM EDTA, and 1 mM dithiothreitol (DTT)] containing 0.25 mM phenylmethylsulfonyl fluoride (PMSF), leupeptin (5 μg/ml), aprotinin (5 μg/ml), and pepstatin A (2 μg/ml). The membranes were removed by centrifugation at 30,000*g* for 30 min. Ammonium sulfate was added to the supernatant to 40% saturation, stirred for 30 min, and centrifuged at 11,300*g*. The supernatant was removed, ammonium sulfate was added to 80% saturation, and the mixture was centrifuged again at 11,300*g*. The pellet, which represented the 40 to 80% ammonium sulfate fraction, was dissolved in 20 ml of TED containing 0.25 mM PMSF, leupeptin (5 μg/ml), and aprotinin (5 μg/ml). CDC42Hs-GDI activity, as assayed by the inhibition of the dissociation of labeled GDP (Fig. 2) (*3*), was present in the 40 to 80% ammonium sulfate fraction. The redissolved pellet was then dialyzed against 1 liter of TED (two changes) for 20 hours. The dialyzed sample was then applied to a DEAE Sephacel (Sigma) column (2.5 cm × 15 cm) equilibrated in TED buffer. The protein was eluted with a 400-ml gradient of 0 to 300 mM NaCl. The peak in GDI activity eluted at approximately 175 mM NaCl. GIP activity was also detected in these peak fractions. The peak in GDI activity was pooled (~60 ml) and concentrated to 10 ml. Half of the concentrate was dialyzed in a solution that contained 350 ml of bis[2-hydroxyethyl]iminotris[hydroxymethyl]methane, 2-bis[2-hydroxyethyl]-amino-2-[hydroxymethy]-1,3 propanediol (Bis-Tris) (pH 7.0), 1 mM EDTA, and 1 mM DTT (BIS-TED) supplemented with 0.1% CHAPS and 5% glycerol. This dialysate was applied to a Pharmacia fast protein liquid chromatography (FPLC)–Mono Q column (HR 5/5) equilibrated in BIS-TED containing 0.1% CHAPS. The protein was eluted with a 30-ml gradient of 0 to 500 mM NaCl. GDI and GIP activity co-eluted at approximately 175 mM NaCl. The peak in GDI-GIP activity was pooled and dialyzed against MED [20 mM Na-2-(*N*-Morpholino)ethanesulfonic acid (Na-MES) (pH 6.1), 1 mM EDTA, and 1 mM DTT] containing 0.1% CHAPS and 5% glycerol and injected onto a Pharmacia FPLC–Mono S (HR 5/5) column equilibrated in MED and 0.1% CHAPS. The protein was eluted with a 25-ml gradient of 0 to 500 mM NaCl. The peak in GDI activity and GIP activity co-eluted at 80 mM NaCl.

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Selecting T Cell Receptors with High Affinity for Self-MHC by Decreasing the Contribution of CD8

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Selective events during T cell repertoire development in the thymus include both the positive selection of cells whose receptors recognize self–major histocompatibility complex (MHC) molecules and negative selection (tolerance) of cells whose interaction with self-MHC is of high affinity. The affinity of T cell interactions with class I MHC molecules includes contributions by both the T cell receptor and the CD8 coreceptor. Therefore, by decreasing the affinity of the interaction with CD8, T cells whose receptors have relatively high affinities for self-MHC may survive negative selection. Such T cells were generated and those T cells reactive with self-MHC plus antigen also displayed low affinity for self.

The affinity of a T cell for class I MHC is attributable to binding by both the clonotypic portion of the T cell receptor (TCR), which binds allele-specific portions of the highly polymorphic α1,α2 domains of class I and its associated peptide ligand, and the CD8 coreceptor that specifically binds a nonpolymorphic region within the α3 domain of the same class I molecule (*1–3*). During maturation in the thymus it is this combined affinity that determines the fate of the developing T cell. In order for a thymocyte to develop into a functional $CD8^+$ T cell, its clonotypic receptor must recognize a self–class I MHC molecule expressed on the thymic epithelium (positive selection) (*4*). Presumably, this maximizes the probability that T cells will recognize foreign antigens presented by self–MHC molecules. However, all T cells that are potentially autoreactive by the criteria that they have sufficiently high affinity for the selecting MHC molecule to permit stimulation in the absence of foreign antigen must be eliminated before maturation (*5*). This paradox has been reconciled by proposing that thymic selection permits maturation of cells with low affinity, yet eliminates cells with no affinity or high affinity for self-MHC. Although numerous experiments using TCR transgenic lines (*4, 5*) and CD8 transgenic lines (*6*) have shown the validity of a number of predictions based on such an affinity model of thymic selection, by definition the affinity of the TCR for the syngeneic restriction molecule must be below the threshold necessary for detection of a response and, therefore, direct evidence for such affinity has been difficult to obtain (*7*).

Now we describe a situation in which a human class I molecule (HLA-A2) for which the affinity of interaction with murine CD8 is suboptimal (*8–11*), has been used to study the effect of decreased CD8 interaction on the resultant A2-restricted T cell repertoire. This is contrasted with the A2-restricted repertoire that develops in response to positive selection by the chimeric $A2/K^b$ molecule that contains a mu-

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