Z5-10:OAc–specific cell type projects to MGC subunit A situated in the medial part of the MGC. In this sensillum type the second cell, responsive to Z5-10:OH, terminates in MGC subunit B, close to the entrance of the antennal nerve into the antennal lobe. The cell responsive to Z7-12:OAc projects to MGC subunit C, situated in the middle of the MGC. In the same sensillum, a second cell of unknown specificity projects to subunit B. In approximately 3000 contacts made with olfactory sensilla during this investigation only one Z9-14:OAc–specific cell was encountered. In this sensillum two cells were filled. One terminates in subunit D, ventral and posterior to the other subunits. As in the other two physiological sensillum types, the second cell projects to subunit B. No correlation was observed between the topographical situation of sensilla on the antenna and their projection into the MGC subunits.

Filling of the second, unstimulated neuron in a sensillum may depend on neural activity induced after prolonged stimulation of the other cell in the sensillum. For example, in the Z5-10:OAc sensillum, buildup of the hydrolysis product of Z5-10:OAc, namely Z5-10:OH, may serve as the stimulus for the nonstimulated cell.

Selective filling of neurons present in the same sensillum has been used to investigate central nervous projections of taste receptors present on the labellum of *Drosophila*. By using horseradish peroxidase cocktails laced with the stimulus for one of the receptor cells present in the sensillum, different cell types were filled (*14*). The response characteristics of the sensilla investigated were, however, not known.

Functional partitioning in the primary olfactory center has been analyzed in rats (*3–5*), crayfish (*15*), cockroaches (*16, 17*), fruit flies (*2, 6*), and moths (*18, 19*). In *Drosophila*, studies with 2-deoxyglucose have until now presented the strongest evidence for a functional partitioning of the antennal lobe (*2, 6*). In the moths *Manduca sexta* (*17*) and *Heliothis virescens* (*18*), single projection neurons of known specificity were identified morphologically, and the dendritic trees of some of these were clearly confined to different glomerular parts of the MGC. However, other types of projection neurons did not confirm the pattern (*17, 18*). Projection of single neurons was also analyzed in the cockroach *Periplaneta*, but no clear structure-function relation was observed (*15, 16*). These studies have indicated that there might be functional significance to the arrangements of glomerular structures in the olfactory pathway.

We show here that specific identifiable olfactory receptor types project to distinctly separated glomerular structures in the MGC of the antennal lobe, thus strongly indicating that these are functionally distinct units.

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20. Either one or two cells were filled in each sensillum. The cell specific for the stimulus applied filled exclusively in about half of the fills. We tested the selectiveness of the filling method by comparing the filling frequencies of the two cells present in the Z5-10:OAc sensillum by Fisher's exact test [R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1981)], which gave a P value of 0.016. The selectiveness was thus statistically significant.

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Association of a 59-Kilodalton Immunophilin with the Glucocorticoid Receptor Complex

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Immunophilins, a family of proteins that exhibit rotamase (peptidyl-prolyl *cis-trans* isomerase) activity in vitro, are expressed in many organisms and most tissues. Although some immunophilins can mediate the immunosuppressive actions of FK506, rapamycin, and cyclosporin A, the physiological role of the unligated proteins is not known. A 59-kilodalton member of the FK506- and rapamycin-binding class was found to associate in the absence of these drugs with two heat shock proteins (hsp90 and hsp70) and the glucocorticoid receptor (GR). Together, these proteins make up the inactive GR, thus biochemically linking two families of proteins proposed to be involved in protein folding and assembly as well as two potent immunosuppressive modalities.

Immunophilins are proteins that bind the immunosuppressants FK506, rapamycin, and cyclosporin A (CsA) (*1*). Immunophilins are comprised of two classes: the FK506- and rapamycin-binding proteins (FKBPs) and the CsA-binding cyclophilins. Certain members of each class mediate the ability of the drugs to block intermediate steps along specific signal transduction pathways in a variety of cell types (*2, 3*). Although all known immunophilins have rotamase (peptidyl-prolyl *cis-trans* isomerase) activity in vitro, the relevance of this activity in vivo is unknown. Thus, the cellular role of these

proteins in the absence of these drugs is unknown. Clues to their function in vivo may be uncovered by the identification of proteins with which they associate. A *Drosophila melanogaster* cyclophilin homolog *ninaA*, which is expressed specifically in the retina, is essential for the trafficking of one isoform of rhodopsin to the membrane (*4*); in addition, a mammalian cyclophilin with restricted tissue distribution has been shown to interact with an unidentified cellular protein (*5*). Here, we report the purification and characterization of a 59-kD member of the FKBP family (FKBP59) and the identification of three associating proteins. These proteins, including FKBP59, make up the inactive GR complex, which contains GR that has not bound its glucocorticoid ligand and cannot bind DNA.

The FKBP59 protein was first identified in human T lymphocytes by affinity chromatography that used either an FK506- or a rapamycin-based matrix (*6*, *7*); FKBP59 is also present in mast cells (*8*). In T lymphocytes both FKBP59 and an 80-kD protein retained by both affinity matrices are phosphorylated in vivo (*6*). FKBP59 was isolated from cell lysates of a rat basophilic leukemia cell line and a human thymus with FK506 and rapamycin affinity matrices and SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (*6*, *8*). After electroblotting to a poly(vinylidine difluoride) membrane, NH_2-terminal sequences of the rat and human proteins were determined (Fig. 1); they share 77% identity. The first 20 amino acids of the human sequence match the NH_2-terminal sequence of a 59-kD protein isolated from IM-9 cells, a human B lymphocyte cell line (*9*). Because other sequences in the database (*10*) were not identical, this region of the protein is probably unique.

The B cell p59 is an antigen of monoclonal antibody (MAb) KN 382/EC1 (EC1) (*11*, *12*), generated by immunization with inactive rabbit progestin hormone receptor. The inactive steroid hormone receptor complex consists of the steroid receptor, the heat shock proteins hsp90 (*13–17*) and hsp70 (*9*, *18*, *19*), and p59 (*9*, *20*, *21*). Binding of the steroid results in dissociation of the heat shock proteins and dimerization and transformation of the steroid receptor to an active (DNA binding) state (*22*). MAb EC1 immunoprecipitates p59 and inactive receptor complexes for estrogen, progesterone, androgen, and glucocorticoid from rabbit uterine cytosol (*20*, *23*). The p59 associates with hsp90 and hsp70 in cytosolic preparations (*9*, *24*) and may itself be a heat shock protein (*25*).

To confirm the identity of FKBP59 and p59, we purified immunophilins from IM-9 cytosol with the FK506 affinity matrix (*6*). After elution of the matrix with FK506, the eluates were analyzed by SDS-PAGE and silver staining (Fig. 2A) or immunoblotting with MAb EC1 (Fig. 2A). The MAb EC1 recognized FKBP59. Anti-hsp90 [MAb AC88 (*26*)], anti-hsp70 [MAb N27 (*18*)], and anti-GR (polyclonal serum PBL135) identified components of the inactive GR complex in the FK506 eluate (Fig. 2A) (*27*). When molybdate, which stabilizes inactive steroid hormone receptors (*20*), was included in the lysis buffer, the amount of FKBP59 retained by the matrix was unaltered, but the amount of hsp70, hsp90, and GR in the eluates increased. Thus, the intact, inactive receptor complex was retained by the FK506 affinity matrix, and FK506 neither activated the receptor complex nor dissociated its components.

Although the inactive receptor complex was retained on the FK506 affinity matrix, the protein responsible for the specific interaction with FK506 was unknown. To identify the location of the binding site, we mixed IM-9 cytosol with the rapamycin affinity matrix (*6*), and the matrix was washed with increasing concentrations of salt to dissociate the GR complex. After four washes with 0.5 M KCl, which dissociates hsp90 from p59 (*23*), and elution by rapamycin, the eluate was analyzed by SDS-PAGE and silver staining (Fig. 2B). Two

Fig. 1. NH_2-terminal sequences of rat and human FKBP59. Lowercase letters in the sequence indicate low confidence residues. Rat FKBP59 was isolated from rat basophilic leukemia cells as described (*8*). For the isolation of human FKBP59, a human thymus (25 g) was frozen in liquid nitrogen and ground in a Waring blender at 4°C (*37*). After resuspension in 75 ml of 20 mM tris HCl (pH 7.5), 250 mM NaCl, 5% glycerol, 1 mM phenylmethylsulfonyl fluoride, 5 mM 2-mercaptoethanol, 0.02% NaN_3, leupeptin (1 μg/ml), and pepstatin (1 μg/ml), the suspension was homogenized with a Potter-Elvehjem motorized homogenizer (Wheaton) at 4°C. The cell lysate was centrifuged at 25,000*g* for 30 min at 4°C. Supernatant (10 ml) was passed twice over 60 μl of FK506–Affigel-10 or rapamycin–Affigel-10 (Affigel-10, Bio-Rad, Richmond, California) (*6*). The matrices were washed twice with 50 mM tris HCl (pH 7.5), 150 mM NaCl, and 1% NP-40; once with 50 mM tris HCl (pH 7.5), 650 mM NaCl, and 1% NP-40; and twice more with 50 mM tris HCl (pH 7.5) and 0.1% NP-40. The matrices were eluted with 100 μl of 10 mM FK506 or rapamycin, respectively, in 1 ml of 10 mM tris HCl (pH 7.5). The eluate was lyophilized and subjected to SDS-PAGE. After electroblotting to a Problott membrane (Applied Biosystems), the NH_2-terminal sequence was determined by automated Edman degradation (*37*). 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.

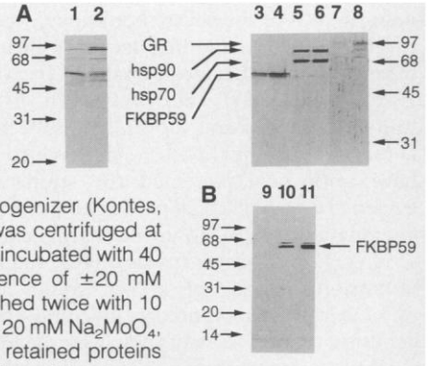

Fig. 2. Isolation of FKBP59, hsp70, hsp90, and GR complex with the FK506 affinity matrix and affinity purification of FKBP59 with the rapamycin affinity matrix. In (**A**), IM-9 lymphocytes were washed three times in cold phosphate-buffered saline and resuspended in 10 mM PG buffer [10 mM sodium phosphate (pH 7.4) and 10% glycerol] with the protease inhibitors listed (Fig. 1) (10^8 cells per milliliter). The cells were disrupted in a Dounce homogenizer (Kontes, Vineland, New Jersey) at 4°C, and the suspension was centrifuged at 100,000*g* for 1 hour at 4°C. Supernatant (0.8 ml) was incubated with 40 μl of FK506–Affigel-10 (*6*) in the absence or presence of ±20 mM Na_2MoO_4 for 3 hours at 4°C. The matrices were washed twice with 10 mM PG ± 20 mM Na_2MoO_4, twice with 100 mM PG ± 20 mM Na_2MoO_4, and twice with 10 mM PG ± 20 mM Na_2MoO_4. The retained proteins were eluted with 1 mM FK506 in 250 μl of 10 mM PG, and the eluates were analyzed by SDS-PAGE and silver staining (*38*); lane 1, without Na_2MoO_4; lane 2, with Na_2MoO_4. The eluted proteins were also electroblotted to nitrocellulose and immunostained with MAb EC1 (*10*) (anti-FKBP59) (lane 3, without Na_2MoO_4; lane 4, with Na_2MoO_4), with MAb N27 (*18*) (anti-hsp70), and MAb AC88 (*26*) (anti-hsp90) (lane 5, without Na_2MoO_4; lane 6, with Na_2MoO_4), or with PBL135 antibodies (anti-human GR) (lane 7, without Na_2MoO_4; lane 8, with Na_2MoO_4). In (**B**), IM-9 cytosol (60 μl) in 10 mM HG buffer [10 mM Hepes (pH 7.4) and 10% glycerol] was incubated with either rapamycin–Affigel-10 (30 μl) or ethanolamine-capped control–Affigel-10 (30 μl) (*6*) for 16 hours at 4°C. The matrices were washed four times with 10 mM Hepes (pH 7.4) with 0.5 M KCl followed by two washes with 10 mM Hepes (pH 7.4). The retained proteins were eluted with 1 mM rapamycin in 10 mM Hepes (pH 7.4) (300 μl) for 1 hour at 4°C (first elution) and 1 mM rapamycin in 10 mM Hepes (pH 7.4) (250 μl) for 12 hours at 4°C (second elution). The eluates were analyzed by SDS-PAGE and silver staining (*38*). Lane 9, control matrix (first elution); lane 10, rapamycin matrix (first elution); lane 11, rapamycin matrix (second elution). Molecular size standards are indicated in kilodaltons.

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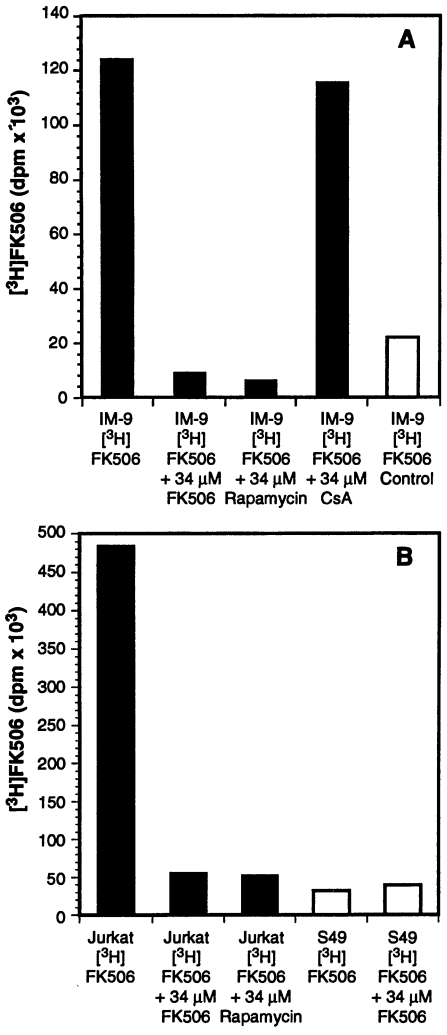

Fig. 3. Determination of binding specificity of FKBP59. (**A**) IM-9 cytosol (0.35 ml) in 10 mM HG was incubated with 100 μl of either MAb EC1–Affigel-10 (black bars) or nonimmune–Affigel-10 (white bar) (which was made up of an antibody that recognized a myeloma surface protein coupled to Affigel-10) (*19*) for 3 hours at 4°C. Nonabsorbed proteins were removed with three washes of 10 mM Hepes (pH 7.4) and 0.5 M KCl and two washes of 10 mM Hepes (pH 7.4). The immunomatrices were resuspended in 10 mM Hepes (pH 7.4) (100 μl) that contained 34 nM [^{3}H]FK506 (*28*) ± 34 μM unlabeled FK506, rapamycin, or CsA and incubated for 12 hours at 4°C. Bound [^{3}H]FK506 was eluted with 300 μl of 50 mM diethylamine in 10 mM Hepes (pH 10.5) (*22*), and the eluate was counted with Scintiverse II (Fisher Scientific, Pittsburgh, Pennsylvania). (**B**) To ensure that MAb EC1–Affigel-10 did not bind [^{3}H]FK506, 100 μl of MAb EC1–Affigel-10 was incubated with either 0.35 ml of Jurkat cytosol, a human T cell line (black bars), or S49 cytosol, a mouse T cell line (white bars). MAb EC1 binds the human isoform of FKBP59 but not the mouse isoform (*23*). After the washing protocol [as in (A)] and incubation with 100 nM [^{3}H]FK506 ± unlabeled competitor, the diethylamine eluates were counted.

bands were observed, both of which cross-reacted with MAb EC1 (*28*). Several isoforms of FKBP59 have been reported (*9*), which may account for the multiple bands. Because only p59 was specifically retained with a stringent washing protocol, it probably provides the binding site for the specific interaction of FK506 and rapamycin with the inactive GR and is the predominant FKBP expressed in IM-9 cells, unlike the human T cell line Jurkat, in which FKBP12 is predominant (*6*).

To investigate the binding of FKBP59 to its ligands in greater detail, we purified it to homogeneity without exposing it to FK506 or rapamycin. The IM-9 cytosol was incubated with MAb EC1 immunoaffinity resin (*20*) and was washed extensively with 0.5 M KCl. The purity of this preparation of FKBP59 was determined by SDS-PAGE and silver staining (*28*). The washed matrix was incubated with [^{3}H]FK506 (*29*) and washed extensively to remove unbound drug, and the FKBP59-[^{3}H]FK506 complexes were eluted with diethylamine (*23*) and analyzed (Fig. 3A). MAb-bound FKBP59 bound [^{3}H]FK506 in a specific manner. Because experiments with mouse lymphocyte cytosol [MAb EC1 does not bind the mouse isoform of FKBP59 (*23*)] resulted in no specific binding of [^{3}H]FK506 (Fig. 3B), the MAb EC1 resin does not specifically bind [^{3}H]FK506—that is, drug binding is dependent on FKBP59. Competition with nonradioactive ligands demonstrated that FKBP59 binds specifically to FK506 and to rapamycin but not to CsA, which is consistent with the relative retention of FKBP59 by affinity matrices of these three drugs (*8*, *30*). With this method, the dissociation constant (K_d) for MAb-bound FKBP59 and [^{3}H]FK506 was determined to be 66 nM (Fig. 4). Scatchard analysis indicated that one high-affinity binding site for [^{3}H]FK506 is present on FKBP59, presumably a result of an FKBP-like domain. Indeed, the sequence of p59 (*31*) reveals the existence of two consecutive FKBP-like domains. The first domain, amino acids 33 through 139, is 50% identical to FKBP12 (all ten amino acids in the drug-binding pocket are conserved), whereas the second domain, amino acids 140 through 254, is 29% identical (only one amino acid in the drug-binding pocket is conserved, but structurally important amino acids, such as particular glycines, are conserved).

The association of an immunophilin with heat shock proteins bridges two classes of proteins implicated in protein folding and assembly (*32*). Like heat shock proteins, immunophilins are evolutionarily conserved and expressed throughout tissues and organisms (*1*). On the basis of their rotamase activity in vitro, immunophilins may catalyze protein folding in vivo, but data from the best studied case—the cyclophilin homolog *ninaA*, which is involved in the assembly and trafficking of rhodopsins in *Drosophila* (*4*, *33*)—are more consistent with a binding role for the *ninaA* gene product. The finding that FKBP59 associates with heat shock proteins (*31*) supports the role of immunophilins as peptidyl-prolyl binding proteins involved in the assembly of proteins and protein complexes (*34*); FKBP59 may be involved in the assembly of the inactive steroid hormone receptor complex.

The association of an immunophilin and steroid hormone receptors raises the possibility of influence by FK506 and rapamycin on steroid hormone receptor function in general and on GR function in particular. Although FK506 and rapamycin seem insufficient to cause activation of GR in vitro (*28*), it is possible that the FKBP59-FK506 or FKBP59-rapamycin complex modulates GR function (*35*, *36*). The association of an FK506- and rapamycin-binding protein with steroid hormone receptors is a provoc-

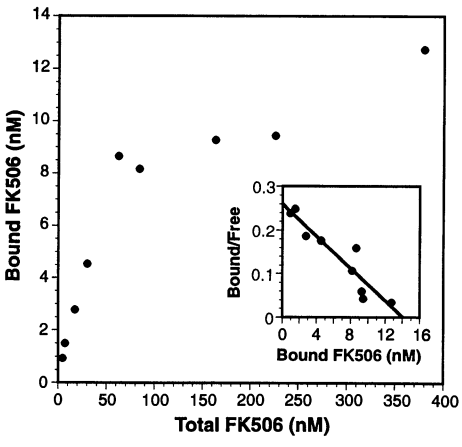

Fig. 4. Saturation curve of [^{3}H]FK506 binding to MAb EC1-FKBP59. Inset: Scatchard plot of [^{3}H]FK506 binding to MAb EC1-FKBP59. IM-9 cytosol (0.2 ml) was incubated with 80 μl of MAb EC1–Affigel-10 (*20*) for 3 hours at 4°C. The immunomatrix was washed three times with 1 ml of 10 mM Hepes (pH 7.4) with 0.5 M KCl and twice with 1 ml of 10 mM Hepes (pH 7.4). Concentrations of [^{3}H]FK506 ranging from 5 nM to 390 nM ± 20 μM unlabeled FK506 were added in a total volume of 200 μl. Free [^{3}H]FK506 and unlabeled FK506 were removed by three quick washes of 1 ml of 10 mM Hepes (pH 7.4) and 0.1 M KCl. The bound drugs were eluted with 50 mM diethylamine (pH 10.5) (*22*) and counted in a biodegradable scintillant (Amersham). The data were analyzed with the LIGAND program for one binding site or molecule of FKBP59 (*39*); the receptor concentration was estimated to be 7.9 nM, and the K_d for antibody-bound FKBP59 and [^{3}H]FK506 was estimated to be 66 nM.

ative connection between two potent regulators of intracellular events.

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34. A more elaborate discussion of this model, including a proposed mechanism of rotamase catalysis, is presented elsewhere (*3*).
35. Although the affinity of FK506 for FKBP59 is less than it is for FKBP12 (K_d = 0.4 nM), the relevant affinity may be that of the immunophilin-drug complex for its target, which is analogous to the way immunosuppressants block signal transduction pathways (*3*). For example, despite having a 100-fold lower affinity for cyclophilin than cyclosporin A, $MeBm_2t^1$-CsA potently inhibits T cell receptor–mediated signal transduction because its cyclophilin complex is a high-affinity ligand to calcineurin (J. Liu *et al.*, *Biochemistry* **31**, 3896 (1992).
36. Okadaic acid (OA), a potent inhibitor of the serine-threonine protein phosphatases PP2A and PP1, activates the progesterone and estrogen receptors in a ligand-independent manner [R. F. Power, S. K. Mani, J. Codina, O. M. Conneely, B. W. O'Malley, *Science* **254**, 1636 (1991)]. OA potentiates the ability of dopamine, acting through the D_1 receptor, to activate these receptors as well. However, neither OA nor dopamine activates the human GR. By a similar mechanism that involves inhibition of calcineurin (*2*) or a related phosphatase, the FKBP59-FK506 complex may activate GR in a ligand-independent manner or potentiate ligand-independent activation by membrane-associated events.
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High-Efficiency Expression and Solubilization of Functional T Cell Antigen Receptor Heterodimers

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The T cell receptor (TCR) ζ chain was attached to the TCR α and β extracellular domains to induce efficient expression of αβ heterodimers that can recognize complexes of antigen with major histocompatibility complex (MHC) molecules. Chimeric constructs expressed in RBL-2H3 cells were efficiently transported to the cell surface uniquely as disulfide-linked heterodimers. Transfectants were activated by specific antigen-MHC complexes, which demonstrated that the expressed αβ was functional and that CD3 was not required for antigen-MHC binding. Constructs with thrombin cleavage sites were efficiently cleaved to soluble disulfide-linked heterodimers. Thus, attachment of TCR ζ domains and protease cleavage sites to TCR α and β induces expression of demonstrably functional heterodimers that can be solubilized.

Determination of the three-dimensional structure of the TCR αβ heterodimer requires the production of large amounts of a soluble form of this complex. However, isolation of the soluble αβ heterodimer presents many difficulties. The α and β chains are both type 1 transmembrane proteins (*1*) and as such are not soluble in the absence of detergent. Furthermore, the αβ heterodimer is not expressed at the cell surface unless associated with the other chains of the TCR complex (*2*). In addition, structural alterations to circumvent the problems of solubility and expression efficiency should not affect the heterodimer combining site. Although soluble forms of the TCR αβ heterodimer have been produced (*3*), it is not clear whether they can bind a specific antigen-MHC complex.

We attached the transmembrane and cytoplasmic domains of the TCR ζ chain to the extracellular domains of the α and β chains to facilitate production of the αβ heterodimer. The cytoplasmic domain of ζ induces cell activation when attached to heterologous proteins that are cross-linked by antibody (*4*, *5*). Thus, the ability of an α-ζ/β-ζ heterodimer to bind antigen-MHC could be tested by exposure of the cells that expressed this heterodimer to antigen-pulsed presenting cells. In addition, the ζ transmembrane domain induces disulfide dimerization when attached to heterologous proteins (*4*). Because cells that express glycosyl-phosphatidylinositol–linked α and β chains have more than 99% of these constructs retained in the endoplasmic reticulum (ER) in a nondisulfide-linked form (*6*), we reasoned that inducing disulfide dimerization could enhance heterodimer expression.

Chimeric constructs made between TCR α and β cDNAs isolated from the T cell hybridoma 2B4 and the murine TCR ζ cDNA (*7*) were stably transfected into the rat basophilic leukemia line RBL-2H3 (*8*). Flow cytofluorometric analysis of stable transfectants revealed that large amounts of the chimeric constructs at the cell surface were obtained only upon expression of both constructs. The mean fluorescence of uncloned transfected cells that expressed both α-ζ and β-ζ constructs after staining with the monoclonal antibody A2B4 (anti-2B4 α chain) (*9*) was substantially greater than that of cells that expressed only an α-ζ construct (Fig. 1A). Clones have been

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