actin primers sequences are available on request. DNA was amplified in a 50 μl of PCR reaction mixture by using 30 cycles at an annealing temperature of 60°, for γc primers and 68°C for actin primers. A sample of the amplified product was separated on a 1% agarose gel and analyzed by ethidium bromide staining. RNA was prepared with the RNA easy kit (Qiagen) and was reverse-transcribed with the Superscript Preamplification System (Gibco-BRL). γc proviral and β-actin cDNA amplification were performed as described above. Quantification of expression was made by comparison with RNA isolated from the same standard curve of diluted cells.

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19. The following monoclonal antibodies (mAbs) were used in immunofluorescence studies: anti-γc chain: Tugh 4 (rat IgG2, PharMingen, San Diego, CA); anti-CD3: Leu 4 (IgG2a, Becton Dickinson, San Diego, CA); anti-CD4: Leu3a (IgG1, Becton Dickinson); anti-CD8: Leu 2a (IgG1, Becton Dickinson); anti-CD19: J4 119 (IgG1 Immunotech, Marseille, France); anti-CD14: Leu M3 (Becton Dickinson); anti-CD16: 3G8 (IgG1, Immunotech); anti-CD56: MY31 (IgG1, Becton Dickinson); anti-CD15 (IgM, PharMingen); anti-TCR αβ: BMA031 (IgG1, Immunotech); anti-TCR γδ: IMMU 515 (IgG1, Immunotech); anti-CD45RO: UCHL1 (IgG2a, Immunotech); anti-CD45RA: 2H4 (IgG1, Coulter Clone, Margency, France); anti-CD34: HPCA-2 (IgG1, Becton Dickinson); anti-TcR Vβ2: MPB2D5 (IgG1, Immunotech); anti-TcR Vβ3: CH92 (IgM, Immunotech); anti-TcR Vβ5.1: IMMU 157 (IgG2a, Immunotech); anti-TcR Vβ5.2: 36213 (IgG1, Immunotech); anti-TcR Vβ5.3: 3D11 (IgG1, Immunotech); anti-TcR Vβ8: 56C5.2 (IgG2a, Immunotech); anti-TcR Vβ9: FIN9 (IgG2a, Immunotech); anti-TcR Vβ13.1: IMMU 222 (IgG2, Immunotech); anti-TcR Vβ13.6: JU74.3 (IgG1, Immunotech); anti-TcR Vβ14: CAS1.13 (IgG1, Immunotech); anti-TcR Vβ17: E17.5F3.15.13 (IgG1, Immunotech); anti-TcR Vβ21.3: IG125 (IgG2, Immunotech). Fluorescence staining was done with phycoerythrin- or fluorescein isothiocyanate–conjugated mAbs. Cells were analyzed on a FACScan flow cytometer (Becton Dickinson).
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32. We thank the medical and nursing staff of the Unité d'Immunologie et d'Hématologie pédiatriques, Hôpital des Enfants-Malades, for patient care. We also thank C. Harré and C. Jacques for technical help; D. Bresson for preparation of the manuscript; N. Wulfraat for patient referral; O. Danos, M. Fougereau, P. Mannoni, C. Eaves, and L. Coulombel for advice; A. Gennery for assistance with English translation; B. Bussière, C. Cailliot, and J. Caraux (Amgen, France) for providing SCF and MGDF; J. Bender and D. Van Epps (Nexell Therapeutics, Irvine, CA) for providing containers; and S. Yoshimura and I. Kato (Takara Shuzo, Shiga, Japan) for providing the CH-296 fibronectin fragment. Supported by grants from INSERM, Association Française des Myopathies, Agence Francaise du Sang, and the Programme Hospitalier de Recherche Clinique (Health Ministry).

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Localization of a Short-Term Memory in *Drosophila*

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Memories are thought to be due to lasting synaptic modifications in the brain. The search for memory traces has relied predominantly on determining regions that are necessary for the process. However, a more informative approach is to define the smallest sufficient set of brain structures. The *rutabaga* adenylyl cyclase, an enzyme that is ubiquitously expressed in the *Drosophila* brain and that mediates synaptic plasticity, is needed exclusively in the Kenyon cells of the mushroom bodies for a component of olfactory short-term memory. This demonstrates that synaptic plasticity in a small brain region can be sufficient for memory formation.

The localization of memory traces has occupied neuroscientists throughout this century (*1*). Approaches have ranged from surgical ablation to mapping localized necessary gene expression in transgenic animals (*2*, *3*). Until recently, attempts to localize a memory trace have relied mainly on determining necessary brain regions (*4*). However, in a highly integrated network, other components besides the one being studied may also be necessary.

In insects, much attention has been paid to the mushroom bodies as the site for olfactory learning (*3*, *5*–*8*). In *Drosophila*, they are made up of about 2500 intrinsic neurons (Kenyon cells), receive multimodal sensory input, preferentially from the antennal lobe to the calyx, and send axon projections to the anterior brain where they bifurcate to form the α/β, α′/β′, and γ lobes (*9*). Noninvasive intervention techniques can provide mushroom body–less flies. In most respects, these flies show remarkably normal behavior but are deficient in olfactory learning (*5*). Genes important for olfactory memory have elevated expression levels in the mushroom bodies (*6*, *8*). Additionally, the mushroom bodies are necessary for context generalization in visual learning at the flight simulator and the control of spontaneous walking activity (*10*, *11*).

The *rutabaga* (*rut*) gene of *Drosophila* encodes a type I Ca^{2+}/calmodulin-dependent adenylyl cyclase (AC). Regulated synthesis of cyclic adenosine 3′,5′-monophosphate by

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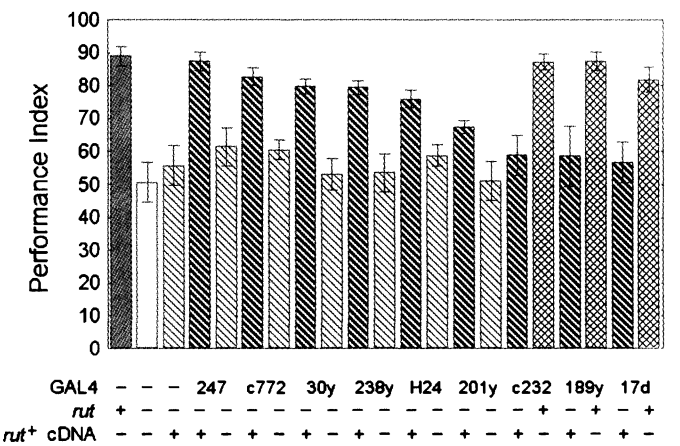

Fig. 1. The *rut* mutant defect in olfactory short-term memory can be rescued with a *rut*$^+$ cDNA in several GAL4 enhancer trap lines. Memory was measured about 2 min after classical conditioning (*17*). Performance indices (PIs) of *rut* mutant flies (white bar) and *rut* mutant flies with either a P[UAS_{GAL4}-*rut*$^+$] or GAL4 enhancer trap element (thin diagonal striped bars) were significantly different from wild-type flies (dark gray bar; P's < 0.0005). There was no significant difference between *rut* mutant flies' PIs rescued with GAL4 enhancer trap elements 247, c772, 30y, 238y, and H24 and the P[UAS_{GAL4}-*rut*$^+$] compared with wild-type flies (dark gray and thick diagonal striped bars, respectively; P's > 0.05). Mutant *rut* flies' performance was rescued with GAL4 element 201y and the P[UAS_{GAL4}-*rut*$^+$] (P < 0.05) but was also significantly lower than the performance of wild-type flies (P < 0.005). GAL4 enhancer trap lines c232, 189y, and 17d with a P[UAS_{GAL4}-*rut*$^+$] did not rescue the *rut* mutation (P's > 0.05). Wild-type flies heterozygous for GAL4 enhancer trap elements c232, 189y, and 17d were not significantly different from wild-type flies (dark gray and cross-hatched bars; P > 0.05). Bars represent mean PIs; errors are SEMs; n = 6 for all genotypes.

a type I AC (through Ca^{2+} and heterotrimeric G-protein signaling) is important for learning and synaptic plasticity throughout the animal kingdom (*4*, *6*, *8*, *12*–*16*). The *Drosophila rut* mutation affects all learning paradigms tested to date and has abnormal synaptic plasticity at the larval neuromuscular junction (*4*, *6*, *8*, *12*, *14*, *15*).

Our approach is built on the assumption that synaptic plasticity is impaired in general in *rut* mutants and that it is this cellular defect that causes the various learning deficits. Restoring *rut* AC in a spatially restricted fashion in a defined set of neurons would furnish synaptic plasticity to only those cells. If in such flies a learning task is rescued, the corresponding memory trace is mapped to the set of neurons expressing the gene, or a subset of these.

Olfactory short-term memory was tested with the apparatus of Tully and Quinn (*6*). In this assay, flies are sequentially exposed to two odorants, one of which is paired with electric shocks (*17*). Shortly after training, ~95% of wild-type flies prefer the odorant not accompanied by punishment. Mutant *rut* flies show significantly lower memory scores (*6*, *8*) (Fig. 1).

To test whether the olfactory learning defect of the *rut* mutant was rescuable, we combined in the *rut* mutant a P-element expressing a wild-type *rut* cDNA under the control of a GAL4-sensitive enhancer P[UAS_{GAL4}-rut^+] with a driver transgene P[*elav*-GAL4] expressing the yeast transcription factor in all neurons (*18*, *19*). This pan-neuronal expression of *rut* AC partially restored olfactory learning in the *rut* mutant (*20*). The incomplete rescue could be due to insufficient expression levels of the P[UAS_{GAL4}-rut^+] transgene, a dominant negative effect of the P[*elav*-GAL4] element, or a negative effect of ectopically expressing this transgene.

Several GAL4 enhancer trap lines were selected for local rescue because of their expression patterns (see below). Mutant *rut* flies with the enhancer trap GAL4 elements 247, c772, 30y, 238y, and H24 in combina-

Table 1. The rescue of the *rutabaga* olfactory phenotype does not alter electroshock or odorant sensitivity. Wild-type, *rutabaga* (*rut*) mutant, and resuced flies were tested for responses to both the electroshock and the odorants used in the learning experiments. There were no significant differences between wild-type flies and any mutant or rescued *rut* flies in either assay. The genotype of the rescued flies is *rut*/Y; GAL4 line/+; P[UAS_{GAL4}-rut^+]/+. Means of six experiments per genotype are shown plus or minus the SEM.

Genotype	Shock avoidance index	Odorant avoidance index
Canton S	82.3 ± 2.2	83.2 ± 4.9
rut	86.0 ± 2.4	86.9 ± 2.0
247	78.6 ± 4.8	83.0 ± 6.7
c772	69.0 ± 5.4	75.9 ± 4.9
30y	80.0 ± 5.7	96.1 ± 1.8
238y	80.4 ± 4.1	92.0 ± 1.8
H24	87.6 ± 3.0	89.6 ± 5.8
201y	79.9 ± 3.1	91.5 ± 2.3
c232	81.7 ± 3.0	82.9 ± 4.5
189y	69.1 ± 7.4	93.1 ± 3.4
17d	67.7 ± 4.4	87.9 ± 2.1

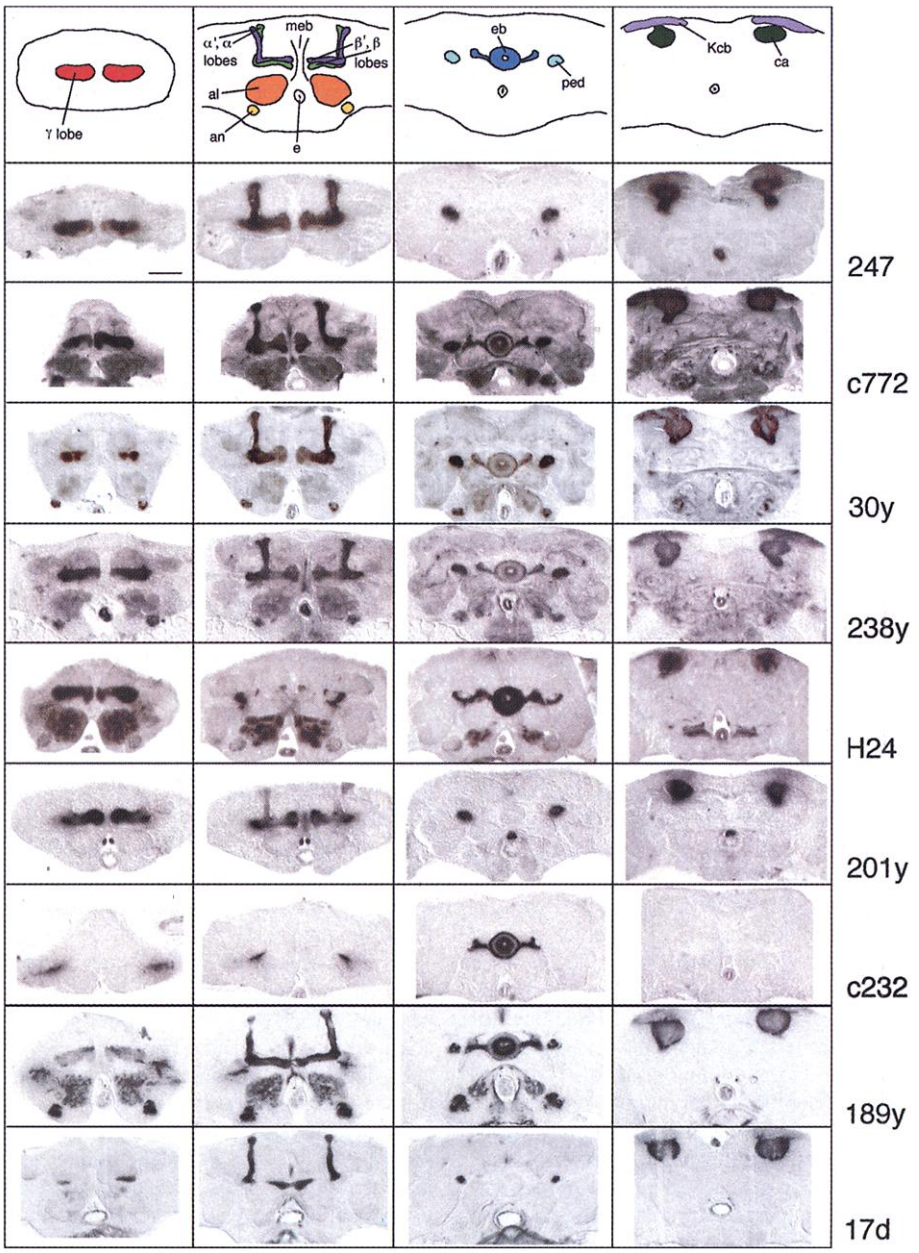

Fig. 2. Spatial expression patterns in the brain of rescuing and nonrescuing GAL4 enhancer trap lines with P[UAS_{GAL4}-TAU] as a reporter (*21*). Serial frontal brain sections were examined. Schematic drawings and sections at the level of the γ lobes, α/β and α′/β′ lobes and median bundle (meb), ellipsoid body (eb) and peduncles (ped), and calyces (ca). The first six lines rescued the *rut* odorant learning defect and showed common expression in the γ lobes of the mushroom body. GAL4 line 247 (*22*) showed detectable expression only in the mushroom bodies. Lines c772, 30y, 238y, H24, and 201y all showed expression outside the mushroom bodies as well. Lines c772, 30y, 238y, and H24 were expressed in the ellipsoid body and antennal lobes (al). Lines c772 and 30y also showed fan-shaped body expression (*20*). The bottom three lines did not rescue the *rut* defect and showed little or no expression in the γ lobe of the mushroom body. Line c232 expression was largely restricted to the ellipsoid body. Line 189y showed expression in the ellipsoid body and the mushroom body α/β lobes and faint expression in the mushroom body γ lobe. Line 17d expression was restricted to the α/β lobes of the mushroom bodies and the median bundle. Some GAL4 enhancer trap lines' expression patterns have been previously described (*4*, *11*, *27*). Dorsal is up; e, esophagus; an, antennal nerve; Kcb, Kenyon cell bodies. Scale bar, 50 μm. The color outside the mushroom bodies and ellipsoid body in lines 247, c232, and 17d is roughly representative of background staining.

tion with the P[UAS$_{GAL4}$-*rut*$^{+}$] transgene showed memory scores statistically indistinguishable from wild-type flies (Fig. 1). The GAL4 line 201y partially rescued the *rut* learning defect. Finally, *rut* mutant flies with three other GAL4 enhancer trap elements (c232, 189y, and 17d) and the P[UAS$_{GAL4}$-*rut*$^{+}$] effector gene had *rut* mutant–like short-term memory scores.

Four of the nine enhancer trap lines were previously used to study olfactory learning after locally expressing a constitutively activated G-protein α subunit (Gαs*) (*3*). In the present experiments, the magnitude of rescue was similar to the suppressive effect of the Gαs* protein in the respective lines. In c232, Gαs* had no effect, in 201y, suppression was about 50%, whereas in c772 [the same expression pattern as c747 in (*3*)] and 238y, suppression was nearly complete.

Neither the rescuing GAL4 enhancer trap lines without P[UAS$_{GAL4}$-*rut*$^{+}$] nor the P[UAS$_{GAL4}$-*rut*$^{+}$] line without driver had a dominant rescue effect. Nor did the nonrescuing GAL4 enhancer trap inserts have a negative effect on wild-type flies (Fig. 1). Thus, it is the specific interaction of the GAL4 enhancer trap element with the P[UAS$_{GAL4}$-*rut*$^{+}$] effector that can rescue the memory defect in *rut* mutant flies.

Control experiments were conducted with naïve wild-type, *rut* mutant, and potentially rescued *rut* mutant flies (Table 1) to assure that none of the memory scores were due to changes in shock reactivity or perception of the odorants. All genotypes avoided electric shocks at similar levels. Although 189y and 17d had somewhat reduced shock reactivity, it was similar to that of c772, which showed a wild-type–like learning score. Thus, these shock reactivity scores cannot be responsible for the low memory scores. In addition, all genotypes tested avoided the aversive odorants used in the training protocol.

To determine what brain structures are minimally sufficient for olfactory short-term memory, we examined the expression patterns of the rescue and nonrescue GAL4 enhancer trap lines (*21*). Serial sections showed that the common structure labeled in all rescuing GAL4 lines was specifically the mushroom bodies (Fig. 2). Indeed, comparing expression patterns of rescuing and nonrescuing lines indicates that the γ lobes may be especially important. In contrast to the GAL4 lines used in (*3*), the rescuing line 247 (*22*) lacks expression in the median bundle. The latter, therefore, is not part of the set of minimally sufficient neurons.

On the basis of the current model of how type I ACs function in synaptic plasticity (*16*) and on the connectivity of the mushroom bodies (*19*, *23*, *24*), the short-term memory trace of odors is localized to a single level in the olfactory pathway: the presynaptic sites in the Kenyon cells contacting extrinsic output neurons and possibly other Kenyon cells in the peduncle and lobes. Modulating neurons carrying the reinforcer must project to the peduncle or lobes and contact presynaptic endings of Kenyon cells there. No *rut*-dependent synaptic plasticity is required in the antennal lobe or calyx for olfactory learning.

Different brain structures are involved in different learning tasks. In the heat box paradigm, the median bundle, antennal lobes, and ventral ganglion are sufficient for rescue of *rut*-dependent short-term memory (*4*, *15*, *25*). To find olfactory and heat box memory at different locations was not unexpected, as mushroom body–less flies do well in heat box learning (*26*). The task-specific rescue in different GAL4 lines strongly supports the claim that it is the spatial distribution of the *rut* AC that matters.

Several open questions remain. Our conclusions refer only to *rut*-dependent synaptic plasticity. Although unlikely, the 60% short-term memory remaining in *rut* mutant flies may reside outside the mushroom bodies. Second, our current understanding of the role of type I ACs in synaptic plasticity and learning is not complete. Third, the P[UAS$_{GAL4}$-TAU] reporter was used to visualize GAL4 expression patterns, and coincidence with P[UAS$_{GAL4}$-*rut*$^{+}$] is inferred. Fourth, temporal control of transgene expression in these lines is not yet possible, leaving the faint possibility that the behavioral rescue in some cases might be due to developmental expression. Finally, whether all memory traces of odors reside in the mushroom bodies and how memory traces of odors are organized within the mushroom bodies await further investigation.

The technique of restoring synaptic plasticity in minimally sufficient brain regions has, in two cases, revealed simple, locally confined memory traces. This result is probably due to the simplicity of the learning tasks, requiring the animal to store a single sensory modality for a binary orientation response. It will be of considerable interest to map memory traces of more complex learning paradigms.

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17. The olfactory discrimination learning paradigm, control experiments, and calculations were used as in (*5*) with some modifications. The odorant cup size for Benzaldehyde and 4-Methylcyclohexanol was 12 mm^2 and 95 mm^2, respectively. One odorant was paired with 12 120-V dc electric shocks (each shock 1.3 s) over 60 s as a negative reinforcer. Olfactory perception controls used odor concentrations of the learning experiments against air. The shock avoidance control experiments used the same shocking protocol as the learning experiment. Standard genetic crosses were used to generate *rut*^{2080} mutant, rescued, and control flies for experiments from stocks described in (*4*). The P[UAS$_{GAL4}$-*rut*$^{+}$] cDNA element is #1 from (*4*). One GAL4 line, c522, had a dominant effect on learning in a wild-type background and was not used in rescue attempts. A general multivariate analysis of variance and, when warranted, the Duncan post hoc test were used to compare genotypic differences in memory performance and control experiments.
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28. D. Armstrong, T. Raabe, and X. Wiggins provided GAL4 lines. We thank B. Mühlbauer for excellent technical assistance. S. Milligan provided helpful comments on the manuscript. Supported in part by a Postdoctoral Research Fellowship from the Alexander von Humboldt Foundation (T.Z.), grants from the Deutsche Forschungsgemeinschaft (He986/10-3) and Fonds der Chemischen Industrie (M.H.), and the NSF (R.S.).

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Control of Homeostasis of CD8$^+$ Memory T Cells by Opposing Cytokines

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Memory T cells maintain their numbers for long periods after antigen exposure. Here we show that CD8$^+$ T cells of memory phenotype divide slowly in animals. This division requires interleukin-15 and is markedly increased by inhibition of interleukin-2 (IL-2). Therefore, the numbers of CD8$^+$ memory T cells in animals are controlled by a balance between IL-15 and IL-2.

Although persistent antigen may help preserve memory T cell numbers (*1*), it is now clear that antigen is not needed for memory T cell survival (*2*, *3*). Thus, memory T cells might not need external stimuli for survival. However, memory T cells and T cells with memory phenotype continue to divide, albeit slowly, in the absence of antigen (*3*, *4*). This suggests that memory T cells might depend on some constantly available factor(s) to preserve themselves.

To investigate the causes of memory T cell division, we characterized the cells and established a system in which we could study the phenomenon. Memory T cells bear high levels of CD44 (*5*) and high levels of IL-2 receptor β (IL-2Rβ), a polypeptide that is shared by the receptors for IL-2 and IL-15 (*6*, *7*). To confirm this phenotype, we measured the levels of IL-2Rβ on CD44^{low} and CD44^{high} CD8$^+$ T cells from normal young or old mice or on antigen-primed T cells bearing a transgenic T cell receptor (TCR) specific for K^b bound to a peptide from ovalbumin (*8*). Almost all of the CD8$^+$ cells that bear high levels of CD44 also bear high levels of IL-2Rβ and vice versa (Fig. 1). Also, as expected, the proportion of CD8$^+$ T cells that were IL-2Rβ^{high}, CD44^{high} increased as the animals aged (*9*). In young mice, exposure to antigen-converted CD44^{low}, IL-2Rβ^{low} TCR transgenic CD8$^+$ T cells into CD44^{high}, IL-2Rβ^{high} cells. These experiments confirmed that both environmentally created and deliberately primed memory CD8$^+$ T cells were CD44^{high} and IL-2Rβ^{high}.

Memory T cells are thought to divide slowly in animals (*3*). To confirm this, we gave mice bromodeoxyuridine (BrdU) in their drinking water for 28 days. CD8$^+$ T cells from the mice were then analyzed for incorporation of BrdU into their DNA, an indication of cell division. The data in Fig. 2, A and B, show that more of the IL-2Rβ^{high} CD8$^+$ T cells had divided than the IL-2Rβ^{low} cells. To find out how frequently the cells were dividing, we sorted IL-2Rβ^{high} or IL-2Rβ^{low} or CD44^{high} or CD44^{low} CD8$^+$ T cells, labeled them with 5-carboxyfluorescein diacetate succininyl ester (CFSE), and transferred them into normal recipients. Many more of the IL-2Rβ^{high} or CD44^{high} cells divided than did their IL-2Rβ^{low} or CD44^{low} counterparts, as demonstrated by dilution of their CFSE stain (Fig. 2, C to F). These experiments confirm that, in animals, CD8$^+$ T cells of memory phenotype divide slowly. Previous experiments by others and our own data suggest that this division is antigen-independent because it occurs in β_2 microglobulin-deficient (β2MKO) mice (*3*, *10*) (see below).

To investigate the idea that cytokines might be driving this proliferation, we tested a number of antibodies against cytokines and cytokine receptors for their ability to affect the process. The combination of antibody to IL-7 (anti–IL-7) and IL-7 receptor α (anti–IL-7Rα) was used to block IL-7 signaling. Anti–IL-2Rβ was used to block signaling by IL-2 and/or IL-15 because the receptors for these cytokines share the IL-2Rβ chain (*7*). To distinguish between the effects of IL-2 and IL-15, we compared the results with anti–IL-2Rβ with those with anti–IL-2 (sometimes combined with anti–IL-2Rα), which blocks IL-2 but not IL-15. These are all rat antibodies so normal rat immunoglobulin G (IgG) was used as a control. To prevent Fc-mediated effects of the antibodies to receptor, we converted them to F(ab′)$_2$'s (*11*). Because the recipients would eventually respond to the rat antibodies, we limited the duration of the experiment to 7 to 9 days. Examples of these

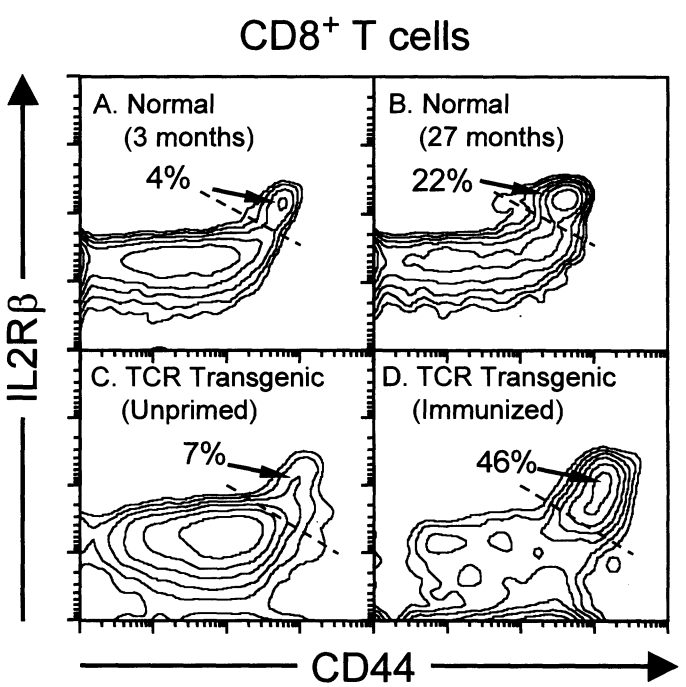

Fig. 1. Memory CD8$^+$ T cells bear high levels of CD44 and IL-2Rβ. (**A** and **B**) Peripheral blood lymphocytes were isolated from C57BL/6 mice, stained with anti-CD8, anti–IL-2Rβ, and anti-CD44, and analyzed (*22*). The data shown are gated on live CD8$^+$ T cells. (**C** and **D**) C57BL/6 mice transgenic for the OT1 TCR (*8*) were untreated (C) or infected with vaccinia virus modified to express chicken ovalbumin (D). Forty-seven days later, T cells were purified by passage over nylon wool, stained, and analyzed as described above, except that cells were also gated to be Vα2$^+$.

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