

then with antibodies to murine IgG-coated immunomagnetic beads. Depletions were confirmed by flow cytometry.

32. H. de la Salle *et al.*, data not shown.

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Gem: An Induced, Immediate Early Protein Belonging to the Ras Family

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A gene encoding a 35-kilodalton guanosine triphosphate (GTP)-binding protein, Gem, was cloned from mitogen-induced human peripheral blood T cells. Gem and Rad, the product of a gene overexpressed in skeletal muscle in individuals with Type II diabetes, constitute a new family of Ras-related GTP-binding proteins. The distinct structural features of this family include the G3 GTP-binding motif, extensive amino- and carboxyl-terminal extensions beyond the Ras-related domain, and a motif that determines membrane association. Gem was transiently expressed in human peripheral blood T cells in response to mitogenic stimulation; the protein was phosphorylated on tyrosine residues and localized to the cytosolic face of the plasma membrane. Deregulated Gem expression prevented proliferation of normal and transformed 3T3 cells. These results suggest that Gem is a regulatory protein, possibly participating in receptor-mediated signal transduction at the plasma membrane.

Genes that are transcribed early after mitogenic activation of resting cells are thought to be crucial for subsequent cell proliferation and expression of differentiated effector functions. We have cloned mitogen-induced genes from human peripheral blood T cells on the basis of differential expression of mRNA between resting and stimulated cells (1). One such clone, pAT 270, is now shown to encode a protein we have termed Gem because it binds GTP and is induced by mitogens. Human Gem is encoded by a single copy gene (2), and its 2127-base pair (bp) complementary DNA (cDNA) was similar in size to the corresponding mRNA (2200 bp) and predicted an open reading frame of 296 amino acids (Fig. 1). The Gem protein contains a core sequence (amino acids 75 to 240) that is highly related to members of the Ras superfamily of small GTP-binding proteins; the flanking NH₂- and COOH-terminal sequences are unrelated to Ras. Gem is most closely related to Rad (~60% identity) (Fig. 1), a protein encoded by a gene that is overexpressed in skeletal muscle from individuals with Type II diabetes relative to skeletal muscle from normal or

Type I diabetic individuals (3). The greatest similarity between Gem and Rad exists in regions that correspond to the guanine nucleotide-binding domains of Ras. Gem and

Rad diverge in the putative effector, or G2, region, suggesting that they interact with distinct targets.

Gem initiates from the first ATG codon (nucleotide 175) whereas Rad has been predicted to initiate from an internal ATG. The predicted start site for Gem was confirmed by immunoprecipitation with antibodies to the predicted NH₂-terminus and by in vitro transcription and translation analyses (2).

Mutational analyses of GTP-binding proteins and crystallographic studies of the H-ras oncogene product have defined regions of sequence consensus that interact with various positions of the guanine nucleotide (4-6). The guanine specificity consensus sequences NKXD and EXSA (X represents any amino acid) are perfectly conserved in Gem, with appropriate spacing, as NKSD (residues 191 to 194) and ETSA (residues 219 to 222), respectively. The consensus sequence GXXXXGK, which participates in interactions with the α and β phosphates of the guanine nucleotide, is also conserved in Gem as the sequence GEQGVGK (residues 82 to 88).

Gem contains an unusual motif in the G3 (DXXG) region, which putatively participates in binding and hydrolysis of the GTP γ phosphate (5). The sequence, ENKG (residues 134 to 137), contains the invariant glycine residue but has a conser-

Fig. 1. Predicted amino acid sequences encoded by cDNAs for human and murine Gem (H- and M-Gem, respectively) and comparison to human Rad (3) and human c-H-Ras1 (18). The open reading frame was determined for two independent human and a single murine Gem cDNA clone. Amino acids conserved in at least three proteins are in bold type and those conserved in all four proteins are bold and underlined. Dots indicate gaps inserted to allow for optimal alignment of the sequences. Numbers on the right indicate residue number. Consensus sequences for GTP-binding regions are indicated in italics (4, 5). Abbreviations for the amino acid residues are as follows: 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. GenBank accession numbers for the human and murine Gem cDNA sequences are O10550 and U10551, respectively.

H-Gem	MTLNNVTMRQGTVMQPPQQRWSIPADGRHLMVQKEPHQYSHRNHRSATP	50
M-Gem	MTLNNVTMRQGTVMQPPQQRWSIPADGRHLMVQKDPHPCNLNRHSTAP	49
Rad	MPVDERDLQAALTPGALT...AAAAGTG	25
c-H-Ras1	MT.....	2
G1		
Phosphate binding		
H-Gem	EDHCRRSSWSSDSTDSVIS...SESNTYIRVVLIGEGGVKSTLANIFAGV	98
M-Gem	EEHCRRTWSSDSTDSVIS...SESNTYIRVVLIGEGGVKSTLANIFAGV	97
Rad	TQGFRLDWPEDSEDSLSGGSDSESVYKVLILGAPGVKSTLANIFAGV	75
c-H-Ras1	EYKLVVVGAGGVGKSALTIQL..I	24
G2		
Effector (GAP)		
H-Gem	HDSMDSDCVGLGDTYERTLMVDGESATIIILLDMWENKGENE...WLHDHC	146
M-Gem	HDSMDSDCVGLGDTYERTLMVDGESATIIILLDMWENKGENE...WLHDHC	145
Rad	EDGPEAE...AGHTYDRSIVVDGEASLMVVDIWEQDQGR...WLPHGC	119
c-H-Ras1	QNHFEVDYDPTIEDSYRKQVVIDGETC...LLDILDITAGQEEYSAMRDQY	71
G3		
Phosphate binding		
H-Gem	MOVGDAYLIYYSITDRASFEKASELRVQLRRARQTEDIPITIIYGNKSDLV	196
M-Gem	MOVGDAYLIYYSITDRASFEKASELRVQLRRARQTEDIPITIIYGNKSDLV	195
Rad	MAMGDAYVIYYSVTDKGSFEKASELRVQLRRARQTDIPITIIYGNKSDLV	169
c-H-Ras1	MRTGEGFLCYFAINNTKSFEDIHQYBEQIKSVKSDSDVPMVLYGNKCDLA	121
G4		
Guanine binding		
H-Gem	RCREYSVSEGRACAVVEDCKFIETSAAVQHNKYKELEFGIVQVQLRRDSK	246
M-Gem	RCREYSVSEGRACAVVEDCKFIETSAAVQHNKYKELEFGIVQVQLRRDSK	245
Rad	RSREYSVSEGRACAVVEDCKFIETSAALHNKYQALFEFGIVQVQLRRDSK	219
c-H-Ras1	ARTVESRQAQDLARSYGIPYIETSAKTROGVDAFYTLVREIRQHLRK	170
G5		
Guanine binding		
H-Gem	EKNERRLAYQKRKESMPKRRFVGKIVAKNNKNMAFKLKSCHDLSQL	296
M-Gem	EKNERRLAYQKRRESIPKRRFVGKIVAKNNKNMASSSKSCHDLSQL	295
Rad	EANARRQAGTRRRESLGGKAKRFLGRIVARNRKNMAFRKSKSCHDLSQL	269
c-H-Ras1	LNPPDESFGGCMSCCKVLS	189

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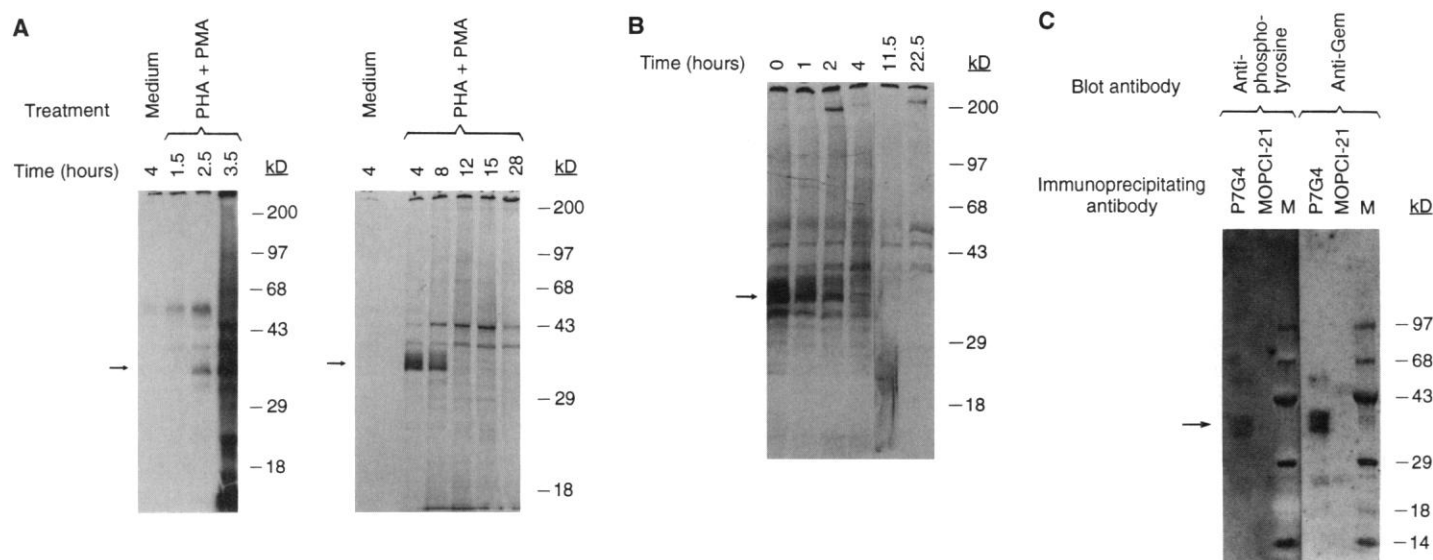
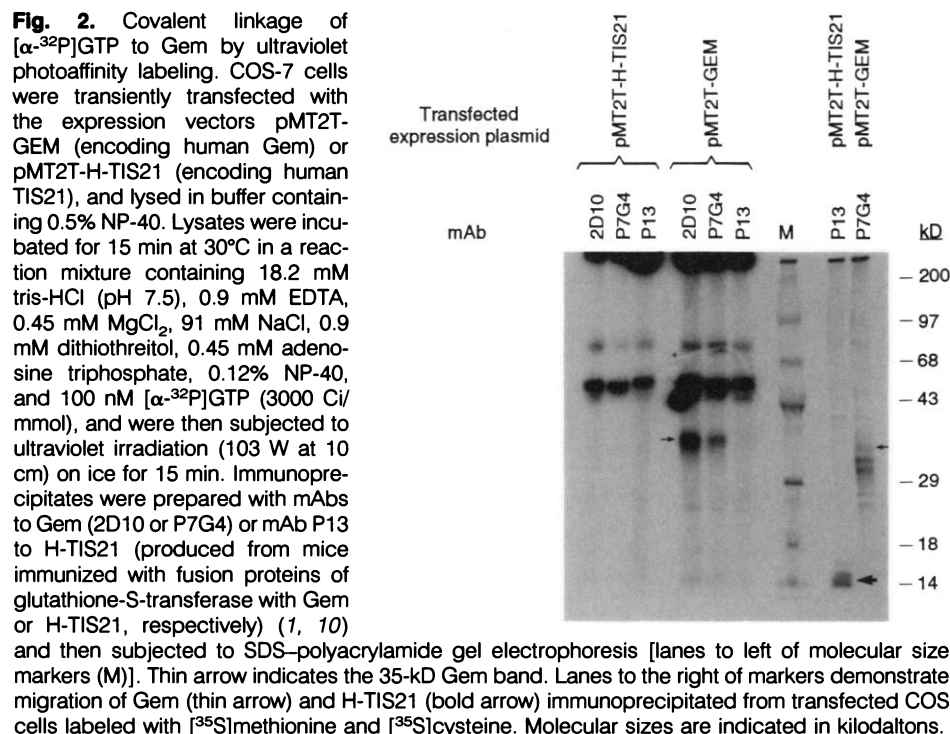
vative substitution (E for D) in the invariant, Mg^{2+} -binding position. The ENKG sequence is the most likely sequence corresponding to DXXG because the invariant

glycine residue is thought to play a central role in inducing conformational changes in guanosine triphosphatases (GTPases) as they cycle between GTP- and guanosine diphos-

phate-bound states (7, 8). Although the sequence EQDG occurs in Rad in a position corresponding to ENKG in Gem, the sequence DIWE has been proposed to correspond to the G3 motif in this protein (3). The amino acid following Gly¹³⁷ in Gem is Glu, corresponding to Gln⁶¹ in H-Ras. Analyses of H-Ras and other GTPases indicate that either Gln or Glu at position 61 can perform similar functions (5, 9).

The ability of Gem to bind guanine nucleotides was confirmed with the recombinant protein. We used ultraviolet photoaffinity labeling to link [α -³²P]GTP to proteins in lysates of COS cells transfected with a Gem expression vector (pMT2T-GEM) (Fig. 2). The major ³²P-labeled protein immunoprecipitated with monoclonal antibodies (mAbs) to Gem migrated at the same molecular size, 35 kD, as similarly immunoprecipitated, metabolically labeled Gem. No specific [α -³²P]GTP cross-linking was apparent with lysates of cells transfected with, and specifically immunoprecipitated with antibodies to, the human homolog of TIS21 (H-TIS21) (1, 10), a protein that does not bind GTP.

The transcription of *ras* superfamily genes is not known to be regulated, although the amount of RhoB mRNA is modulated by growth factors (11). We analyzed the synthesis, degradation, and modification of Gem in human CD4⁺ T cells stimulated with phyto-



10⁶ cpm)] (A) or equivalent cell numbers (35 $\times$ 10⁶ cells) (B) were subjected to immunoprecipitation with mAb 2D10 to Gem. Gem (arrows) was detected by electrophoresis and autoradiography. (C) Proteins immunoprecipitated with mAb P7G4 to Gem or the control mAb MOPC-21 (Cappel) from lysates of COS-7 cells transfected with pMT2T-GEM were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and subjected to immunoblot analysis with mAb 4G10 to phosphotyrosine (1 μ g/ml) (UBI) or a 1:1000 dilution of rabbit polyclonal antibodies to a Gem peptide (Gem-A). The blot was subsequently incubated with ¹²⁵I-labeled protein A (72 μ Ci/ μ g; 10⁵ cpm/ml) (Dupont Biotechnology Systems) and subjected to autoradiography. Arrow indicates Gem. M, molecular size markers.

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hemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA) (Fig. 3). Gem was first synthesized between 1.5 and 2.5 hours after the onset of mitogenic stimulation; synthesis was increased at 3.5 hours but had declined by 8 hours after treatment, with little protein being produced after 12 hours (Fig. 3A). Approximately 50% of newly synthesized, ^{35}S -labeled Gem was degraded during

the first hour after the label was removed from the medium, and the labeled protein was barely detectable after 4 hours (Fig. 3B). Thus, in mitogen-activated T cells, Gem is expressed during the G1 phase of the cell cycle. This transient pattern of Gem expression is unusual among Ras-related proteins.

Gem mRNA was induced in primary T cells, B cells, monocytes, and fibroblasts after activation with PHA plus PMA, *Staphylococcus aureus*, PMA, or fetal bovine serum, respectively (12). In contrast, no detectable Gem mRNA was observed in a variety of transformed cell lines including T cell, B cell, pre-B cell, plasmacytoma, myeloid, erythroid, and epithelial cell lines (13). A small amount of Gem mRNA, but no protein, was detected in U266 plasmacytoma and U1242 glioma cell lines. However, NTERA-2, a human embryonic carcinoma cell line (14), expressed both Gem mRNA and protein. A Northern (RNA) blot of various murine tissues revealed Gem mRNA to be most abundant in thymus, spleen, kidney, lung, and testis, with relatively few or no transcripts in heart, brain, liver, and skeletal muscle (Fig. 4). The tissue-specific expression of *gem* contrasts with that of *rad*; *Rad* mRNA was detected in large amounts in cardiac and skeletal muscle and was undetectable in kidney (3).

Gem appeared as a diffuse band after denaturing polyacrylamide gel electrophoresis (Fig. 3A). Treatment of immunoprecipitated Gem from T cells with potato acid phosphatase resulted in a single sharp band that migrated at a position corresponding to the leading edge of the nontreated protein (2). Immunoblot analysis with antibodies to phosphotyrosine of Gem immunoprecipitated from COS cells overexpressing Gem established

that Gem was phosphorylated on tyrosine (Fig. 3C). Phosphorylation is thus a potential mechanism by which Gem function may be modulated.

Immunofluorescence analyses of CV-1 cells transfected with a Gem expression vector showed that full-length Gem localized predominantly to the plasma membrane (Fig. 5A). Vesicular staining surrounding the nucleus was also observed. The lack of staining in live, nonpermeable transfected cells, indicated that Gem is localized on the cytoplasmic face of the plasma membrane. A Gem mutant in which the NH_2 -terminal 70 amino acids were replaced by a 14-amino acid tag was associated with the inner face of the plasma membrane (Fig. 5B). In contrast, a Gem mutant lacking the COOH-terminal 61 amino acids was localized in the nucleus, where it appeared in aggregates (Fig. 5C). Therefore, a sequence within the COOH-terminal 62 amino acids appears to be necessary for membrane localization. The COOH-terminus of Gem contains a single cysteine (position 290 in the human protein) that could serve as a target for lipid modification. However, because this cysteine is not part of a CAAX motif, a mechanism distinct from those used for other members of the Ras family likely determines membrane association of Gem (6, 15). The COOH-terminal cysteine is embedded within 11 amino acids that are identical in Gem and Rad, suggesting a conserved function in Rad.

Endogenously expressed Gem in NTERA-2 cells localized to the inner face of the plasma membrane (Fig. 5D). Cross-reactive binding of mAbs to Gem to centromeres in NTERA-2 (Fig. 5D) and other human cells was observed that appeared to be unrelated

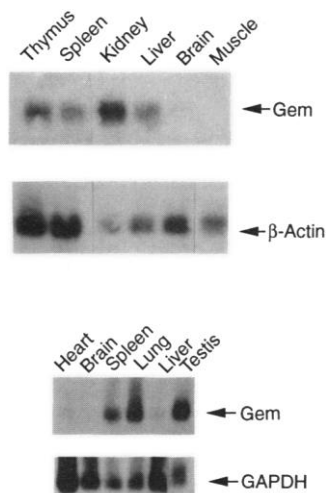
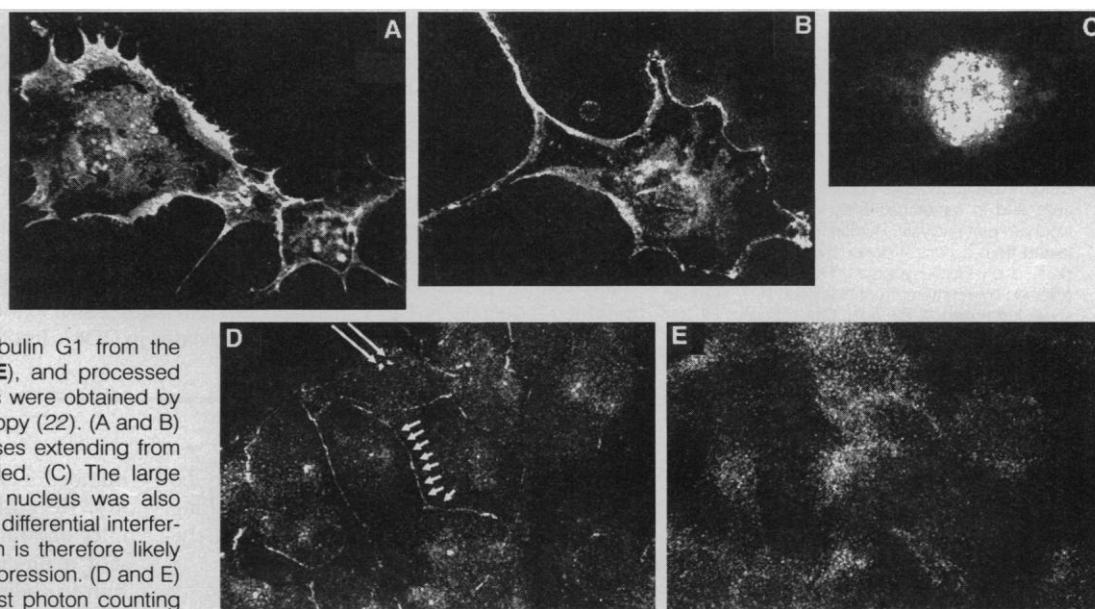


Fig. 4. Northern blot analysis of Gem mRNA in murine tissues. Total RNA was extracted from tissues obtained from adult BALB/c mice, except thymus, which was from a 4-week-old mouse. Total RNA (10 µg, upper panel) or polyadenylated RNA (2 µg, lower panel) (Clontech) was analyzed on Northern blots probed with a full-length murine *gem* cDNA and either a murine β -actin cDNA (upper panel) (21) or a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (lower panel) (Clontech).

Fig. 5. Localization of Gem in transfected CV-1 cells and untransfected NTERA-2 cells. CV-1 cells were transiently transfected with pMT2T-GEM (A), pMT2T-delGEM[amino acids 71 to 296] (B), or pMT2T-delGEM[amino acids 1 to 234] (C) and processed for Gem immunofluorescence analysis 48 hours after transfection. NTERA-2 cells were labeled with mAb 2D10 to Gem (D) or control immunoglobulin G1 from the MOPCI-21 myeloma (Cappel) (E), and processed for immunofluorescence. Images were obtained by laser scanning confocal microscopy (22). (A and B) Note the long and short processes extending from the cell that are intensely labeled. (C) The large concentration of antigen in the nucleus was also visible with transmitted light and differential interference contrast optics; the protein is therefore likely aggregated as a result of overexpression. (D and E) Images were acquired in the fast photon counting mode with the same settings, which were chosen to ensure linearity of fluorescence intensity with pixel intensity. Staining of the plasma membrane (short arrows) and centromeres (long arrows) is indicated in (D).



to expression of the Gem protein.

The effect of deregulated expression of Gem on cell growth and morphology was investigated by permanently transfecting NIH 3T3 cells with a mycophenolic acid-selectable mammalian expression vector (pMSG) containing the entire Gem coding region (pMSG-GEM). Deregulated expression of Gem reduced the number of selectable colonies to <0.1% of that obtained with cells transfected with the vector alone (16). Similarly, the number of selectable colonies obtained after cotransfection of 3T3 cells with pMT2T-GEM and pSV2-neo was ~20% of that obtained after transfection with pSV2-neo alone (2). Transfection of pMSG-GEM into 3T3 cells previously transformed by *v-fms*, *v-H-ras*, or *v-raf* did not yield viable colonies (16). Because signals transduced through Raf act subsequent to those transduced through Ras (17), these results suggest that Gem did not inhibit growth simply by competing with Ras for substrates or regulatory factors. Rather, Gem must inhibit growth or induce cell death by some other mechanism. Gem thus appears to be a tightly regulated protein that may function in receptor-mediated signal transduction.

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12. Gem mRNA abundance in primary, nontransformed cells and in tumor cell lines was determined by Northern blot analysis. Human monocytes were obtained from a normal donor by leukaphoresis and purified on Ficoll-Hypaque (Pharmacia) gradients followed by countergradient elutriation [T. L. Gerrard, C. H. Jurgensen, A. S. Fauci, *Cell. Immunol.* **82**, 394 (1983)]. Monocytes were either unstimulated or were stimulated with PMA (50 ng/ml) for 22 hours before harvesting. Normal human B cells were from tonsillar mononuclear cells stimulated with *S. aureus* for 0, 2, 8, 16, 24, 48, or 96 hours; the highest Gem mRNA concentration in B cells was apparent at 2 and 8 hours. Serum-deprived human embryonic fibroblasts were stimulated with 20% fetal bovine serum as described previously (1).
13. The transformed cell lines that were analyzed included CEM, YT, Molt 4, Jurkat, Molt 3, H9, EL-4, ARH77, Raji, EW, BJAB, HPBALL, Nalm 6, Rch, U937, THP, HL60, K562, HEL, and HeLa. Jurkat, Molt 3, and H9 were analyzed before and after activation with PHA (1 μ g/ml) and PMA (10 ng/ml). EL-4 was analyzed before and after activation with PMA (10 ng/ml).
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16. 3T3 cells (10^5) or their transformed derivatives—the *v-fms*— and *v-H-ras*—transformed lines [M. Noda, Z. Selinger, E. M. Scolnick, R. Bassin, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5602 (1983)] were obtained from R. Bassin, and the *v-raf*—transformed line was obtained from U. Rapp (both of the National Cancer Institute)—were transfected with 1 μ g of either pMSG (Pharmacia) or pMSG-GEM (produced by inserting a full-length human Gem cDNA fragment into the Sma I site of pMSG) by a calcium phosphate procedure [M. Barbacid, *J. Virol.* **37**, 518 (1981)]. The number of surviving colonies was determined between 10 and 21 days after selection. Approximately 100 colonies per 10^5 cells were obtained after transfection of the various cell lines with pMSG. At least 10 independent transfections (10^5 cells per transfection) were analyzed for each cell line transfected with pMSG-GEM.
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22. CV-1 cells were fixed with cold methanol:acetone (50:50) on ice for 10 min and rinsed overnight in phosphate-buffered saline at 4°C. Fixed cells were labeled sequentially with mAb 2D10, fluorescein-conjugated rabbit antibodies to mouse immunoglobulin (DAKO), and fluorescein-conjugated goat antibodies to rabbit immunoglobulin (Boehringer Mannheim). NTERA-2 cells were grown on glass cover slips in Dulbecco's minimum essential medium containing 10% fetal bovine serum, and processed for fluorescence microscopy as for CV-1 cells. pMT2T-GEM was constructed by inserting the Eco RI fragment (nucleotides 1 to 1786) of clone 270-4, containing the entire open reading frame of human Gem, into the pMT2T vector (20). The deleted constructs were produced with nucleotide modifications to the human *gem* cDNA engineered with the polymerase chain reaction and were inserted into the pBluescript vector (Stratagene); the constructs were subsequently characterized by nucleotide sequencing of the polymerase chain reaction-generated regions and cloned into pMT2T. Transfection of COS-7 cells with pMT2T expression vectors containing either full-length or deleted *gem* cDNAs, metabolic labeling of the cells, and immunoprecipitation of cell lysates with the 2D10 mAb demonstrated the expected sizes for the engineered Gem constructs. The leader sequence for the pMT2T-delGEM[amino acids 71 to 296] was derived from T7.TagTM (Novagen). Confocal microscopy was performed with an MRC 600 Bio-Rad laser confocal scanning system and a Zeiss Axioplan microscope. Images were acquired with a 63 $\times$ Zeiss planapo objective in the fast photon counting mode, enhanced with Adobe Photoshop software, and printed with a Kodak XL7700 printer.
23. We thank G. A. van Seventer for purification of CD4⁺ T cells, D. Webb for providing elutriated peripheral blood monocytes, J. Kehrl for a Northern blot of activated B cells, P. Scherle for a Northern blot of multiple tumor lines, and P. Davis, D. Borman, and C. Washington for technical assistance; and R. A. Kahn for discussions and advice.

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Ability of HIV to Promote a T_H1 to T_H0 Shift and to Replicate Preferentially in T_H2 and T_H0 Cells

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Both interferon γ (IFN- γ) produced by T helper 1 (T_H1) lymphocytes and interleukin-4 (IL-4) produced by T_H2 lymphocytes were reduced in either bulk circulating mononuclear cells or mitogen-induced CD4⁺ T cell clones from the peripheral blood of individuals infected with human immunodeficiency virus (HIV). There was a preferential reduction in clones producing IL-4 and IL-5 in the advanced phases of infection. However, enhanced proportions of CD4⁺ T cell clones producing both T_H1-type and T_H2-type cytokines (T_H0 clones) were generated from either skin-infiltrating T cells that had been activated in vivo or peripheral blood T cells stimulated by antigen in vitro when cells were isolated from HIV-infected individuals. All T_H2 and most T_H0 clones supported viral replication, although viral replication was not detected in any of the T_H1 clones infected in vitro with HIV. These results suggest that HIV (i) does not induce a definite T_H1 to T_H2 switch, but can favor a shift to the T_H0 phenotype in response to recall antigens, and (ii) preferentially replicates in CD4⁺ T cells producing T_H2-type cytokines (T_H2 and T_H0).

Defects in T_H immune function can be detected in HIV-infected individuals long before the occurrence of a decline in the

number of circulating CD4⁺ T lymphocytes (1). Recently, it has been shown in both mice and humans that CD4⁺ T cells represent a functionally heterogeneous population in their profile of cytokine production (2). T_H1 cells produce IFN- γ , IL-2, and tumor necrosis factor (TNF)- β ; these cells promote macrophage activation (which re-

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