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10. In our experimental approach, the mRNA differential display technique (*25*) was modified by the use of degenerate primers related to known gene families (family-specific primers) for polymerase chain reaction (PCR). RNAs and oligo(dT)-primed cDNAs were prepared from NK, B, T, and myeloid cells by standard techniques. Ig-SF– and C-type lectin–related primers used for cDNA amplification were designed around consensus sequence positions derived from alignments of Ig- (*26*) and C-type lectin (*27*) amino acid sequences. Primers were 18 to 20 nucleotides (nt) long and included degenerate nucleotides of up to 516-fold degeneracy to ensure efficient priming of unidentified sequences. PCR reactions were done as described (*28*). The cDNA fragments selectively amplified from NK cDNA were gel-purified, cloned into pCRII (Invitrogen), and sequenced by dideoxynucleotide chain termination. The identified sequences were compared with the DNA sequence databases. Selective expression of cDNA clones on NK cells was determined by Northern blot analysis with a panel of RNAs obtained from several tissues and hematopoietic cells. This strategy led to the identification of several genes differentially expressed either in NK cells, in B cells, or in myeloid and dendritic cells.
11. Ig-SF degenerate primers used to amplify this fragment were CCHTGGARCTKGTRRTSACAG-5′ and CCRTAGCAYCYGTAKRTYCC-3′.
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14. Hybridizing transcripts were also detected in the spleen after a long exposure. Polyadenylated [poly(A)$^+$] RNAs from human tissues were purchased from Clontech (Palo Alto, CA). Poly(A)$^+$ RNA from cell lines and cell clones were extracted by standard procedures. RNA samples of 1 μg were fractionated in 1% agarose–2.2 M formaldehyde gel, transferred to nylon membrane by capillary transfer in 10× SSC [1× SSC is 0.15 M sodium chloride–0.015 M sodium citrate (pH 7.2)], and hybridized with ^{32}P-labeled probe. Hybridizations were in 6× SSC, 5 mM EDTA, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.2% SDS, 10% dextran sulfate, and sonicated salmon sperm DNA (100 μg/ml) for 16 hours at 65°C. Membranes were washed two times at room temperature for 30 min each with 3× SSC–0.1% SDS and three times at 65°C for 30 min each with 3× SSC–0.1% SDS, 0.3× SSC–0.1% SDS, and 0.1× SSC–0.1% SDS, respectively, and exposed to Kodak X-Omat AR film at −70°C with intensifying screens.
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18. NKAT1-related transcripts were amplified from cDNA by RT-PCR. cDNA synthesis was as described (*10*). PCR was carried out for 35 cycles, each consisting of 1-min steps at 94°C, 62°C, and 72°C, according to a standard procedure. The primers used for cDNA amplification were CGGCAGCACCATGTCGCTC-5′ (nt 23 to 41) and GTGCTGCGTTAAGAGGGAG-3′ (nt 1441 to 1423). PCR yielded products of ~1.4 kb and ~1.7 kb, which were gel-purified, subcloned into pCRII (Invitrogen), and sequenced by dideoxynucleotide chain termination.
19. To assess the expression and the possible polymorphism of NKAT genes, NKAT transcripts were amplified from NK clones derived from three unrelated donors (*18*) and sequenced. All four NKAT genes were expressed in at least one NK clone for each donor. The sequence of one transcript revealed a variant of NKAT2 (designated NKAT5, accession number L41347), with a charged residue in the transmembrane portion and a shorter cytoplasmic tail without Tyr-x-x-Leu pairs. This variant may have different pairing and signaling properties.
20. For oligonucleotide typing of NKAT genes, an NKAT fragment was amplified by RT-PCR as described (*18*). The primers used for amplification were TTCCCTCCTGGCCCACCCA-5′ (nt 119 to 137 of NKAT1)′ and TCCCTGGATAGATGGTACA-3′ (nt 520 to 502 of NKAT1). Amplified fragments of 402 bp were separated by electrophoresis in a 1.8% agarose gel, transferred to a nylon membrane by capillary blot in 0.4% NaOH, and hybridized with the following ^{32}P-labeled oligonucleotides: TCGCATGACGCAAGACCTGGCAG (NKAT1-specific), CATGATGCTTGCCCTTGCAG (NKAT2-specific), CATGATGCTTGCCCTTGCAG (NKAT3-specific), and CCCTTGATGCCTGTCCTTGCA (NKAT4-specific). Hybridization was carried out for 2 hours at 42°C in 5× SSPE [1× SSPE is 0.18 M sodium chloride–0.01 M sodium phosphate (pH 7.4)–0.001 M EDTA], 0.5% SDS, and 5× Denhardt's solution. Membranes were washed in 6× SSC–0.5% SDS for 10 min at room temperature and for 10 min at 65°C, 60°C, 62°C, and 65°C, respectively.
21. NKAT1 was amplified by RT-PCR, cloned into pCRIII (Invitrogen), and transfected by electroporation into Jurkat cell line. Stable transfectants were selected in G418-containing medium and analyzed for cell surface expression of p58 molecules with mAb HP3E4 (*8*). HP3E4-positive cells were sorted on a FACStar plus sorter (Becton Dickinson) and expanded.
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29. We thank M. Lopez-Botet (Hospital de la Princesa, Madrid, Spain) for the gift of mAb HP3E4; M. Cella, K. Karjalainen, J. Kaufman, and G.-k. Sim (Basel Institute for Immunology, Basel, Switzerland) for reviewing the manuscript; and A. Lanzavecchia for helpful discussions and advice. The Basel Institute for Immunology was founded and is supported by Hoffmann–La Roche Ltd, CH-4002 Basel.

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The Role of Igβ in Precursor B Cell Transition and Allelic Exclusion

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Lymphocytes express multicomponent receptor complexes that mediate diverse antigen-dependent and antigen-independent responses. Despite the central role of antigen-independent events in B cell development, little is known about the mechanisms by which they are initiated. The association between the membrane immunoglobulin (Ig) M heavy chain (mμ) and the Igα-Igβ heterodimer is now shown to be essential in inducing both the transition from progenitor to precursor B cells and subsequent allelic exclusion in transgenic mice. The cytoplasmic domain of Igβ is sufficient to induce these early antigen-independent events by a mechanism that requires conserved tyrosine residues in this protein.

Membrane-bound Ig mediates several physiological responses in both developing and mature B lymphocytes (*1*). These responses can be divided into two categories: One set of events is triggered by antigen, whereas a second group is antigen-independent. The antigen-independent responses occur early in the B cell developmental pathway and can be induced by mμ even in the absence of light chain synthesis. The first of these events is a discreet developmental transition from progenitor B cell (pro–B cell) to precursor B cell (pre–B cell). Disruption of either the transmembrane domain–encoding region of the mμ gene or the recombinase activating genes (*RAG*s) that are required for Ig gene assembly results in lymphocytes that fail to develop beyond the pro–B cell stage (*2*, *3*). This developmental deficiency can be specifically complemented by the transgenic addition of a rearranged mμ gene (*4*, *5*). B cells that develop to the pre–B cell stage then undergo heavy-chain allelic exclusion, a second antigen-independent, mμ-mediated response (*6*). Expression of mμ in pre–B cells

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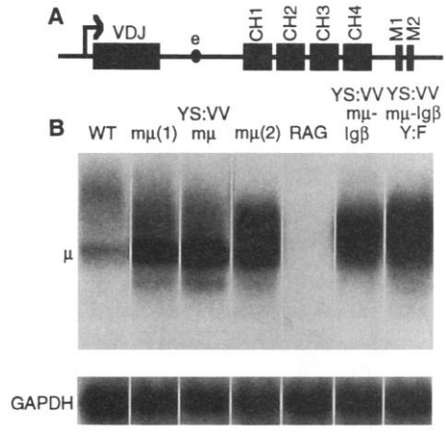

Fig. 1. Immunoglobulin transgene expression in *RAG-1*$^{-/-}$ mice. (**A**) The mouse-human hybrid mμ transgene (*19*). VDJ, variable region coding exon; e, mouse heavy chain enhancer; CH1 to CH4, human constant region coding exons; M1 and M2, transmembrane domain coding exons. (**B**) Human mμ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA abundance in bone marrow samples from *RAG-1*$^{-/-}$ transgenic mice and their *RAG-1*$^{-/-}$ littermate controls. Two independently derived strains of mμ transgenic mice are indicated as mμ(1) and mμ(2). The mμ(1) transgenic strain was used throughout. Bone marrow RNA samples were also tested by ribonuclease protection and all strains were shown to produce only the membrane form of μ mRNA. WT, wild-type mouse; RAG, *RAG-1*$^{-/-}$ mouse; YS:VV mμ, mμ transgene with Tyr^{587} and Ser^{588} in the transmembrane domain replaced by Val;YS:VV mμ-Igβ Y:F, Tyr^{195} and Tyr^{206} in the cytoplasmic tail of the YS:VV mμ-Igβ chimera were replaced with Phe; μ, position of the human mμ mRNA; and GAPDH, position of the control GAPDH mRNA.

inhibits V to DJ recombination resulting in the activation of only one of two antibody alleles in a given cell (*7*, *8*). Analogous events have also been described in developing T cells (*9*).

Although much is known about how B and T cell receptors activate antigen-driven responses, the molecular mechanisms by which earlier antigen-independent events occur have not been determined. In mature B cells, exposure to antigen produces two categories of responses: activation and antigen presentation. The signals that produce these responses require receptor cross-linking and are mediated by two closely related Ig-associated proteins—Igα (*10*) and Igβ (*11*)—that form a disulfide-linked heterodimer (Igα-Igβ). Both Igα and Igβ belong to a larger family of antigen receptor–associated signal transducers that share a tyrosine-containing cytoplasmic sequence motif, termed ARH1, TAM, or ARAM (*12*). B cell activation by Igα-Igβ involves triggering the Src and Syk family kinases and requires receptor tyrosine phosphorylation (*13*). A second pathway that may be activated by Igα-Igβ results in internalization and antigen processing and does not require phosphorylation of the conserved ARH1 tyrosine residues (*14*). Additional levels of regulation, as well as independent signaling roles for Igα and Igβ, have been suggested by the observations that Igα and Igβ bind to different sets of tyrosine kinases (*15*) and that the two proteins stimulate different biochemical activities in transfected lymphoid cell lines (*16*, *17*).

Igα and Igβ are produced early by developing B cells (*10*, *11*, *18*), and, by analogy to more mature B cells, it has been proposed that either one or both molecules mediate mμ-induced antigen-independent events. To test this hypothesis and to examine the mechanism by which Ig influences the antigen-independent phase of B cell development, we created strains of transgenic mice that carried specifically altered human mμ genes (Fig. 1). Nineteen independent transgenic strains that carry a human-mouse hybrid mμ gene were created (*19*), and all strains tested showed high concentrations of membrane IgM mRNA (Fig. 1B); normal variation between strains is illustrated by mμ(1) and mμ(2).

To determine whether the induction of the pro–B cell to pre–B cell transition requires interaction between mμ and Igα-Igβ, we produced a strain of transgenic mice that carried a mμ transgene in which codons encoding Tyr^{587} and Ser^{588} in the transmembrane domain were replaced by Val (YS:VV mμ) (*20*). We chose this mutation because transfection experiments have shown that it results in a normally anchored mμ protein (*21*) that fails to associate with endogenous Igα-Igβ (*17*, *22*), is unable to trigger either antigen presentation or B cell activation (*20*) and does not require Igα or Igβ for transport to the cell surface (*17*, *22*). The YS:VV mμ and control mμ transgenes were transferred into a *RAG-1*–deficient background (*RAG-1*$^{-/-}$) in which B cell development is blocked at the pro–B cell stage (*2*). Complementation was assessed by staining of bone marrow cells with anti-B220 and either anti-CD43 or anti-CD2 antibodies (*5*, *23*) (Fig. 2). Pro–B cells are B220$^+$, CD43$^+$, and CD2$^-$, whereas pre–B cells are B220$^+$, CD43$^-$, and CD2$^+$; the latter are missing in the bone marrow of *RAG-1*$^{-/-}$mice (*2*). Expression of the control mμ transgene in the *RAG-1*$^{-/-}$ background restored the B220$^+$CD43$^-$ and B220$^+$CD2$^+$ pre–B cell pools (Fig. 2) (*4*, *5*), but transgenic mμ was not expressed on the cell surface and did not allow B cells to develop beyond the pre–B cell stage (*4*, *5*). The extent of complementation achieved with the mouse-human hybrid transgene was similar to that described for other mμ transgenes, despite a two- to fivefold difference in the amount of mμ expressed in the different strains (*4*, *5*) (Fig. 1); both wild-type mμ strains showed similar biological activities in a *RAG-1*$^{-/-}$ pre–B cell reconstitution assay. In contrast, the mutant YS:VV mμ transgene had no effect on developing B cells in three independently derived transgenic strains (Fig. 2). Lack of complementation was not due to insufficient YS:VV mμ transgene expression because the mRNA abundance was greater

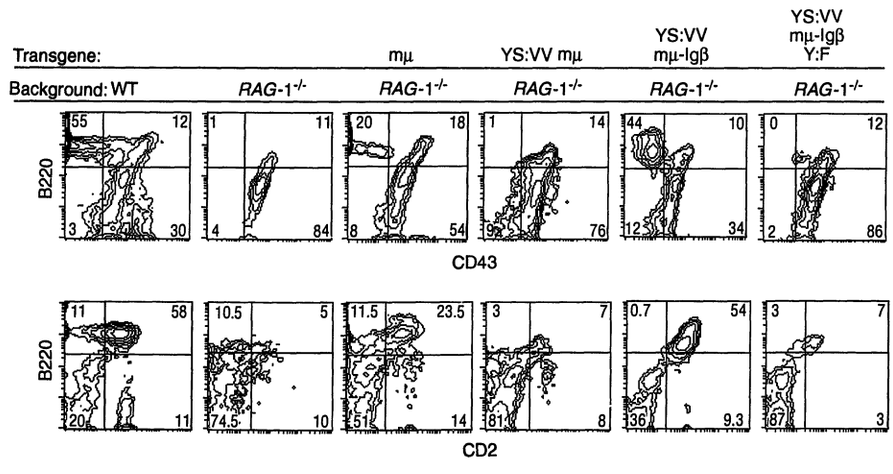

Fig. 2. B cell development in the bone marrow of *RAG-1*$^{-/-}$ mμ transgenic mice. Bone marrow cells from 6- to 8-week-old transgenic mice were analyzed on a Becton Dickinson FACScan fluorescence-activated cell sorter with CELLQuest software. Cells were stained with phycoerythrin-labeled anti-B220 (Pharmingen) and either fluorescein isothiocyanate–labeled anti-CD43 (S7 hybridoma; American Type Culture Collection) or anti-CD2 (Pharmingen) antibodies. The lymphocyte population was gated according to standard forward- and side-scatter values. The numbers in each quadrant represent the percentages of gated lymphocytes. The absolute numbers of bone marrow cells recovered were 2×10^7 for wild type (WT) and between 1×10^7 and 1.5×10^7 for all transgenic strains. The number of cells in the CD2$^-$B220$^+$ pool was not consistently increased in the *RAG-1*$^{-/-}$ and *RAG-1*$^{-/-}$ mμ mice.

than or equal to that of mμ transgenic strains that did reconstitute the pre–B cell pool (Fig. 1); moreover, similar amounts of intracellular staining with fluorescent antibodies to human mμ protein were detected in bone marrow samples of all strains. Interaction between mμ and Igα-Igβ thus appears essential for mμ to induce the pro–B cell to pre–B cell transition.

To explore the mechanism by which Ig induces the transition to pre–B cells, we produced a chimeric mμ transgene that encodes the external and transmembrane domains of the inactive YS:VV mμ and the cytoplasmic domain of Igβ (YS:VV mμ-Igβ) (*17*). In tissue culture experiments, YS:VV mμ-Igβ mediates Ca^{2+} and phosphorylation responses, although it does not associate with endogenous Igα-Igβ (*17*). Two independently derived strains that carried the YS:VV mμ-Igβ transgene in a *RAG-1*$^{-/-}$ background differed from mice that carried the parent YS:VV mμ transgene in that $B220^{+}CD2^{+}CD43^{-}$ pre–B cells developed in the bone marrow (Fig. 2). Thus, the addition of the cytoplasmic tail of Igβ to the inactive YS:VV mμ was sufficient to complement the *RAG-1* deficiency and restore the pro–B cell to pre–B cell transition.

To determine whether this transition is activated through a tyrosine-dependent or tyrosine-independent signaling pathway, we replaced Tyr^{195} and Tyr^{206} in the ARH1 domain of the cytoplasmic tail of the YS:VV mμ-Igβ chimera with Phe (YS:VV mμ-Igβ Y:F) (*17*). High-level expression of the YS:VV mμ-Igβ Y:F transgene failed to complement the pre–B cell deficiency in the bone marrow of *RAG-1*$^{-/-}$ mice in two independently derived strains (Fig. 2). Thus, the cytoplasmic domain of Igβ appears sufficient to induce the antigen-independent pro–B cell to pre–B cell transition and the mechanism that mediates this response is similar to B cell activation in that it requires receptor tyrosine phosphorylation.

Inhibition of V to DJ recombination, resulting in heavy chain allelic exclusion, is another antigen-independent event triggered by expression of mμ (6). To examine the role of Igα-Igβ in the inhibition of V to DJ rearrangements, we introduced the various mμ transgenes into a genetic background that supported normal VDJ recombination. Polymerase chain reaction (PCR) assays with DNA from bone marrow cells were used to measure both V to DJ, and DJ rearrangements (Fig. 3). The controls included wild-type and *RAG-1*$^{-/-}$ mouse bone marrow samples, as well as nonrecombining intervening sequence primers. In the bone marrow samples from wild-type mice, both V to DJ and D to J recombination was detected, whereas neither of these events

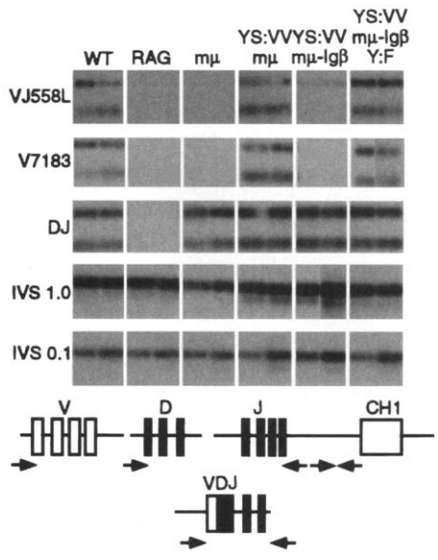

Fig. 3. Immunoglobulin gene rearrangements in transgenic mice assayed by PCR. Recombination of VJ558L family, V7183 family, or D region segments with J segments was assayed by PCR amplification (*24*). Bone marrow DNA samples were obtained from two independent mice for each of the strains indicated. The genetic backgrounds were identical for all the strains, and all were wild type for recombination except the indicated control samples from *RAG-1*$^{-/-}$ (RAG) mice. Amplified DNA was visualized by Southern hybridization with an Eco RI DNA fragment that covers the mouse J region. Control primers were from the J-CH1 intervening sequence (*24*). The germline and rearranged μ variable region and the primers (indicated by arrows) used in the PCR experiments are shown below. Primer sequences and PCR conditions were as previously described (*24*).

was observed in *RAG-1*$^{-/-}$ bone marrow samples. Introduction of the control mμ transgene into the wild-type background inhibited V to DJ but not D to J recombination, consistent with results obtained with other mμ transgenes (*24*). In contrast, the YS:VV mμ transgene had no effect on either V to DJ or DJ recombination. Thus, inhibition of V to DJ recombination by transgenic mμ requires contact between transgenic mμ and Igα-Igβ. The YS:VV mμ mutant can be restored to wild-type competence by addition of the cytoplasmic domain of Igβ, and replacement of ARH1 tyrosines in the Igβ tail with phenylalanine abrogates this activity. Thus, the cytoplasmic tail of Igβ contains sufficient information to mediate both the pro–B cell to pre–B cell transition and the inhibition of V to DJ recombination. Furthermore, both of these antigen-independent events require receptor tyrosine phosphorylation.

Our experiments demonstrate that mμ induces antigen-independent responses and B cell activation by similar mechanisms. As in B cell activation, both allelic exclusion and the induction of the pro–B cell to pre–B cell transition require contact between mμ and the Igα-Igβ heterodimer. The observation that the cytoplasmic domain of Igβ, and specifically the ARH1 tyrosine residues, was required for antigen-independent responses indicates that these events are more closely related to B cell activation than to antigen presentation, because the latter does not require receptor tyrosine phosphorylation (*14*).

In all instances in which signaling by antigen receptor–associated proteins has been analyzed, receptor aggregation has been shown to be essential for cellular activation (*25*). The observation that conserved ARH1 tyrosine residues are important in antigen-independent processes in B cells indicates either that aggregation is an intrinsic property of nascent Ig or, more likely, that a specific receptor cross-links mμ to trigger early developmental transitions.

In developing B cells, the B cell receptor (BCR) consists of a complex of proteins that include mμ, the surrogate light chains (*26*), and Igα-Igβ (*27, 28*). Our chimeric antibody experiments show that the external domains of Igα and Igβ are dispensable for triggering allelic exclusion and the transition to pre–B cells. In addition, the V region of mμ may be dispensable for pre-BCR signaling, because membrane-bound Dμ proteins are sufficient to regulate VDJ recombination and B cell development (*29*). Transgenic mμ interacts with the surrogate light chains (*28*) and these molecules are important in directing early B cell development (*30*). Thus, the surrogate chains may interact with the putative cross-linking receptor or, alternatively, they may be required for delivery of the pre-BCR to the appropriate cellular compartment for activation.

ARH1 motifs and their tyrosine residues are present in a variety of immune recognition receptors, including the T cell receptor (TCR) (*12*), and genetic experiments have shown that ARH-containing proteins and their associated kinases are important in regulating early T cell development (*31*). However, targeting individual elements of the CD3 complex is likely to interfere with the assembly of the entire TCR (*32*), and thus the contributions of individual receptor components would be difficult to evaluate. Indeed, each of these signal transducers could have either independent or overlapping functions in regulating the many developmental transitions required to complete lymphocyte development (*15–17, 33*). The chimeric antigen receptor approach described here should facilitate the evaluation of the role of individual TCR and BCR components in regulating lymphocyte development in vivo. Furthermore, the observation that the cytoplasmic do-

main of Igβ is sufficient to trigger two antigen-independent responses by a tyrosine-mediated mechanism establishes a direct link between mμ, Igβ, and tyrosine kinases in developing B cells.

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19. Bone marrow total RNA (5 μg) was subjected to electrophoresis in formaldehyde-containing agarose gels, transferred to nylon membranes, and blotted with antisense RNA probes. The μ probe covers the VD and J regions of the transgenic V region exon (*34*), and glyceraldehyde-3-phosphate dehydrogenase mRNA was used as a loading control [M. Piechaczyk *et al.*, *Nucleic Acids Res.* **12**, 6951 (1984)]. The starting construct for transgene production was a Hind III–Bam HI fragment of the human μ gene that contained constant and membrane exons modified to direct the synthesis of only the membrane form of human μ (*8*). A murine V region and enhancer (*34*) were added to increase the efficiency of transgene expression. In addition, Bam HI and Not I unique sites were placed around the membrane exons to allow for rapid replacement of the wild-type membrane coding sequence with specifically modified transmembrane domains (*17*). Transgenic mice were created by injection of purified DNA into *RAG-1*$^{-/-}$ × FVB/n F1 oocytes [R. R. Palmiter and R. L. Brinster, *Annu. Rev. Genet.* **20**, 465 (1986)], and founders were backcrossed to *RAG-1*$^{-/-}$ mice (*4, 5*).
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FAP-1: A Protein Tyrosine Phosphatase That Associates with Fas

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Fas is a cell surface receptor that controls a poorly understood signal transduction pathway that leads to cell death by means of apoptosis. A protein tyrosine phosphatase, FAP-1, capable of interacting with the cytosolic domain of Fas, was identified. The carboxyl terminal 15 amino acids of Fas are necessary and sufficient for interaction with FAP-1. FAP-1 expression is highest in tissues and cell lines that are relatively resistant to Fas-mediated cytotoxicity. Gene transfer–mediated elevations in FAP-1 partially abolished Fas-induced apoptosis in a T cell line. These findings are consistent with an inhibitory effect of FAP-1 on Fas signal transduction.

Fas (also known as APO-1 and CD95) is a cell surface receptor that is expressed on a variety of normal and neoplastic cells. It shares significant amino acid sequence homology with several members of the tumor necrosis factor receptor (TNFR) family, including p55-TNFR, CD40, and the p75–nerve growth factor receptor (NGFR), which have been shown to act as either inhibitors or inducers of cell death (*1*). The ligand for Fas is expressed predominantly on cytolytic T cells (*2*), suggesting that Fas plays a role in the effector branch of cellular immune responses. Mutations in the genes encoding Fas or its ligand have been associated with lymphoproliferative and autoimmune disorders in mice (*3*). Furthermore, alterations in Fas production have been associated with autoimmune disease in humans and susceptibility to induction of apoptosis of T cells in human immunodeficiency virus–infected persons (*4*).

Monoclonal antibodies specific against Fas induce apoptosis in many types of cells (*5*). However, in some cases antibodies to Fas stimulate cell proliferation (*6, 7*), suggesting that the intracellular signal transduction pathways used by this receptor are subject to regulation. The cytosolic domain of Fas contains no similarity to known kinases or other enzymes that might transduce signals into cells. Deletion mapping analysis has identified a domain that is required for induction of apoptosis, which is called the "death domain." This domain shares homology with sequences located in the cytosolic domains of p55-TNFR1, CD40, and p75-NGFR (*8*). For some cells, apoptosis induced by antibodies to Fas is dependent on protein synthesis inhibitors such as cycloheximide (*8*). This implies either the existence of a labile protein that suppresses Fas-generated signals leading to cell death or the induction of proteins that inhibit the ability of Fas to trigger apoptosis. A negative regulatory domain has been mapped to the COOH-terminal 15 amino acids of Fas that is not homologous to other TNFR-like proteins; deletion of this domain can abrogate the dependence on protein synthesis inhibitors for apoptosis induced by antibody to Fas (*8*).

To identify complementary DNAs (cDNAs) encoding proteins that can potentially modulate the activity of Fas, we used a yeast two-hybrid system for cDNA library screening. We used the cytosolic domain of human Fas fused to a LexA DNA binding domain (*9, 10*) and random cDNAs fused in frame with a VP16 transactivation domain. Using a His synthetase gene (*HIS3*) under the control of LexA operators as a reporter, we identified 395 His^+ colonies from an initial screen of 3×10^8 transformants. Of these, 84 were also positive when a *lacZ* gene (β-galactosidase) under the control of a LexA operator was used as an alternative reporter. Mating tests were then performed (*11*); only 2 of the 84 candidate clones (numbers 31 and 43) reacted with the cytosolic domain of Fas (Ta-

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