cium measurements support this view (*10*).

In normal cells, both the SR and Na^+-Ca^{2+} exchange will contribute to relaxation by removing Ca^{2+} from the cytosol. However, the Na^+-Ca^{2+} will be effective only when membrane potential (E_m) is negative to the Na^+-Ca^{2+} exchange reversal potential (E_{rev}). Owing to the inferred voltage dependence of Na^+-Ca^{2+} exchange, Ca^{2+} extrusion increases as E_m becomes increasingly negative to E_{rev}. Thus, during a single twitch the trajectory of the quantity $E_m - E_{rev}$ will determine both the onset and variation of Ca^{2+} efflux with time.

The foregoing facts will have important consequences for the regulation of contraction in heart muscle. If Ca^{2+} extrusion is abruptly delayed or reduced by prolonged membrane depolarization (for example, a prolonged action potential in which E_m spends less time negative to E_{rev}), the SR could sequester Ca^{2+} normally removed by the exchanger. Alternatively, accumulation of internal Na^+ as a result of glycoside applications would collapse $E_m - E_{rev}$, thereby reducing Ca^{2+} extrusion via the exchanger, with resulting increases in the SR Ca^{2+} pool. This enlarged SR Ca^{2+} store would presumably strengthen the subsequent contraction. In contrast, a brief depolarization (for example, shortened action potential) would have the opposite effect. Thus, the trajectory of $E_m - E_{rev}$ can regulate competition between the SR and Na^+-Ca^{2+} exchange for cytosolic Ca^{2+}. This suggests voltage-dependent control of sarcolemmal Ca^{2+} extrusion via Na^+-Ca^{2+} exchange can provide an effective and delicate mechanism for regulating the SR Ca^{2+} available for contraction.

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Cloning and Expression of the Human Interleukin-6 (BSF-2/IFNβ 2) Receptor

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Interleukin-6 (IL-6/BSF-2/IFNβ 2) is a multifunctional cytokine that regulates the growth and differentiation of various tissues, and is known particularly for its role in the immune response and acute phase reactions. A complementary DNA encoding the human IL-6 receptor (IL-6–R) has now been isolated. The IL-6–R consists of 468 amino acids, including a signal peptide of ~19 amino acids and a domain of ~90 amino acids that is similar to a domain in the immunoglobulin (Ig) superfamily. The cytoplasmic domain of ~82 amino acids lacks a tyrosine/kinase domain, unlike other growth factor receptors.

B CELL STIMULATORY FACTOR–2 (BSF-2) was originally identified as a T cell–derived factor that causes the terminal maturation of activated B cells to Ig-producing cells (*1*). After the cDNAs were cloned, BSF-2 was found to be identical to the 26-kD protein, IFN-β 2, myeloma-plasmacytoma growth factor and hepatocyte stimulating factor (*2–6*). It is established that BSF-2, now called IL-6, has many biological functions, which include growth and differentiation activities on B cells (*1, 2, 7*), T cells (*8*), myeloma-plasma-

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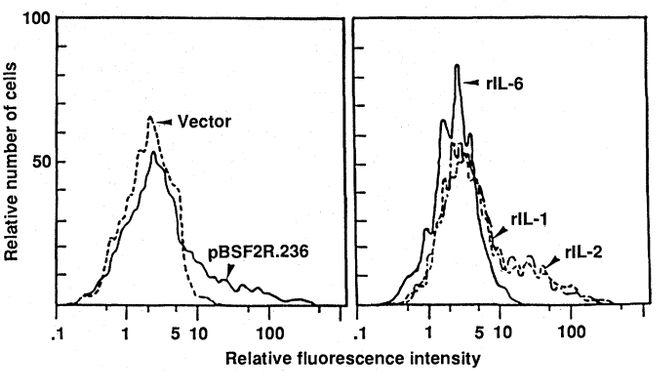


Fig. 1. Flow cytofluorometry analysis of COP cells transfected with pBSF2R.236 DNA. Left panel: murine COP cells (*24*) transfected with pBSF2R.236 DNA (——) or CDM8 vector DNA (- - -) were stained with B–rIL-6 and FITC-A as described (*13*). Right panel: COP cells transfected with pBSF2R.236 DNA were stained with ~10 ng of B–rIL-6 and FITC-A in the presence fo 200 ng of either rIL-6 (——), rIL-1 (— - — - —), or rIL-2 (- - -).

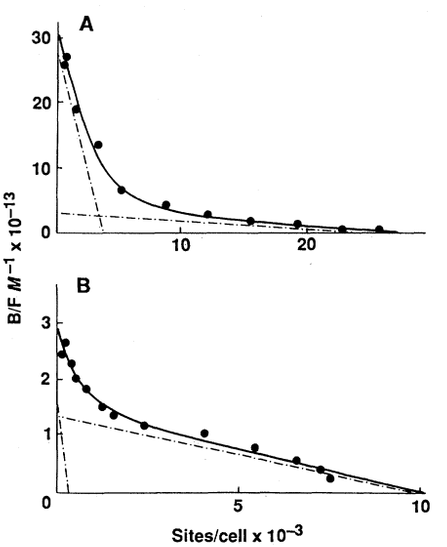


Fig. 2. Scatchard plot analysis of the IL-6–R encoded by the insert cDNA of pBSF2R.236, as well as the IL-6–R expressed on U266 cells. The IL-6–R negative human T cell line, Jurkat, was transfected with pZipNeoSVB2R [constructed by introducing the insert cDNA of pBSF2R.236 at the Bam HI site of pZipNeoSV(X)1 (*25*)] and transfectant (JBSF2R) was cloned. The IL-6 binding was assayed in both U266 (**A**) JBSF2R (**B**) as described (*14*), with ^{125}I-labeled rIL-6 (specific activity of 6.4×10^{13} cpm/g). U266, K_{d1} = 9.8 ± 2.1 p*M*, K_{d2} = 740 ± 170 p*M*, R_1 = 3000 ± 480 sites per cell, R_2 = 24,000 ± 1400 sites per cell; JBSF2R, K_{d1} = 17 ± 14 p*M*, K_{d2} = 710 ± 110 p*M*, R_1 = 240 ± 190 sites per cell, R_2 = 12,000 ± 680 sites per cell.

cytomas (*4, 5, 9*), hepatocytes (*6*), hematopoietic stem cells (*10*), and nerve cells (*11*).

To elucidate how one cytokine can mediate multiple functions, the structure of their receptor molecules must be determined. However, the low number of cytokine receptors on target cells (10^2 to 10^3 per cell) makes their isolation and characterization difficult. We now report the cloning of the cDNA for IL-6 receptor (IL-6–R) utilizing a high-efficiency COS7 cell expression system with the CDM8 vector (*12*). The expressed receptors were detected with biotinated-recombinant IL-6 (B–rIL-6) and fluorescein-conjugated avidin (FITC-A).

We constructed a cDNA library from polyadenylated [poly(A)$^+$] RNA of a human NK-like cell line, YT (*12*). Plasmid DNA was transfected into monkey COS7 cells, and the cells were stained with B–rIL-6 and FITC-A. Cells expressing the IL-6–R were obtained with a fluorescence-activated cell sorter (FACS), resulting in the identification of a candidate plasmid clone, pBSF2R.236 (*13*). To confirm that this clone contained the cDNA encoding IL-6–R, we used murine COP cells for efficient expression. More than 10% of pBSF2R.236 transfected COP cells expressed IL-6–R as measured by B–rIL-6 binding when compared to the control cells transfected with CDM8 vector DNA. Moreover, the binding of B–rIL-6 was competitively inhibited by excess amounts of rIL-6, but not rIL-1β or rIL-2 (Fig. 1).

JBSF2R, a stable transfectant expressing

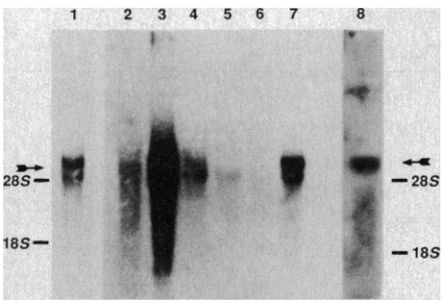
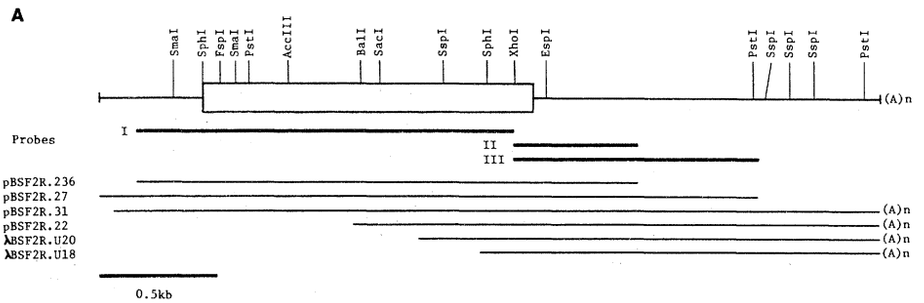


Fig. 3. RNA blot analysis of IL-6–R mRNA. Poly(A)$^+$ RNA was isolated from U266 (lanes 1, 3, and 8), U937 (lane 2), CESS (lane 4), BL29 (lane 5), Jurkat (lane 6), and YT (lane 7). RNA (10 μg) was denatured and subjected to blotting analysis (*26*) using the insert cDNA of pBSF2R.236 as a probe (lanes 1 to 7). The filter was exposed for 20 min (lane 1) or 16 hours (the other lanes). The arrow indicates the specific band hybridized to pBSF2R.236 cDNA. The band corresponding to ~28*S* is not specific, because it disappears when the Fsp I–Esp I fragment (see legend to Fig. 4A) was used as a probe (lane 8). The number of binding sites per cell, estimated by Scatchard analysis, were 29,000, 2,800, 2,700, and 3,700 for U266, U937, CESS, and YT cells, respectively. BL29 and Jurkat cells showed no detectable IL-6 binding sites.



B

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-437                                                                                                                GGCGGTCCCCTGTTCTCCCCGCTCAGGTGCGGCGCTGTGGCAGGAAGCCACCCCCTCGGTCGGCCGGTGCGCGGGGCTGT

-357  TGCGCCATCCGCTCCGGCTTTCGTAACCGCACCCTGGGACGGCCCAGAGACGCTCCAGCGCGAGTTCCTCAAATGTTTTCCTGCGTTGCCAGGACCGTCCGCCGCTCTGAGTCATGTGC

-238  GAGTGGGAAGTCGCACTGACACTGAGCCGGGCCAGAGGGAGAGGAGCCGAGCGCGGCGCGGGGCCGAGGGACTCGCAGTGTGTGTAGAGAGCCGGGCTCCTGCGGATGGGGGCTGCCCC

-119  CGGGGCCTGAGCCCGCCTGCCCGCCCACCGCCCCGCCCCGCCCCTGCCACCCCTGCCGCCCGGTTCCCATTAGCCTGTCCGCCTCTGCGGGACCATGGAGTGGTAGCCGAGGAGGAAGC

   1  ATG CTG GCC GTC GGC TGC GCG CTG CTG GCT GCC CTG CTG GCC GCG CCG GGA GCG GCG CTG GCC CCA AGG CGC TGC CCT GCG CAG GAG GTG
   1  Met Leu Ala Val Gly Cys Ala Leu Leu Ala Ala Leu Leu Ala Ala Pro Gly Ala Ala Leu Ala Pro Arg Arg Cys Pro Ala Gln Glu Val

  91  GCA AGA GGC GTG CTG ACC AGT CTG CCA GGA GAC AGC GTG ACT CTG ACC TGC CCG GGG GTA GAG CCG GAA GAC AAT GCC ACT GTT CAC TGG
  31  Ala Arg Gly Val Leu Thr Ser Leu Pro Gly Asp Ser Val Thr Leu Thr Cys Pro Gly Val Glu Pro Glu Asp Asn Ala Thr Val His Trp
                                                                                                  *** *** ***

 181  GTG CTC AGG AAG CCG GCT GCA GGC TCC CAC CCC AGC AGA TGG GCT GGC ATG GGA AGG AGG CTG CTG CTG AGG TCG GTG CAG CTC CAC GAC
  61  Val Leu Arg Lys Pro Ala Ala Gly Ser His Pro Ser Arg Trp Ala Gly Met Gly Arg Arg Leu Leu Leu Arg Ser Val Gln Leu His Asp

 271  TCT GGA AAC TAT TCA TGC TAC CGG GCC GGC CGC CCA GCT GGG ACT GTG CAC TTG CTG GTG GAT GTT CCC CCC GAG GAG CCC CAG CTC TCC
  91  Ser Gly Asn Tyr Ser Cys Tyr Arg Ala Gly Arg Pro Ala Gly Thr Val His Leu Leu Val Asp Val Pro Pro Glu Glu Pro Gln Leu Ser
          *** *** ***

 361  TGC TTC CGG AAG AGC CCC CTC AGC AAT GTT GTT TGT GAG TGG GGT CCT CGG AGC ACC CCA TCC CTG ACG ACA AAG GCT GTG CTC TTG GTG
 121  Cys Phe Arg Lys Ser Pro Leu Ser Asn Val Val Cys Glu Trp Gly Pro Arg Ser Thr Pro Ser Leu Thr Thr Lys Ala Val Leu Leu Val

 451  AGG AAG TTT CAG AAC AGT CCG GCC GAA GAC TTC CAG GAG CCG TGC CAG TAT TCC CAG GAG TCC CAG AAG TTC TCC TGC CAG TTA GCA GTC
 151  Arg Lys Phe Gln Asn Ser Pro Ala Glu Asp Phe Gln Glu Pro Cys Gln Tyr Ser Gln Glu Ser Gln Lys Phe Ser Cys Gln Leu Ala Val

 541  CCG GAG GGA GAC AGC TCT TTC TAC ATA GTG TCC ATG TGC GTC GCC AGT AGT GTC GGG AGC AAG TTC AGC AAA ACT CAA ACC TTT CAG GGT
 181  Pro Glu Gly Asp Ser Ser Phe Tyr Ile Val Ser Met Cys Val Ala Ser Ser Val Gly Ser Lys Phe Ser Lys Thr Gln Thr Phe Gln Gly

 631  TGT GGA ATC TTG CAG CCT GAT CCG CCT GCC AAC ATC ACA GTC ACT GCC GTG GCC AGA AAC CCC CGC TGG CTC AGT GTC ACC TGG CAA GAC
 211  Cys Gly Ile Leu Gln Pro Asp Pro Pro Ala Asn Ile Thr Val Thr Ala Val Ala Arg Asn Pro Arg Trp Leu Ser Val Thr Trp Gln Asp
                                  *** *** ***

 721  CCC CAC TCC TGG AAC TCA TCT TTC TAC AGA CTA CGG TTT GAG CTC AGA TAT CGG GCT GAA CGG TCA AAG ACA TTC ACA ACA TGG ATG GTC
 241  Pro His Ser Trp Asn Ser Ser Phe Tyr Arg Leu Arg Phe Glu Leu Arg Tyr Arg Ala Glu Arg Ser Lys Thr Phe Thr Thr Trp Met Val
                  *** *** ***

 811  AAG GAC CTC CAG CAT CAC TGT GTC ATC CAC GAC GCC TGG AGC GGC CTG AGG CAC GTG GTG CAG CTT CGT GCC CAG GAG GAG TTC GGG CAA
 271  Lys Asp Leu Gln His His Cys Val Ile His Asp Ala Trp Ser Gly Leu Arg His Val Val Gln Leu Arg Ala Gln Glu Glu Phe Gly Gln

 901  GGC GAG TGG AGC GAG TGG AGC CCG GAG GCC ATG GGC ACG CCT TGG ACA GAA TCC AGG AGT CCT CCA GCT GAG AAC GAG GTG TCC ACC CCC
 301  Gly Glu Trp Ser Glu Trp Ser Pro Glu Ala Met Gly Thr Pro Trp Thr Glu Ser Arg Ser Pro Pro Ala Glu Asn Glu Val Ser Thr Pro

 991  ATG CAG GCA CTT ACT ACT AAT AAA GAC GAT GAT AAT ATT CTC TTC AGA GAT TCT GCA AAT GCG ACA AGC CTC CCA GTG CAA GAT TCT TCT
 331  Met Gln Ala Leu Thr Thr Asn Lys Asp Asp Asp Asn Ile Leu Phe Arg Asp Ser Ala Asn Ala Thr Ser Leu Pro Val Gln Asp Ser Ser
                                                  *** *** ***

1081  TCA GTA CCA CTG CCC ACA TTC CTG GTT GCT GGA GGG AGC CTG GCC TTC GGA ACG CTC CTC TGC ATT GCC ATT GTT CTG AGG TTC AAG AAG
 361  Ser Val Pro Leu Pro Thr Phe Leu Val Ala Gly Gly Ser Leu Ala Phe Gly Thr Leu Leu Cys Ile Ala Ile Val Leu Arg Phe Lys Lys

1171  ACG TGG AAG CTG CGG GCT CTG AAG GAA GGC AAG ACA AGC ATG CAT CCG CCG TAC TCT TTG GGG CAG CTG GTC CCG GAG AGG CCT CGA CCC
 391  Thr Trp Lys Leu Arg Ala Leu Lys Glu Gly Lys Thr Ser Met His Pro Pro Tyr Ser Leu Gly Gln Leu Val Pro Glu Arg Pro Arg Pro

1261  ACC CCA GTG CTT GTT CCT CTC ATC TCC CCA CCG GTG TCC CCC AGC AGC CTG GGG TCT GAC AAT ACC TCG AGC CAC AAC CGA CCA GAT GCC
 421  Thr Pro Val Leu Val Pro Leu Ile Ser Pro Pro Val Ser Pro Ser Ser Leu Gly Ser Asp Asn Thr Ser Ser His Asn Arg Pro Asp Ala
                                                                  *** *** ***

1351  AGG GAC CCA CGG AGC CCT TAT GAC ATC AGC AAT ACA GAC TAC TTC TTC CCC AGA TAG CTGGCTGGGTGGCACCAGCAGCCTGGACCCTGTGGATGACAAA
 451  Arg Asp Pro Arg Ser Pro Tyr Asp Ile Ser Asn Thr Asp Tyr Phe Phe Pro Arg ---

1451  ACACAAACGGGCTCAGCAAAAGATGCTTCTCACTGCCATGCCAGCTTATCTCAGGGGTGTGCGGCCTTTGGCTTCACGGAAGAGCCTTGCGGAAGGTTCTACGCCAGGGGAAAATCAGC

1570  CTGCTCCAGCTGTTCAGCTGGTTGAGGTTTCAAACCTCCCTTTCCAAATGCCCAGCTTAAAGGGGTTAGAGTGAACTTGGGCCACTGTGAAGAGAACCATATCAAGACTCTTTGGACAC

1689  TCACACGGACACTCAAAAGCTGGGCAGGTTGGTGGGGGCCTCGGTGTGGAGAAGCGGCTGGCAGCCCACCCCTCAACACCTCTGCACAAGCTGCACCCTCAGGCAGGTGGGATGGATTT

1808  CCAGCCAAAGCCTCCTCCAGCCGCCATGCTCCTGGCCCACTGCATCGTTTCATCTTCCAACTCAAACTCTTAAAACCCAAGTGCCCTTAGCAAATTCTGTTTTTCTAGGCCTGGGGACG

1927  GCTTTTACTTAAACGCCAAGGCCTGGGGGAAGAAGCTCTCTCCTCCCTTTCTTCCCTACAGTTCAAAAACAGCTGAGGGTGAGTGGGTGAATAATACAGTATGTCAGGGCCTGGTCGTT

2046  TTCAACAGAATTATAATTAGTTCCTCATTAGCAGTTTGCCTAAATGTGAATGATGATCCTAGGCATTTGCTGAATACAGAGGCAACTGCATTGGCTTTGGGTTGCAGGACCTCAGGTG

2165  AGAAGCAGAGGAAGGAGAGGAGGGGCACAGGGTCTCTACCATCCCCTGTAGAGTGGGAGCTGAGTGGGGGATCACAGCCTCTGAAAACCAATGTTCTCTCTTCTCCACCTCCCACAA

2284  AGGAGAGCTAGCAGCAGGGAGGGCTTCTGCCATTTCTGAGATCAAAACGGTTTTACTGCAGCTTTGTTTGTTGTCAGCTGAACCTGGGTAACTAGGGAAGATAATATTAAGGAAGACAA

2403  TGTGAAAAGAAAAATGAGCCTGGCAAGAATGCGTTTAAACTTGGTTTTTAAAAAACTGCTGACTGTTTTCTCTTGAGAGGGTGGAATATCCAATATTCGCTGTGTCAGCATAGAAGTAA

2522  CTTACTTAGGTGTGGGGGAAGCACCATAACTTTGTTTAGCCCAAAACCAAGTCAAGTGAAAAAGGAGGAAGAGAAAAAATATTTTCCTGCCAGGCATGGAGGCCCACGCACTTCGGGAG

2641  GTCGAGGCAGGAGGATCACTTGAGTCCAGAAGTTTGAGATCAGCCTGGGCAATGTGATAAAACCCCATCTCTACAAAAAGCATAAAAATTAGCCAAGTGTGGTAGAGTGTGCCTGAAGT

2760  CCCAGATACTTGGGGGGCTGAGGTGGGAGGATCTCTTGAGCCTGGGAGGTCAAGGCTGCAGTGAGCCGAGATTGCACCACTGCACTCCAGCCTGGGGTGACAGAGCAAGTGAGACCCTG

2879  TCTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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Fig. 4. (**A**) Restriction endonuclease cleavage map of the insert cDNAs of pBSF2R.236 and other five clones. (**B**) Combined nucleotide sequence and deduced amino acid sequence of the insert cDNAs of pBSF2R.236 and other five clones. Numbers at the left margin of the sequence show positions of nucleotides and amino acids, respectively. The asteriks show potential *N*-glycosylation sites (Asn-X-Ser/Thr); the underlined region is a presumed signal peptide; the box encircles a presumed transmembrane domain; the dots identify a possible poly(A) addition signal. Sequencing was performed by the chain termination method (*2*).

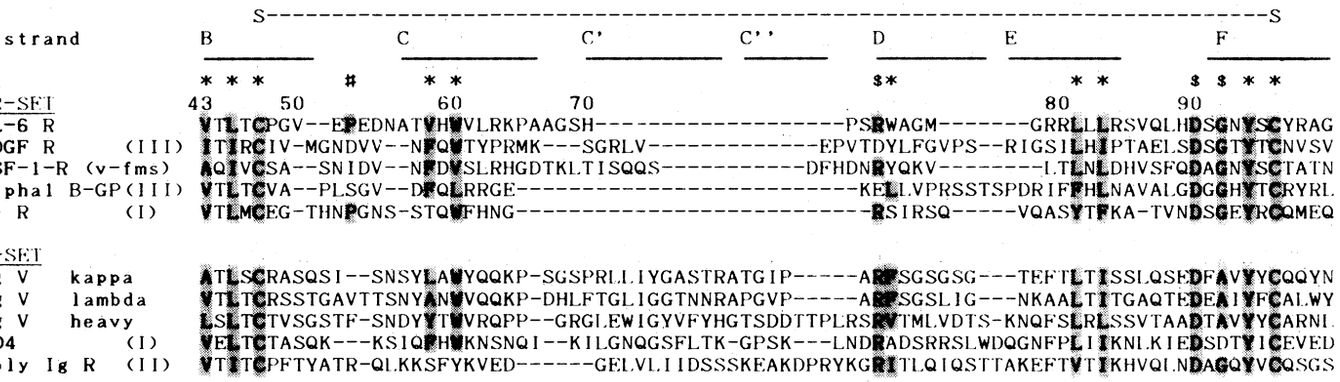

Fig. 5. Alignment of IL-6–R domain to Ig superfamily protein domains. IL-6–R, CSF-1–R (*27*) and human Ig V kappa chain V-III region (*28*) sequences were aligned to several proteins of Ig superfamily (*20*) by inspection. (*) Conserved patterns common to the V, C1, and C2 sets; ($) common to the V and C2 sets; (#) common to the C1 and C2 sets (*20*). The known locations of β-strands in Ig V domains are marked with bars and capital letters above the bars. The numbers above the aligment represent positions of amino acids of IL-6–R sequence. The position of the putative disulfide bridge within IL-6–R domain is indicated by S - - - S.

the IL-6–R from pBSF2R.236, was established from an IL-6–R negative human T cell line, Jurkat. Scatchard plot analysis was performed by the nonlinear least squares method in the AOS/VS (Data General Corp. Operating System) version of SAS release 4.07 (SAS Institute Inc., Cary, North Carolina) and the result was consistent with two classes of IL-6–R: a high-affinity binding site [dissociation constant (K_{d1}) $\sim 10^{-11} M$, number of sites per cell (R1) ~240 ± 190 (SE)] and a low-affinity binding site (K_{d2} $\sim 10^{-9} M$, R2 ~12,000 ± 680) (Fig. 2B). The myeloma cell line, U266, Scatchard plot was also consistent with there being two classes of IL-6–R with approximately the same K_d values as the IL-6–R of the JBSF2R transfectant cells (Fig. 2A). The pBSF2R.236 cDNA, therefore, can code for both high- and low-affinity binding sites, although the mechanism that determines the affinity of the IL-6–R remains to be elucidated.

The expression of IL-6–R mRNA was analyzed by RNA blots (Fig. 3). The pBSF2R.236 cDNA probe hybridized to a single species of mRNA of approximately 5000 nucleotides (nt), extracted from the YT cell line. Similar length IL-6–R mRNA was also detected in RNA extracts of the myeloma cell line U266, the histiocytic leukemia cell line U937, and the Epstein-Barr virus–transformed B cell line CESS. In fact, these cell lines had been shown to express IL-6–R (*14*). However, the T cell line Jurkat and the Burkitt's lymphoma cell line BL29, both of which had no detectable IL-6–R (*14*), had no mRNA that hybridized with the probe. The relatively high concentration of IL-6–R mRNA in U266 cells may indicate that IL-6 functions as an autocrine growth factor for myeloma cells (*5*).

Although pBSF2R.236 contained the insert cDNA for the coding region of the IL-6–R mRNA, it did not contain the full mRNA sequence, as it was only 2200 nt long. To obtain the entire sequence cDNA, we probed the same cDNA library with different labeled fragments (Fig. 4A). Three additional clones contained insert cDNAs corresponding to several parts of IL-6–R mRNA. Two other cDNA clones were also isolated from a λgt10 cDNA library from U937 cells as described (*2*). Four of the insert cDNAs have the same 3′ end followed by the poly(A) sequence (Fig. 4a). Possible poly(A) addition signals (ATAAAA) are at positions corresponding to residues 161 and 186, 200 nt upstream of the poly(A) sequence, a position that corresponds to those previously described (*15*).

The nucleotide sequence (Fig. 4B) was confirmed by data on the independent clones. There is a single open reading frame which is included in the cDNA of pBSF2R.236. In this frame, the initiator ATG, which conforms to the described criteria (*16*), is followed by 467 codons before the termination to triplet TAG. A hydropathy plot (*17*) of the deduced amino acid sequence of IL-6–R showed two major hydrophobic regions, one located between residues 1 and 20, and the other located in the region of residues 359 to 386. The former is presumably a typical signal peptide, with the predicted cleavage site between Ala^{19} and Leu^{20} (*18*). The latter, the putative transmembrane domain, is immediately followed by positively charged residues (Arg-Phe-Lys-Lys), which may represent the stop transfer signal anchoring the IL-6–R in the membrane during biosynthesis (*19*). There are six potential *N*-linked glycosylation sites (Asn-X-Ser/Thr) (five in the extracellular domain and one in the intracellular domain).

The comparative sequence data were obtained from the National Biomedical Research Foundation database (Bolt Beranek and Newman, Inc.) and Genetic Sequence Data Bank (Los Alamos National Laboratory). Homologies were found with several members of the Ig superfamily, including the Ig light chain variable (V) region, the rabbit poly–Ig receptor, the CD4 molecule and the alpha-1-B–glycoprotein (alpha 1 B-GP). Furthermore, the IL-6–R sequence between position ~20 and ~110 fulfills the criteria proposed by Williams and Barclay (*20*) for the constant 2 (C2) set of Ig superfamily as shown in Fig. 5. The IL-6–R sequence does not contain the loop (C′ and C″) between the β-strands C and D, which is characteristic of the V set. The distance between Cys^{47} and Cys^{96} is 50 residues, as described for the C2 set (*20*). The C2 set includes several adhesion molecules, the platelet-derived growth factor (PDGF) receptor, the colony-stimulating factor 1 (CSF-1) receptor, the Fc γ receptor, and the alpha 1 B-GP (*20*). The receptors for polypeptide growth factors, such as PDGF, CSF-1, and IL-6, could then be grouped in the C2 set.

The IL-6–R lacks tyrosine kinase domains, unlike some other growth factor receptors, although IL-6 has been found to be a potent growth factor for myeloma-plasmacytoma cells (*4, 5, 9*). Receptors for nerve growth factor (*21*) and growth hormone (*22*) also lack the tyrosine kinase domain. The mechanism (or mechanisms) of its signal transduction for growth and differentiation could be mediated through an unknown biochemical pathway.

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13. Monkey COS7 cells were transfected with purified plasmid DNA (2.5 μg/ml) by the DEAE dextran method (*2*). The cells were stained with B–rIL-6 after culture for 2 days. For the preparation of B–rIL-6, rIL-6 (5 μg) (*12*) in 10 μl of 0.1*M* biocarbonate buffer (*p*H 8.4) was incubated with 1.3 μg of biotin-O-succinimide ester (Biosearch #B-1000) in dimethyl sulfoxide (DMSO) (2 mg/ml) at room temperature for 3 hours, and free biotin was separated by Sephadex G-50 column. COS7 cells (5×10^5) transfected with DNA were incubated with ~10 ng of B–rIL-6 in 10 μl of biotin-free RPMI 1640 (Deficient RPMI 1640, Irvine Scientific) at 4°C for 90 min. The cells were washed by passing through fetal calf serum and further incubated with FITC-A at 4°C for 20 min. Positively stained cells were sorted out with a FACS 440 (Becton Dickinson) as described (*23*).

Escherichia coli MC1061/P3 was transformed with episomal DNA collected from the sorted cells as described (*12*). Plasmid DNA was extracted and subjected to a second round of sorting as described above Single colonies (250) were randomly picked after four rounds of selection, and plasmid DNA was extracted from each colony. Plasmid DNA with insert cDNAs greater than 2 kb was individually transfected into COS7 cells and the expression of the IL-6–R was analyzed by flow cytofluorometry. COS 7 cells transfected with plasmid DNA from two colonies were found to express the IL-6–R. One of them, pBSF2R.236, was extensively analyzed.
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A Monoclonal Antibody to the α Subunit of G_k Blocks Muscarinic Activation of Atrial K^+ Channels

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The activated heterotrimeric guanine nucleotide binding (G) protein G_k, at subpicomolar concentrations, mimics muscarinic stimulation of a specific atrial potassium current. Reconstitution studies have implicated the α and βγ subunits as mediators, but subunit coupling by the endogenous G protein has not been analyzed. To study this process, a monoclonal antibody (4A) that binds to α_k but not to βγ was applied to the solution bathing an inside-out patch of atrial membrane; the antibody blocked carbachol-activated currents irreversibly. The state of the endogenous G_k determined its susceptibility to block by the antibody. When agonist was absent or when activation by muscarinic stimulation was interrupted by withdrawal of guanosine triphosphate (GTP) in the presence or absence of guanosine diphosphate (GDP), the effects of the antibody did not persist. Thus, monoclonal antibody 4A blocked muscarinic activation of potassium channels by binding to the activated G protein in its holomeric form or by binding to the dissociated α subunit.

COUPLING BETWEEN THE ATRIAL muscarinic acetylcholine receptor and the potassium channel current (K^+_{ACh}) that it activates (*1*) is thought to be independent of a cytoplasmic second messenger pathway (*2*). G proteins were implicated as coupling agents because whole-cell K^+_{ACh} currents required guanosine triphosphate (GTP), were decreased by pertussis toxin (PTX) (*3*), and became independent of ligand in the presence of the nonhydrolyzable GTP analog 5′-guanylylimidodiphosphate (GppNHp) (*4*). The muscarinic effects on the atrial membrane potential and atrial pacing were also blocked by PTX (*5*). Taken together with the results in (*2*), the possibility of G protein mediation independent of cyclic nucleotide second messengers was noted (*3*). Analysis of the whole-cell currents may be complicated, however, by the presence of a PTX-insensitive nonselective cation current related to phosphoinositide hydrolysis (*6*). The demonstration that another nonhydrolyzable GTP analog, gua-

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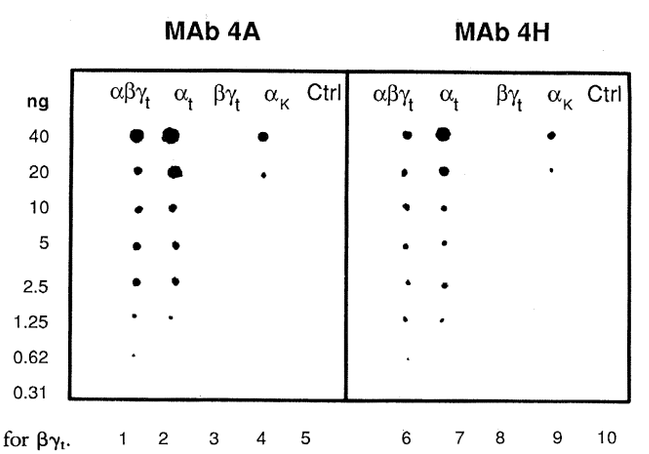

Fig. 1. "Dot blots" of the reaction between mAbs 4A and 4H (*13*) and transducin holoprotein $G_t(\alpha\beta\gamma_t)$ (lanes 1 and 6), α_t-GTPγS (lanes 2 and 7), the βγ subunit of transducin ($\beta\gamma_t$) (lanes 3 and 8), α_k-GTPγS (lanes 4 and 9), and control protein (trypsin inhibitor) (lanes 5 and 10). Dot blots were performed as described in (*12*), and antibody binding was detected by ^{125}I-labeled protein A binding and autoradiography of dot blots. The mAb 4A has a similar affinity for $\alpha\beta\gamma_t$ and α_t, lower affinity for α_k, and no affinity for $\beta\gamma_t$.