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A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas

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B cells and primary carcinomas express a surface molecule, Bp50 (CDw40), absent from other hematopoietic cells and from normal epithelium, and thought to play a regulatory role in B-cell maturation and epithelial neoplasia. In this work the sequence of a cDNA clone encoding Bp50 was analyzed by a newly derived transition matrix method. Among several interesting relationships with known receptors was found an extensive homology with the nerve growth factor receptor. The mRNA is induced by γ-interferon in both B cells and epithelial neoplasms, suggesting a role for the molecule in the development of carcinomas at sites of chronic inflammation.

Key words: B cells/hematopoietic cells/epithelium/nerve growth factor receptor/γ-interferon/neoplasms

Introduction

B lymphocyte activation and differentiation are controlled by a complex interplay of soluble factors and cell surface molecules at specific restriction points in the cell cycle (Howard *et al.*, 1984; Melchers and Andersson, 1986; Kishimoto and Hirano, 1988). Among the known factors acting on B cells are IL-1α, IL-2, IL-4, IL-5, IL-6, BCGFI, $BCGF_{low}$, α-IFN and λ-IFN (Howard *et al.*, 1985; Kishimoto *et al.*, 1984; Mehta *et al.*, 1985; Kishimoto and Hirano, 1988; O'Garra *et al.*, 1988), all of which exert regulatory influences on other target cells. However, B cells also express lineage-specific surface molecules which presumably represent targets for lineage-specific ligands. The best known of these is surface immunoglobulin (sIg). sIg triggers B-cell activation following crosslinking by antigen or anti-immunoglobulin antibody (Kishimoto and Ishizaka, 1975; Parker, 1980). Cells activated in this way become responsive to growth factors such as BCGF which enable them to traverse the cell cycle.

Recently two mAbs, G28-5 (Clark and Ledbetter, 1986b) and S2C6 (Paulie *et al.*, 1985), which recognize a 50 kd surface polypeptide on B cells (Bp50, CDw40) were shown to have similar functional activity (Gordon *et al.*, 1987). Although treatment of resting B cells with anti-CDw40 does not induce activation, treatment with anti-CDw40 in the presence of anti-IgM, the anti-CD20 mAb 1F5 or phorbol esters allows the cells to enter S phase (Clark and Ledbetter, 1986b; Gordon *et al.*, 1987). In this respect anti-CDw40 is akin to a low mol. wt B-cell growth factor ($BCGF_{low}$) (Ambrus and Fauci, 1985; Mehta *et al.*, 1985) but recent studies have shown that the effects of $BCGF_{low}$ and anti-CDw40 differ on acute B-lymphoblastic leukemia cells, some of which respond to one but not the other of the stimuli (Ledbetter *et al.*, 1987a). Anti-CDw40 in conjunction with IL-4 has been shown to mediate the release of soluble CD23 (Kikutani *et al.*, 1986) (a low affinity IgE receptor and a putative autocrine B cell growth factor) from B cells (Gordon and Guy, 1987; Cairns *et al.*, 1988).

Unlike other B-cell surface antigens, CDw40 is also expressed in carcinomas but not in normal epithelial cells (Paulie *et al.*, 1985; Clark and Ledbetter, 1986a; Ledbetter *et al.*, 1987b). These data suggest an additional role for CDw40 in the progression to or maintenance of the neoplastic state in cells of epithelial origin.

In this study a cDNA clone encoding CDw40 was isolated from a mammalian cell expression library. The cDNA encodes a polypeptide with structural similarities to several growth factor receptors and an extensive relatedness to nerve growth factor (NGF) receptor (Johnson *et al.*, 1986; Redeke *et al.*, 1987). Expression studies showed that CDw40 can be induced by a variety of activators in B cells and by γ-interferon in carcinomas. Because carcinomas in general and human hepatomas in particular are predisposed to arise in the context of chronic inflammation, the presence and cytokine modulation of CDw40 may reflect an important link in the chain of events leading to epithelial malignancy.

Results

Isolation of the cDNA

Initial attempts to use a COS cell expression system (Aruffo and Seed, 1987b; Seed and Aruffo, 1987) proved unsuccessful when COS cells treated with antibody G28-5 were found to adhere to dishes coated with goat anti-mouse immunoglobulins, despite the absence of a detectable immunofluorescence reaction. Accordingly, mouse WOP3027 cells (Dailey and Basilico, 1985) were used; these cells support the replication of plasmids containing polyoma virus origins, and do not react with G28-5. To exploit the WOP cell system, a cDNA library prepared from the Burkitt lymphoma (BL) line, Raji, in vector πH3M (Aruffo and Seed, 1987b) was cleaved with *Not*I and *Sfi*I, and the fragment containing the vector CDM8, which includes a polyoma virus origin (Seed, 1987).

The library was introduced into WOP cells by spheroplast fusion and subjected to three rounds of panning and transformation into *Escherichia coli* as described (Seed and Aruffo, 1987; Aruffo and Seed, 1987b). Following the third round of panning, two out of six plasmids tested positive for CDw40 expression by indirect immunofluorescence in WOP cells. Both cDNA clones contained an insert of

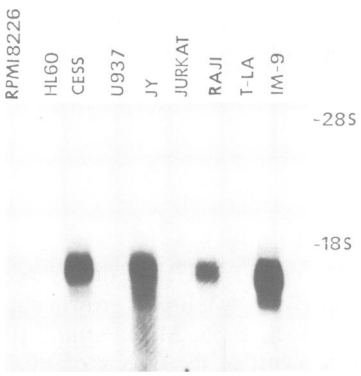

Fig. 1. RNA blot analysis of CDw40 transcripts. Total RNA (20 μg) from different sources was fractionated on a 1% agarose gel, transferred to a nylon filter and hybridized with a CDw40 probe. Samples included B-LCL (CESS, JY, IM-9), a BL (Raji), myeloid lines (HL60 and U937), a T cell leukemia (Jurkat) and lymphokine-activated T cells (T-LA).

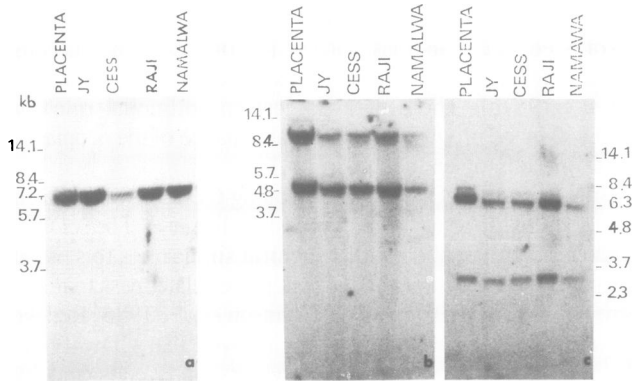

Fig. 2. DNA blot analysis of CDw40. DNA (20 μg) from each cell source was digested with (**a**) *Eco*RI, (**b**) *Hin*dIII and (**c**) *Bam*HI. Restriction enzyme-digested DNA was fractionated on a 0.8% agarose gel, transferred to nylon filters and hybridized to a CDw40 probe. No differences were noted between placental DNA and DNA obtained from B-LCL (JY, CESS) or BL (Raji, Namalwa) lines. Less CESS DNA was loaded in blot (a).

1050 bp. COS cells transfected with either plasmid reacted with G28-5 by indirect immunofluorescence but failed to react with other members of the B-cell panel of antibodies obtained from the Third International Leukocyte Typing Workshop (Ling and Ledbetter, 1987) (data not shown).

Characterization of the cDNA

RNA blots showed that CDw40 is expressed in B-cell lines representing different stages of differentiation (Figure 1). A single 1.3-kb message was detected in all B-cell lines tested. Expression was highest in the B-lymphoblastoid (B-LBL) lines JY, IM-9 and CESS, intermediate in the Burkitt lymphoma Raji and barely detectable in the plasmacytoma RPMI 8226. No expression was detected in activated peripheral T cells, a T-cell leukemia (Jurkat) or in the myeloid cell lines U937 (promonocytic leukemia) and HL60 (promyelocytic leukemia).

DNA blot hybridization gave a simple pattern consistent with a single copy gene. Analysis of DNA from various B-cell lines showed that the gene encoding CDw40 is not rearranged as a consequence of either normal or neoplastic development (Figure 2).

The nucleotide sequence of the cDNA insert contains an open reading frame of 831 bp starting with an ATG embedded in the initiation consensus sequence (Kozak, 1984) (Figure 3A). The predicted polypeptide consists of 277 amino acids with a M_r 30 600. The first methionine is followed by 19 predominantly hydrophobic residues consistent with a secretory signal peptide. The proposed site of cleavage of this peptide in agreement with the rules of von Heijne (1986) would yield a mature protein of M_r 28 300. The deduced amino acid sequence conforms to the typical features of an integral membrane protein (Figure 3B). The extracellular domain consists of 193 amino acids which include 22 cysteine residues (11.4% cysteine). The 71 amino acids preceding the transmembrane region (residues 112–182) contain nine serine and seven threonine residues. There are two potential N-linked glycosylation sites (As-Xaa-Ser/Thr), at positions 153 and 180. The extracellular domain is followed by a transmembrane region consisting of 22 hydrophobic residues immediately preceded and followed by charged residues. The cytoplasmic region is composed of 62 amino acids. The combination of a cysteine-rich N-terminal domain and a serine- and threonine-rich region preceding the transmembrane domain is characteristic of several growth factor receptors (Downward *et al.*, 1984; Ullrich *et al.*, 1984, 1985; Ebina *et al.*, 1985; Bargman *et al.*, 1986; Yarden *et al.*, 1986).

A novel method for comparing distantly related proteins

Initial comparisons of the CDw40 peptide sequence turned up only limited homology with entries in the NBRF database. A search for distant homology was then conducted with a new family of scoring matrices based on a rederivation of the matrix of Dayhoff and collaborators (Dayhoff *et al.*, 1978, 1983; Schwartz and Dayhoff, 1978). The new approach relies on the observation that the transition matrix for 1% accepted mutation (1 PAM), **M**, can be used to compose an easily computed exponential representation (Feller, 1966). We demonstrate this as follows. Consider a model protein of 100 residues, and choose the units of evolutionary time so that t is the average number of exchanges in time t. Then **M** is the transition matrix for one exchange (1% accepted mutation). The probability that there will be exactly n exchanges after time t is $t^n e^{-t}/n!$. Since the transition matrix for n successive exchanges is $\mathbf{M}^n$, the cumulative transition matrix for all possible numbers of exchange in time t is $\Sigma_{n=0}^{\infty} (\mathbf{M}t)^n e^{-t}/n! = e^{t(\mathbf{M}-\mathbf{I})}$, where **I** is the identity matrix. Mathematically $(\mathbf{M}-\mathbf{I})$ plays the role of an infinitesimal generator (Feller, 1966). $e^{t(\mathbf{M}-\mathbf{I})}$ is not only a more rigorous representation, but also much easier to compute than the Dayhoff matrix $\mathbf{M}^t$ for large t. Because the eigenvalues of the generator $\mathbf{M}-\mathbf{I}$ are all real, distinct and nonpositive (as they should be), the eigenvectors can be used to constitute a diagonal representation $e^{t(\mathbf{M}-\mathbf{I})} = \mathbf{C}^{-1}e^{t\mathbf{D}}\mathbf{C}$, where **C** is the matrix of eigenvectors, and $e^{t\mathbf{D}}$ is the diagonal matrix with entries $d_{ii} = e^{t\lambda_i}$ where λ_i are the eigenvalues. Once the eigenvectors and eigenvalues are known, $e^{t(\mathbf{M}-\mathbf{I})}$ is easily calculated for any t, and an evolutionary simulation can be quickly performed. Log odds scoring matrices (Schwartz and Dayhoff, 1978; Dayhoff *et al.*, 1983) constructed in this way are presented in Figure 4 for 100, 200, 400 and 800% accepted mutation. For large t the matrix is dominated by Cys and Trp residue contributions. Taking either both Cys and Trp or only Cys dominated matrices, a reexamination of growth factor and

A

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   1 GCCTCGCTCGGGCGCCCAGTGGTCCTGCCGCCTGGTCTCACCTCGCCATGGTTCGTCTGCCTCTGCAGTGCGTCCTCTGGGGCTGCTTGCTGACCGCTGTCCATCCAGAACCACCCACTG
   1                                                  M  V  R  L  P  L  Q  C  V  L  W  G  C  L  L  T  A  V  H  P  E  P  P  T  A

 121 CATGCAGAGAAAAACAGTACCTAATAAACAGTCAGTGCTGTTCTTTGTGCCAGCCAGGACAGAAACTGGTGAGTGACTGCACAGAGTTCACTGAAACGGAATGCCTTCCTTGCGGTGAAA
  26   C  R  E  K  Q  Y  L  I  N  S  Q  C  C  S  L  C  Q  P  G  Q  K  L  V  S  D  C  T  E  F  T  E  T  E  C  L  P  C  G  E  S

 241 GCGAATTCCTAGACACCTGGAACAGAGAGACACACTGCCACCAGCACAAATACTGCGACCCCAACCTAGGGCTTCGGGTCCAGCAGAAGGGCACCTCAGAAACAGACACCATCTGCACCT
  66   E  F  L  D  T  W  N  R  E  T  H  C  H  Q  H  K  Y  C  D  P  N  L  G  L  R  V  Q  Q  K  G  T  S  E  T  D  T  I  C  T  C

 361 GTGAAGAAGGCTGGCACTGTACGAGTGAGGCCTGTGAGAGCTGTGTCCTGCACCGCTCATGCTCGCCCGGCTTTGGGGTCAAGCAGATTGCTACAGGGGTTTCTGATACCATCTGCGAGC
 106   E  E  G  W  H  C  T  S  E  A  C  E  S  C  V  L  H  R  S  C  S  P  G  F  G  V  K  Q  I  A  T  G  V  S  D  T  I  C  E  P

 481 CCTGCCCAGTCGGCTTCTTCTCCAATGTGTCATCTGCTTTCGAAAAATGTCACCCTTGGACAAGCTGTGAGACCAAAGACCTGGTTGTGCAACAGGCAGGCACAAACAAGACTGATGTTG
 146   C  P  V  G  F  F  S  N  V  S  S  A  F  E  K  C  H  P  W  T  S  C  E  T  K  D  L  V  V  Q  Q  A  G  T  N  K  T  D  V  V
                          --CHO--                                                                                    --CHO--
 601 TCTGTGGTCCCCAGGATCGGCTGAGAGCCCTGGTGGTGATCCCCATCATCTTCGGGATCCTGTTTGCCATCCTCTTGGTGCTGGTCTTTATCAAAAAGGTGGCCAAGAAGCCAACCAATA
 186   C  G  P  Q  D  R  L  R  A  L  V  V  I  P  I  I  F  G  I  L  F  A  I  L  L  V  L  V  F  I  K  K  V  A  K  K  P  T  N  K
                          ================================================================
 721 AGGCCCCCCACCCCAAGCAGGAACCCCAGGAGATCAATTTTCCCGACGATCTTCCTGGCTCCAACACTGCTGCTCCAGTGCAGGAGACTTTACATGGATGCCAACCCGTCACCCAGGAGG
 226   A  P  H  P  K  Q  E  P  Q  E  I  N  F  P  D  D  L  P  G  S  N  T  A  A  P  V  Q  E  T  L  H  G  C  Q  P  V  T  Q  E  D

 841 ATGGCAAAGAGAGTCGCATCTCAGTGCAGGAGAGACAGTGAGGCTGCACCCACCCAGGAGTGTGGCCACGTGGGCAAACAGGCAGTTGGCCAGAGAGCCTGGTGCTGCTGCTGCAGGGGT
 266   G  K  E  S  R  I  S  V  Q  E  R  Q

 901 GCAGGCAGAAGCGGGGAGCTATGCCCAGTCAGTGCCAGCCCCTC
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B

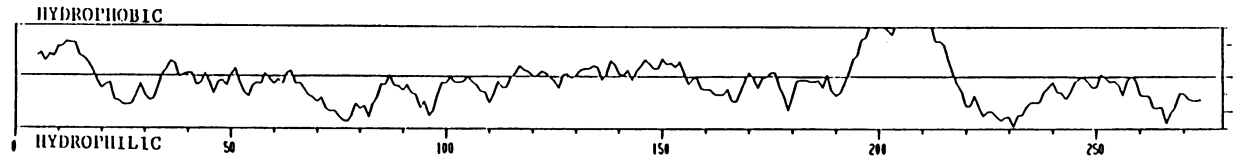

Fig. 3. (**A**) Nucleotide sequence of the CDw40 cDNA. The nucleotide sequence and translated open reading frame are numbered at left. Sites of potential N-linked glycosylation are marked 'CHO' and the transmembrane region is underscored. (**B**) Hydropathicity plot of the sequence in (a).

100 PAM LOG ODDS MATRIX

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	X
A	6	-5	-1	-1	-5	-2	0	1	-5	-2	-5	-4	-3	-7	1	2	2	-11	-7	0	0
R	-5	10	-3	-6	-6	1	-5	-8	1	-4	-7	4	-2	-7	-2	-1	-4	1	-10	-6	0
N	-1	-3	7	4	-8	-1	1	-1	2	-4	-6	1	-5	-6	-3	1	0	-8	-3	-5	0
D	-1	-6	4	8	-11	1	5	-1	-1	-6	-9	-2	-8	-11	-4	-1	-2	-13	-9	-6	0
C	-5	-6	-8	-11	14	-11	-11	-9	-6	-5	-12	-11	-11	-10	-6	-1	-5	-13	-2	-4	0
Q	-2	1	-1	1	-11	9	4	-5	4	-5	-4	0	-2	-10	-1	-3	-4	-10	-9	-5	0
E	0	-5	1	5	-11	4	8	-2	-2	-5	-7	-2	-6	-11	-3	-2	-3	-14	-8	-5	0
G	1	-8	-1	-1	-9	-5	-2	8	-7	-7	-8	-5	-8	-8	-3	1	-3	-13	-11	-4	0
H	-5	1	2	-1	-6	4	-2	-7	11	-7	-5	-3	-7	-4	-2	-4	-5	-8	-1	-6	0
I	-2	-4	-4	-6	-5	-5	-5	-7	-7	9	2	-4	2	0	-6	-4	-1	-12	-4	5	0
L	-5	-7	-6	-9	-12	-4	-7	-8	-5	2	9	-6	4	0	-5	-7	-5	-9	-5	1	0
K	-4	4	1	-2	-11	0	-2	-5	-3	-4	-6	8	1	-11	-4	-2	-1	-8	-10	-6	0
M	-3	-2	-5	-8	-11	-2	-6	-8	-7	2	4	1	13	-2	-6	-4	-2	-11	-8	1	0
F	-7	-7	-6	-11	-10	-10	-11	-8	-4	0	0	-11	-2	12	-9	-6	-6	-2	6	-5	0
P	1	-2	-3	-4	-6	-1	-3	-3	-2	-6	-5	-4	-6	-9	10	0	-1	-11	-11	-4	0
S	2	-1	1	-1	-3	-2	1	-4	-4	-7	-2	-4	-6	0	0	6	2	-3	-6	-4	0
T	2	-4	0	-2	-5	-4	-3	-3	-5	-1	-5	-1	-2	-6	-1	2	7	-10	-6	-1	0
W	-11	1	-8	-13	-13	-10	-14	-13	-8	-12	-9	-8	-11	-2	-11	-3	-10	19	-2	-14	0
Y	-7	-10	-3	-9	-2	-9	-8	-11	-1	-4	-5	-10	-8	6	-11	-6	-6	-2	13	-6	0
V	0	-6	-5	-6	-4	-5	-5	-4	-6	5	1	-6	1	-5	-4	-4	-1	-14	-6	8	0
X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

400 PAM LOG ODDS MATRIX

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	X
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R	-1	3	0	0	-2	1	0	-1	1	-1	-1	2	0	-2	0	0	0	2	-3	-1	0
N	0	0	1	1	-2	1	1	0	1	-1	-2	1	-1	-2	0	0	0	-3	-1	-1	0
D	0	0	1	2	-3	1	2	1	1	-1	-2	0	-1	-3	0	0	0	-4	-3	-1	0
C	-1	-2	-2	-3	10	-3	-3	-2	-2	-1	-3	-3	-3	-2	-1	0	-1	-5	1	-1	0
Q	0	1	1	1	-3	2	1	0	2	-1	-1	1	-1	-2	0	0	0	-3	-2	-1	0
E	0	0	1	2	-3	1	2	0	1	-1	-2	0	-1	-3	0	0	0	-4	-3	-1	0
G	1	-1	0	1	-2	0	0	3	-1	-1	-2	-1	-1	-3	0	1	0	-4	-3	-1	0
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I	0	-1	-1	-1	-1	-1	-1	-1	-1	2	2	-1	2	1	-1	-1	0	-3	0	2	0
L	-1	-1	-2	-2	-3	-1	-2	-2	-1	2	4	-1	3	2	-1	-1	-1	-2	0	2	0
K	0	2	1	0	-3	1	0	-1	0	-1	-1	3	0	-3	0	0	0	-2	-3	-1	0
M	-1	0	-1	-1	-3	-1	-1	-1	-1	2	3	0	3	0	-1	-1	0	-3	-1	1	0
F	-2	-2	-2	-3	-2	-2	-3	-3	-1	1	2	-3	0	7	-3	-2	-2	2	6	0	0
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X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

200 PAM LOG ODDS MATRIX

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	X
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R	-2	7	0	-2	-4	1	-2	-4	2	-2	-4	4	-1	-5	-1	-1	-1	2	-6	-3	0
N	0	0	3	3	-5	1	1	0	2	-2	-4	1	-2	-4	-1	1	0	-5	-2	-2	0
D	0	-2	3	5	-6	2	4	0	0	-3	-5	0	-4	-7	-1	0	0	-8	-5	-3	0
C	-3	-4	-5	-6	13	-7	-7	-5	-4	-3	-7	-7	-6	-5	-3	0	-3	-8	0	-2	0
Q	-1	1	1	2	-7	5	3	-2	3	-3	-2	1	-1	-6	0	-1	-1	-6	-5	-3	0
E	0	-2	1	4	-7	3	5	0	0	-3	-4	0	-3	-6	-1	0	-1	-9	-5	-2	0
G	1	-4	0	0	-5	-2	0	6	-3	-3	-5	-3	-4	-5	-1	1	0	-8	-6	-2	0
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T	1	-1	0	0	-3	-1	-1	0	-2	0	-2	0	-1	-4	0	2	4	-6	-4	0	0
W	-7	2	-5	-8	-8	-6	-9	-8	-4	-7	-5	-4	-6	0	-7	-2	-6	18	0	-8	0
Y	-4	-6	-2	-5	0	-5	-5	-6	0	-2	-2	-6	-4	7	-6	-4	-4	0	11	-3	0
V	0	-3	-2	-3	-2	-3	-2	-2	-3	4	2	-3	2	-2	-2	-1	0	-8	-3	5	0
X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

800 PAM LOG ODDS MATRIX

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	X
A	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	-1	-1	0	0
R	0	1	0	0	-1	0	0	0	0	0	0	0	0	-1	0	0	0	1	-1	0	0
N	0	0	0	0	-1	0	0	0	0	0	0	0	0	-1	0	0	0	-1	-1	0	0
D	0	0	0	0	-1	0	0	0	0	0	0	0	0	-1	0	0	0	-1	-1	0	0
C	0	-1	-1	-1	6	-1	-1	-1	-1	0	-1	-1	-1	0	0	0	0	-2	1	0	0
Q	0	0	0	0	-1	0	0	0	0	0	0	0	0	-1	0	0	0	-1	-1	0	0
E	0	0	0	0	-1	0	0	0	0	0	0	0	0	-1	0	0	0	-1	-1	0	0
G	0	0	0	0	-1	0	0	0	0	0	0	0	0	-1	0	0	0	-2	-1	0	0
H	0	0	0	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0
I	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	-1	0	0
L	0	0	0	0	-1	0	0	0	0	1	1	0	1	1	0	0	0	-1	1	0	0
K	0	0	0	0	-1	0	0	0	0	0	0	0	0	-1	0	0	0	-1	-1	0	0
M	0	0	0	0	-1	0	0	0	0	0	1	0	0	-1	0	0	0	-1	-1	0	0
F	-1	-1	-1	-1	0	-1	-1	-1	0	0	1	-1	0	3	-1	-1	0	1	3	0	0
P	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	-1	-1	0	0
S	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	-1	-1	0	0
T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	-1	0	0
W	-1	1	-1	-1	-2	-1	-1	-2	-1	-1	-1	-1	-1	1	-1	-1	-1	12	2	-1	0
Y	-1	-1	-1	-1	1	-1	-1	-1	0	0	1	-1	0	3	-1	-1	-1	2	4	0	0
V	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0
X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Fig. 4. Log odds scoring matrices for different periods of divergence. The matrices were constructed from the transition matrix derived in the text by the method outlined previously (Dayhoff *et al.*, 1987). Matrices representing 100, 200, 400 and 800% accepted mutation (incorporated changes) are shown.

related receptors revealed relationships not recognizable with the commonly used mutation data matrix for 250 PAMs. The distant matrices were particularly useful in these studies for establishing the existence and endpoints of homologies which could not be detected otherwise. In most cases, once the endpoints were identified the homologies could also be demonstrated with the less divergent matrices, including the canonical 250 PAM matrix.

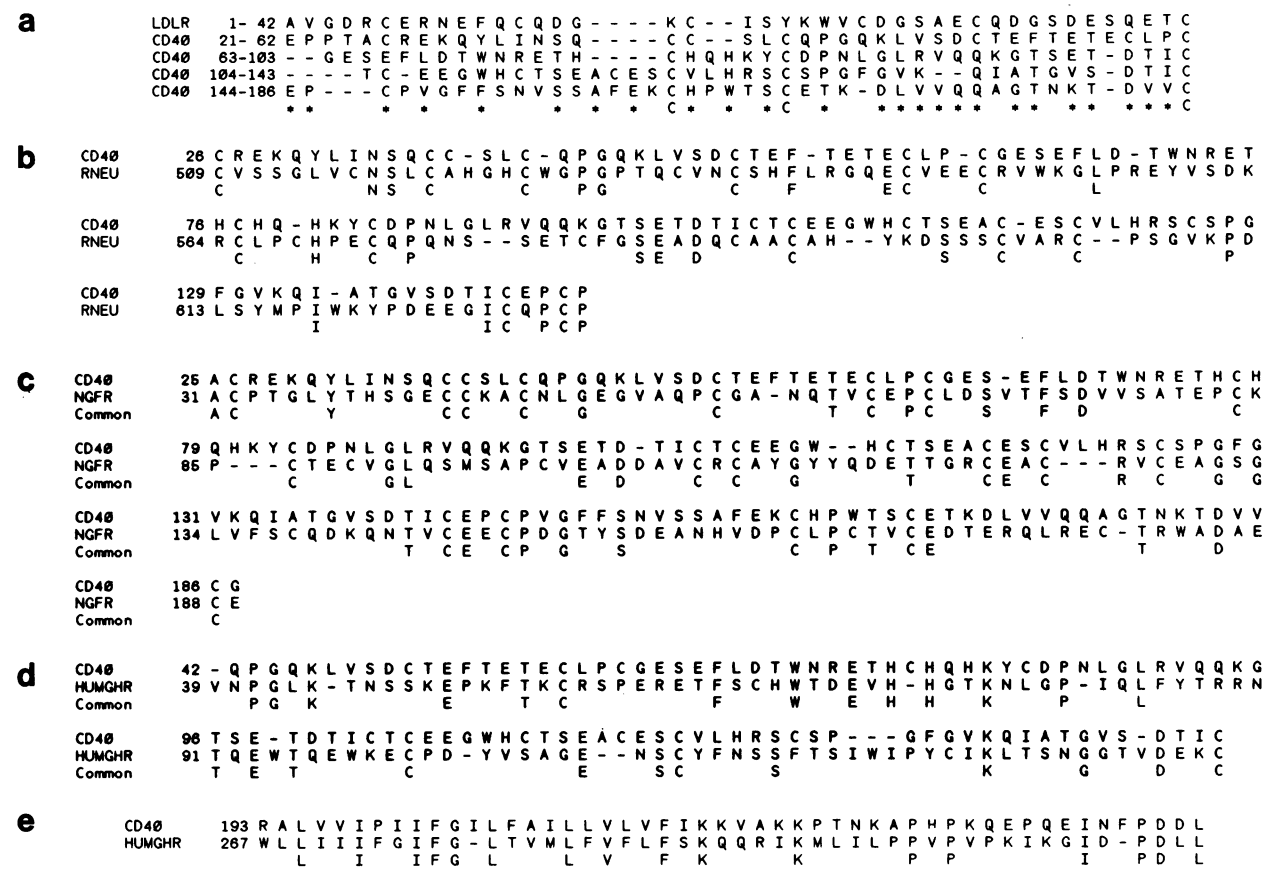

Fig. 5. Sequence alignment of CDw40. (**a**) Alignment of CDw40 subdomains with the first domain of the LDL receptor. Using the newly derived matrix, alignment of CDw40 to LDL receptor decomposed the molecules into their respective domains, which in the case of LDL receptor respect the exon boundaries. Asterisks denote residues shared among two or three domains. (**b**) Optimal alignment of CDw40 and neu oncogene. (**c**) Optimal alignment of the extracellular domains of CDw40 and human growth factor hormone receptor (HUMGHR). (**e**) Optimal alignment of transmembrane and cytoplasmic domains of CDw40 and HUMGHR.

CDw40 is the closest known relative of NGF receptor

Using the distant matrices to compare entire molecules, or entire extracellular domains, a striking decomposition of the external domain of CDw40 was observed when CDw40 was aligned with the neu oncogene or LDL, insulin and EGF receptors. In the optimal alignments, CDw40 was found to be divided into halves, or quarters, depending on the molecule to which it was compared. Subsequent internal comparisons using either the less divergent (100 or 200% divergence) matrices, or the canonical 250 PAM matrix showed that CDw40 possesses a repeating extracellular domain of four approximately equal subdomains (Figure 5a). Although the exonic structure of the related insulin and EGF receptors is not known, the optimal alignments with LDL receptor matched the first two CDw40 domains with the first two LDL exons (Sudhof *et al.*, 1985), which share the same length and domain endpoints as the CDw40 quarters but show little relatedness apart from cysteine residues (Figure 5a). The same was true for the comparison of CDw40 to neu oncogene, although the relatedness between the corresponding domains was more significant (Figure 5b).

Similarly, although an initial alignment of the entire CDw40 molecule with NGF receptor gave a score of slightly >3 standard deviations (SD) above the mean score for an ensemble of randomly permuted molecules of the same lengths and amino acid composition, alignment with the distant matrices gave scores 12 and 16 SD above the mean. Using the endpoints established with the distant matrices, subsequent comparison of CDw40 and NGF receptor using the Dayhoff matrix gave an ALIGN score of 10.10 SD above the mean (Figure 5c), corresponding to a probability of 2×10^{-24} for an equally good or better match between two unrelated proteins of identical composition. The relationship between CDw40 and NGF receptor was first noted by Paulie and coworkers (Braesch-Andersen *et al.*, 1989), based on alignment of a partial N-terminal amino acid sequence. Like CDw40, NGF receptor shows a four domain extracellular structure and a short intracellular portion (Johnson *et al.*, 1986; Redeke *et al.*, 1987). Although the total number of cysteine residues is higher in the extracellular domain of NGFR than in CDw40, the positions of the cysteine residues are conserved. Furthermore, although conservation of cysteine placement is the salient similarity between the two molecules, several other residues are shared accounting for an overall 34.5% homology in the extracellular domains. The similarity between CDw40 and NGFR suggests that these molecules may be part of a distinct family of growth factor receptors.

Two related regions were also found by conventional searches between CDw40 and the human growth hormone receptor (GHR) (Leung *et al.*, 1987). The first spans residues 42–143 and 39–140 in the extracellular domain of CDw40 and GHR respectively (Figure 5d). The second spans residues 193–250 and 267–320 of CDw40 and GHR respectively which comprise the transmembrane and part of the cytoplasmic domains of each molecule (Figure 5e). The

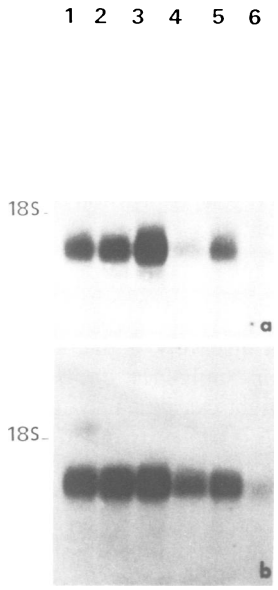

Fig. 6. RNA blot analysis of CDw40 (**a**) and HLA-DR-β transcripts (**b**) in tonsillar B cells. 20 μg of RNA from each sample was loaded per lane. **Lane 1**, γ-IFN-stimulated dense B cells; **lane 2**, anti-IgM-stimulated dense B cells; **lane 3**, IF5-stimulated dense B cells; **lane 4**, unstimulated buoyant B cells; **lane 5**, PMA-stimulated dense B cells; **lane 6**, unstimulated dense B cells. The same filter was used for CDw40 and HLA-DR hybridizations.

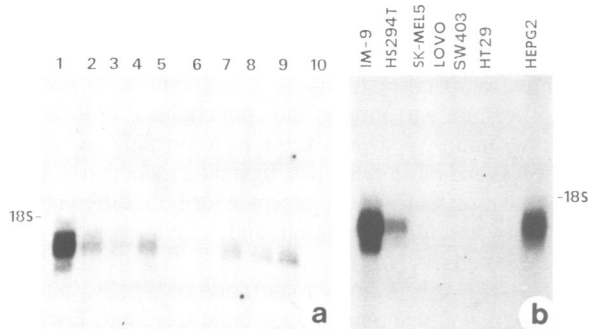

Fig. 7. RNA blot analysis of CDw40 transcripts in primary carcinomas (**a**) and carcinoma cell lines (**b**). 20 μ of total RNA from each sample was loaded per lane. (**a**) **Lane 1**, IM-9 B-LCL; **lane 2**, pulmonary adenocarcinoma; **lane 3**, mesothelioma; **lane 4**, colon carcinoma 1; **lane 5**, normal colonic epithelium; **lane 6**, normal duodenal epithelium; **lane 7**, colon carcinoma 2; **lane 8**, esophageal carcinoma; **lane 9**, thyroid carcinoma; **lane 10**, lymphokine-activated T cells. **b**) Cell lines from which the RNA samples were derived are indicated.

scores for these two alignments were 3.78 and 4.99 SD above the mean respectively. However, unlike the growth factor receptors above, GHR is not a cysteine-rich protein. Whether the relatedness reflects a selected structural motif or an evolutionary link between these two classes of seemingly unrelated proteins is not clear at present.

Regulation of expression of CDw40 in normal B cells

Previous studies have shown that surface expression of CDw40 varies among different sub-populations of B cells (Clark and Ledbetter, 1986b; Ledbetter *et al.*, 1987a). Several B-cell activators that induce expression of class II major histocompatibility complex (MHC) antigens have been shown to induce CDw40 surface expression in B cells (Ledbetter *et al.*, 1987a; E.A.Clark, unpublished data). Furthermore, CDw40 and MHC class II antigens have a similar expression pattern in lymphoid tissues (Ling and Ledbetter 1987). On the basis of these observations it has been suggested that CDw40 and MHC class II antigens may be under a common regulatory control (Ledbetter *et al.*, 1987a). To assess CDw40 induction and expression at the transcriptional level, B cells were purified from human tonsils. After depleting the T-cell and monocyte fractions by anti-CD3/complement lysis and panning on plastic dishes respectively, buoyant B cells were separated from dense B cells on a Percoll step gradient (Dagg and Levitt, 1981). The dense B-cell population was aliquoted into five equal fractions one of which was maintained in medium only, while the other four were stimulated with γ-interferon (γ-IFN) (1 μg/ml), anti-IgM antibody (50 μg/ml), 1F5 (anti-CD20 mAb) (5 μg/ml) and phorbolmyristate acetate (PMA) (20 ng/ml). RNA was extracted from each fraction 24 h after stimulation. All four mediators induced CDw40 mRNA (Figure 6a). CDw40-specific RNA in buoyant cells was slightly more abundant than in unstimulated dense cells. To compare the effect of the mediators on HLA class II antigen expression, the filter was stripped and reprobed with an HLA DR-β chain cDNA probe (Long *et al.*, 1983). Increased DR expression was observed in all four stimulated samples as well as in the buoyant cell fraction (Figure 6b). While anti-CD20 had a roughly similar effect on CDw40 and class II gene induction, γ-IFN, anti-IgM and PMA showed a stronger effect on class II than on CDw40 expression (Figure 6b).

Expression and regulation of CDw40 in carcinomas

Anti-CDw40 mAb have been shown to react with most carcinomas and with several carcinoma lines (Paulie *et al.*, 1985; Ledbetter *et al.*, 1987b). To study the transcription pattern, total RNA was prepared from several primary carcinomas and carcinoma cell lines and subjected to RNA blot hybridization. All the primary tumors tested, including a pulmonary adenocarcinoma, a mesothelioma, two colon carcinomas, an esophageal carcinoma and a thyroid carcinoma, showed roughly the same CDw40 mRNA abundance, substantially lower than that observed in several of the B-cell lines (Figure 7a). Samples from normal colonic (Figure 7a, lane 5) and duodenal (Figure 7a, lane 6) epithelium contained barely detectable levels of CDw40 transcripts. Histologic examination of both the carcinoma samples and the normal epithelium did not show significant lymphoid infiltrates, rendering unlikely the possibility that the observed message was due to B cells. Of six cell lines tested, including two melanomas (SKMel-5 and HS294T) three colon carcinomas (Lovo, SW403 and HT29) and a hapatocarcinoma (HepG2), only HS294T and HepG2 showed CDw40 expression (Figure 7b). The level of expression, as in the primary tumors, was lower than in the B lymphoblastoid line IM-9.

To assess the regulation of CDw40 expression in tumors, HS294T, SKMel-5 and HepG2 cells were treated with γ-IFN (1 μg/ml), PMA (20 ng/ml), interleukin-1 (5 U/ml) and tumor necrosis factor (TNF) (200 U/ml). Cells were harvested for RNA extraction 24 and 48 h following stimulation. Both γ-IFN-induced HS294T and HepG2 showed an increase in CDw40 mRNA at 24 h (Figure 8a) whereas no expression was observed in SKMel-5 (data not shown). While increased CDw40 expression persisted at

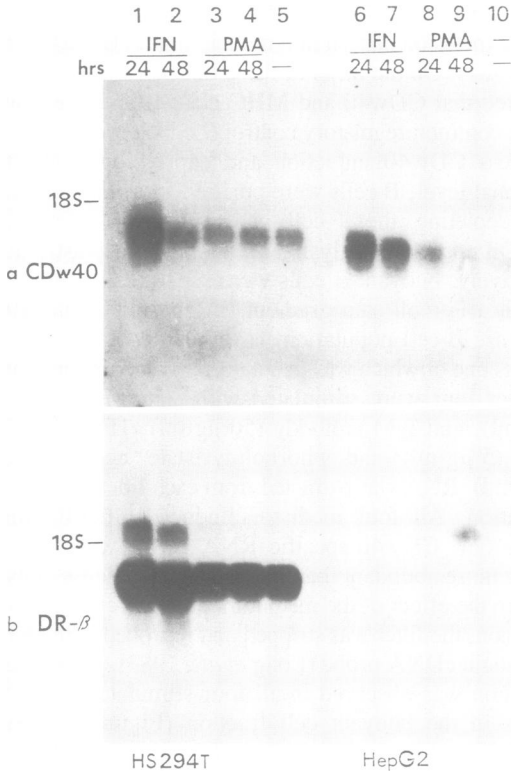

Fig. 8. RNA blot analysis of CDw40 (**a**) and HLA-DR-β (**b**) induction in the HS294T melanoma (**lanes 1–5**) and the HepG2 hepatoblastoma (**lanes 6–10**) cells. **Lanes 1** and **6**, 24 h after γ-IFN treatment; **lanes 2** and **7**, 48 h after γ-IFN treatment; **lanes 3** and **8**, 24 h after PMA treatment; **lanes 4** and **9**, 48 h after PMA treatment; **lanes 5** and **10**, no treatment, 24 h in media.

48 h in HepG2 cells, it subsided to pre-stimulation levels in HS294T. γ-IFN failed to induce CDw40 expression in tumor lines that did not constitutively express it. No induction in any of the lines was observed with PMA, TNF and interleukin-1 (Figure 8a and data not shown). To test whether induction by γ-IFN could be achieved in other cells that do not constitutively express CDw40 but that do show induction of class II antigens (Trinchieri and Perussia, 1985), RNA from U937 and HL50 cells stimulated by γ-IFN, IL-1, TNF and PMA was hybridized to the CDw40 probe. No CDw40 induction was observed (data not shown). When filters were stripped and rehybridized with the HLA DR-β-chain cDNA probe, γ-IFN-induced HS294T cells showed increased amounts of class II transcripts at 24 and 48 h (Figure 8b) while no class II gene transcription was observed in HepG2 cells.

Expression of CDw40 in mutant cell lines defective in HLA-DR expression

Previous reports suggested that the expression of CDw40 and HLA-DR may be coordinately regulated (Ledbetter *et al.*, 1987b). The present expression studies in B cells and in carcinomas show that the same mediators that induce CDw40 expression also induce class II antigen expression. However, HepG2 cells which express CDw40 do not express DR, and SKMel-5 cells which express class II do not express CDw40 (data not shown). Further correlation was sought by examining CDw40 expression in mutant cell lines that have lost DR expression. The mutant B cell line 6.16 (Gladstone and Pious, 1978) which has a defect in class II gene expression showed conserved expression of CDw40 when compared with the parent cell line T5-1 (data not shown). Another mutant line RJ 2.2.5, derived from the Burkitt lymphoma Raji and similarly defective for class II gene expression (Accolla, 1983; Accolla *et al.*, 1985), showed normal CDw40 expression. Thus at least one factor required for class II gene expression is not also required for CDw40 expression.

Discussion

One prediction of this work is that an NGF-like cytokine exerts a regulatory influence on B cells and epithelial tumors. What insight can be gained from studies on NGF and its receptor? NGF is required for the survival of embryonic sensory and sympathetic neurons following axonal innervation of the neuronal target fields during development (Davies, 1988). The survival-promoting action of NGF follows its binding to receptors at the axon termini, internalization of the ligand–receptor complex, and transport to the soma. NGF also directly promotes growth of post-embryonic and regenerating sensory sympathetic nerve fibers. Interestingly, NGF has recently been shown to be produced by epithelial cells as well as Schwann cells (Davies *et al.*, 1987), leaving open the possibility that the CDw40 ligand may also be a neurotrophic factor.

Production of NGF and expression of CDw40 are both mediated by cytokines, the former induced by interleukin-1 (Lindholm *et al.*, 1987) and the latter by γ-IFN. Like NGF, the CDw40 ligand may be required to regulate cellular responses to specific stimuli, e.g. B-lymphocyte proliferation, in response to antigen and epithelial cell proliferation following injury. γ-IFN exerts pleiomorphic effects on lymphocyte and other cell types, including induction of MHC antigens and high affinity Fc receptor for IgG, differentiation of B cells and differentiation and activation of myelomonocytic cells.

Since γ-IFN can promote maturation of B cells, inducing phenotype changes and immunoglobulin secretion (Trinchieri and Perussia, 1985), it is not surprising that it should also induce the expression of a receptor for a cell cycle progression signal. Just as NGF is necessary for neuronal survival at certain stages of development, the CDw40 ligand may be required for B-cell survival after initial stimulation, allowing, for example, a fraction of committed cells to become memory cells.

While γ-IFN-mediated CDw40 induction can be readily explained in B lymphocytes, how can one account for γ-IFN induction of a putative growth factor receptor in carcinomas, and what light, if any, can this shed on the possible role of CDw40 in tumor development? The various known functions of γ-IFN suggest that it plays a major role in protecting the organism against infection. Just as NGF receptor and NGF synthesis are increased in nerve injury (Taniuchi *et al.*, 1986; Heumann, 1987), CDw40 expression may be required to promote regeneration of damaged tissues. By increasing MHC and CDw40 expression in areas of tissue injury, as in a viral infection, γ-IFN would play the dual role of facilitating lysis of infected cells by cytotoxic lymphocytes and promoting the proliferation of healthy cells.

Such a scenario may also help explain why tumors frequently arise at sites of chronic inflammation (Simon *et al.*, 1981; Gerber and Thung, 1985; Schein and Levin, 1985;

Weitzman *et al.*, 1985). The carcinomas most frequently associated with chronic inflammation are cervical squamous cell carcinomas arising in the context of herpes virus infection and hepatocellular carcinoma associated with chronic active hepatitis and cirrhosis. In each case, although the incidence of carcinoma closely follows the incidence of the underlying virus infection, there is no evidence that integration of viral DNA is responsible for transformation. Rather, it appears that chronic viral infection creates a milieu of inflammation and regeneration which prepares the bed from which the tumor grows (Blumberg and London, 1971; Gerber and Thung, 1985). It is known, for example, that cytotoxic T lymphocytes recognizing HBV core antigen expressed on the surface of infected hepatocytes are present among the inflammatory infiltrates of chronic active hepatitis (Mondelli *et al.*, 1982; Gerber and Thung, 1985). Since activated T cells are potent sources of γ-IFN, the regenerating and/or partially transformed hepatocytes could be expected to have high levels of CDw40 expression. Some evidence suggesting a subsequent autocrine proliferative mechanism has recently been uncovered (E.A.Clark, unpublished data).

Materials and methods

cDNA library construction

Total RNA was extracted from 10^9 Raji cells by the guanidinium thiocyanate method, poly(A)$^+$ RNA was prepared and a cDNA library was constructed in the πH3M vector (Seed and Aruffo, 1987; Aruffo and Seed, 1987b). In order to screen the library using WOP3027 cells, 10 μg of the resulting library was cleaved with *Not*I and *Sfi*I and fractionated on a 5–20% potassium acetate gradient. The disperse high mol. wt fraction was recovered and ligated to a complementary *Not*I–*Sfi*I fragment similarly prepared from the vector CDM8 (Seed, 1987).

RNA, DNA blots and sequencing

RNA and DNA blots were performed as reported elsewhere (Aruffo and Seed, 1987a,b; Seed and Aruffo, 1987). Total RNA (20 μg) were loaded onto each lane, electrophoresed, transferred onto nylon filters (Gene Screen Plus, Dupont, Boston, MA), hybridized with a CDw40 cDNA probe, washed and exposed for autoradiography for 12–24 h. For DNA blots, 20 μg of DNA digested with the appropriate restriction enzyme were loaded per lane, size fractionated through a 0.8% agarose gel, transferred onto a nylon filter and hybridized with the CDw40 cDNA probe. Following washing, the filter was exposed for autoradiography for 48 h. Sequencing was done by the method of Sanger *et al.* (1977) using specific oligonucleotide primers.

Probes

Single-stranded DNA from the CDM8/CDw40 construct was prepared as described (Levinson *et al.*, 1984). Probes were constructed by primer extension using an oligonucleotide homologous to a portion of the vector 3′ to the insert and labeled with [^{32}P]dATP or dCTP.

Cell lines and tissue specimens

The B-lymphoblastoid lines IM-9 and CESS, the Burkitt lymphoma lines Raji, Daudi and Namalwa, the plasmacytoma line RPMI 8226, the T-cell leukemia line Jurkat, the myeloid lines HL60 and U937, the melanoma lines HS294T and SKMel-5 and the colon carcinoma lines Lovo, HT29 and SW403 were obtained from the ATCC. The B-lymphoblastoid line JY, peripheral T blasts and the hepatoblastoma line HepG2 were kindly provided by Jack Strominger, Jim Kurnick and Barbara Knowles respectively. The T5-1 line and 6.16 variant of T5-1 were provided by Donald Pious. The RJ 2.2.5 subline of Raji was kindly provided by Robert Accolla. Tissue specimens were obtained at surgery and were immediately processed for RNA extraction. The diagnosis was obtained from the Department of Pathology, Massachusetts General Hospital after routine histologic analysis.

Cell preparations

Tonsillar cells were prepared by teasing the tissue in sterile phosphate-buffered saline (PBS). The cells were washed several times and T cells were removed by incubating with anti-CD3 mAb at 4°C followed by goat anti-rabbit complement (Pelfreez) lysis at 37°C for 45 min in serum-free IMDM medium (Gibco, Grand Island, NY). Monocytes were removed by panning on Petri dishes for 2 × 30 min. Of the resulting cells, 80–85% were sIgM positive. The buoyant and dense fractions were separated on a Percoll (Pharmacia) step gradient as described (Dagg and Levitt, 1981) and resuspended at a concentration of 5 × 10^5/ml in IMDM supplemented with 2 mM glutamine, 15 μg/ml gentamicin sulfate and 10% fetal bovine serum (FBS).Tumor cell lines were grown in 10-cm tissue-culture dishes (Falcon) to confluence in IMDM supplemented with antibiotics, glutamine and FBS.

Growth and stimulatory factors

Recombinant γ-IFN was obtained from Hoechst AG. Anti-human IgM was obtained from Cooper Biomedical (Malvern, PA). PMA was a kind gift from Jim Kurnick; recombinant TNF and IL1 were kindly provided by Michael Bevilacqua.

Stimulation of cells

Dense tonsillar B cells were stimulated with either recombinant γ-IFN (1 μg/ml), anti-IgM (50 μg/ml), IF5 (5 μg/ml) or PMA (20 ng/ml) for 6 h. Cells were washed, resuspended in fresh media and harvested for RNA extraction 24 h after stimulation. Carcinoma cell lines were stimulated with either γ-IFN (1 μg/ml), PMA (20 ng/ml), TNF (200 U/ml) or interleukin-1 (5 U/ml) for 6 h, washed and resuspended in fresh media for 24 and 48 h.

Indirect immunofluorescence

COS cells transfected with CDw40 cDNA were trypsinized 24 h after transfection and passaged into new plates. Cells were incubated with antibodies at 1:1000 dilution in PBS containing 0.5 mM $CaCl_2$ and 5% FBS for 45 min, washed with 0.15 M NaCl and treated in the same buffer with a fluorescein-labeled affinity-purified second antibody (Capel, Malvern, PA). Results were assessed by fluorescence microscopy.

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References

Accolla,R.S. (1984) *J. Exp. Med.*, **159**, 378–393.
Accolla,R.S., Carra,G. and Guardola,J. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 5145–5149.
Ambrus,J.L., Jr and Fauci,A.S. (1985) *J. Clin. Invest.*, **74**, 732–740.
Aruffo,A. and Seed,B. (1987a) *EMBO J.*, **6**, 3313–3316.
Aruffo,A. and Seed,B. (1987b) *Proc. Natl. Acad. Sci. USA*, **84**, 8573–8577.
Bargmann,C.I., Hung,M.-C. and Weinberg,R.A. (1986) *Nature*, **319**, 226–230.
Blumberg,B.S. and London,W.T. (1981) *N. Engl. J. Med.*, **304**, 782–784.
Braesch-Andersen,S., Pauli,S., Koho,H., Nika,H., Aspenstrom,P. and Perlmann,P. (1989) *J. Immunol.*, **142**, 562–567.
Clark,E.A. and Ledbetter,J.A. (1986a) *Immunology Today*, **7**, 267–270.
Clark,E.A. and Ledbetter,J.A. (1986b) *Proc. Natl. Acad. Sci. USA*, **83**, 4494–4498.
Cairns,J., Flores-Romo,L., Millsum,M.J., Guy,G.R., Gillis,S., Ledbetter, J.A. and Gordon,J. (1988) *Eur. J. Immunol.*, **18**, 349–353.
Dagg,M.K. and Levitt,D. (1981) *Clin. Immunol. Immunopathol.*, **21**, 39–49.
Dailey,L. and Basilico,C. (1985) *J. Virol.*, **54**, 739–749.
Davies,A.M. (1988) *Trends Genet.*, **4**, 139–143.
Davies,A.M., Bandtlow,C., Heumann,R., Korsching,S., Rohrer,H. and Thoenen,H. (1987) *Nature*, **326**, 353–358.
Dayhoff,M.O., Barker,W.C. and Hunt,L.T. (1983) *Methods Enzymol.*, **91**, 524–545.
Dayhoff,M.O., Schwartz,R.M. and Orcutt,B.C. (1978) In Dayhoff,M.O. (ed.), *Atlas of Protein Sequence and Structure*. National Biomedical Research Foundation, Silver Spring, MD, Vol. 5, suppl. 3, pp. 345–352.
Downward,J., Yarden,Y., Mayes,E., Scrace,B., Totty,N., Stockwell,P., Ullrich,A., Schlessinger,J. and Waterfield,M.D. (1984) *Nature*, **307**, 521–527.
Ebina,Y., Leland,E., Jarnagin,K., Ederin,M., Graf,L., Clauser,E., Jing-hsuing,O., Masiarz,F., Kan,Y.W., Golfine,I.D., Roth,R.A. and Rutter,W.J. (1985) *Cell*, **40**, 747–758.

Feller,W. (1966) In Feller,W. (ed.), *An Introduction to Probability Theory and its Applications.* Wiley, New York, Vol. II, pp. 311–345.
Gerber,M.A. and Thung,S.N. (1985) *Lab. Invest.*, **52**, 572–590.
Gladstone,P. and Pious,D. (1978) *Nature*, **271**, 459–461.
Gordon,J. and Guy,G.R. (1987) *Immunol. Today*, **8**, 339–343.
Gordon,J., Webb,A.J., Walker,L., Guy,G.R., Paulie,S., Ehlin-Henriksson, B. and Rosen,A. (1987) In McMichael,A.J. (ed.), *Leukocyte Typing III.* Oxford University Press, Oxford, pp. 426–430.
Heumann,R. (1987) *J. Exp. Biol.*, **132**, 133–150.
Howard,M., Nakanishi,K. and Paul,W.E. (1984) *Immunol. Rev.*, **78**, 185–210.
Johnson,D., Lanahan,A., Buck,C.R., Sehgal,A., Morgan,C., Mercer,E., Bothwell,M. and Chao,M. (1986) *Cell*, **47**, 545–554.
Kikutani,H., Inui,S., Sato,R., Barsumian,E.L., Owaki,H., Yamasaki,K., Kaisho,T., Uchibayashi,N., Hardy,R.R., Hirano,T., Tsunasawa,S., Sakiyama,F., Suemura,M. and Kishimoto,T. (1986) *Cell*, **47**, 657–665.
Kishimoto,T. and Hirano,T. (1988) *Annu. Rev. Immunol.*, **6**, 485–512.
Kishimoto,T. and Ishizaka,K. (1975) *J. Immunol.*, **129**, 1367–1372.
Kishimoto,T., Yoshizaki,K., Kimoto,M., Okada,M., Kuritani,T., Kikutani, H., Shimizu,K., Nakagawa,T., Miki,Y., Kishi,H., Fukunaga,K., Yoshikubo,T. and Taga,T. (1984) *Immunol. Rev.*, **78**, 97–118.
Kozak,M. (1984) *Nucleic Acids Res.*, **12**, 857–870.
Ledbetter,L.A., Shu,G., Gallagher,M. and Clark,E.A. (1987a) *J. Immunol.*, **138**, 788–794.
Ledbetter,J.A., Clark,E.A., Norris,N., Shu,G. and Hellstrom,I. (1987b) In McMichael,A.J. (ed.), *Leukocyte Typing III.* Oxford University Press, pp. 432–435.
Leung,D.W., Spencer,S.A., Cachianes,G., Hammonds,R.G., Collins,C., Henzel,W.J., Barnard,R., Waters,M.J. and Wood,W.I. (1987) *Nature*, **330**, 537–543.
Levinson,A., Silver,D. and Seed,B. (1984) *J. Mol. Appl. Genet.*, **2**, 507–517.
Lindholm,D., Heumann,R., Meyer,M. and Thoenen,H. (1987) *Nature*, **330**, 658–659.
Ling,N.R. and Ledbetter,J.A. (1987) In McMichael,J.A. (ed.) *Leucocyte Typing III.* Oxford University Press, Oxford, p. 336.
Long,O., Wake,C.T., Gorski,J. and Mach,B. (1983) *EMBO J.*, **2**, 389–394.
Melchers,F. and Andersson,J. (1986) *Annu. Rev. Immunol.*, **4**, 13–36.
Mehta,S.R., Conrad,D., Sander,R., Morgan,J., Montagna,R. and Maizel,A.L. (1985) *J. Immunol.*, **135**, 3298–3302.
Mondelli,M., Mieli-Vergani,G., Albert,A., Vergani,D., Portmann,B., Eddleston,A. and Williams,R. (1982) *J. Immunol.*, **129**, 2773–2778.
O'Garra,A., Umland,S., De France,T. and Christiansen,J. (1988) *Immunol. Today*, **9**, 45–54.
Parker,D.C. (1980) *Immunol. Rev.*, **52**, 115–139.
Paulie,S., Ehin-Henriksson,B., Mellstedt,H., Koho,H., Ben-Aissa,H. and Perlmann,P. (1985) *Cancer Immunol. Immunother.*, **20**, 23–30.
Redeke,M.J., Misko,T.P., Hsu,C., Herzenberg,L.A. and Shooter.E.M. (1987) *Nature*, **325**, 593–597.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.
Schein,P.S. and Levin,D. (1985) In Calabresi,P., Schein,P.S. and Rosenberg,S.A. (eds), *Medical Oncology. Basic Principles and Clinical Management of Cancer.* Macmillan Publishing Co., New York, pp. 852–853; 887–888.
Schwartz,R.M. and Dayhoff,M.O. (1978) In Dayhoff,M.O. (ed.), *Atlas of Protein Sequence and Structure.* National Biomedical Research Foundation, Silver Spring, MD, Vol. 5, suppl. 3, pp. 352–358.
Seed,B. (1987) *Nature*, **329**, 840–842.
Seed,B. and Aruffo,A. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 3365–3369.
Simon,R.H., Scoggin,C.H. and Patterson,D. (1981) *J. Biol. Chem.*, **256**, 7181–7186.
Sudhof,T.C., Goldstein,J.L., Brown,M.S. and Russell,D.W. (1985) *Science*, **228**, 815–822.
Taniuchi,M., Clarke,H.B. and Johnson,E.M. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 4094–4098.
Trinchieri,G. and Perussia,B. (1985) *Immunol. Today*, **6**, 131–136.
Ullrich,A., Coussens,L., Hayflick,J.S., Dull,T.J., Gray,A., Tam,A.W., Lee,J., Yarden,Y., Libermann,T.A., Schlessinger,J., Downward,J., Mayes,E.L.V., Whittle,N., Waterfield,M.D. and Seeburg,P.H. (1984) *Nature*, **309**, 418–425.
Ullrich,A., Bell,J.R., Chen,E.Y., Herrera,R., Petruzzelli,L.M., Dull,T.J., Gray,A., Coussens,L., Liao,Y.-C., Tsubokawa,M., Mason,A., Seeburg, P.H., Grunfeld,C., Rosen,O.M. and Ramachandran,J. (1985) *Nature*, **313**, 756–761.
von Heijne,G. (1986) *Nucleic Acids Res.*, **14**, 4683–4690.
Weitzman,S.A., Weitberg,A.B., Clark,E.P. and Stossel,T.P. (1985) *Science*, **227**, 1231–1233.
Yarden,Y., Escobedo,J.A., Kuang,W.J., Yang-Feng,T.L., Daniel,T.O., Tremble,P.M., Chen,E.Y., Ando,M.E., Harkins,R.N., Francke,U., Fried,V.A., Ullrich,A. and Williams,L.T. (1986) *Nature*, **323**, 226–232.

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