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B cell growth modulating and differentiating activity of recombinant human 26-kd protein (BSF-2, HuIFN-β_2, HPGF)

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The human '26-kd protein' is a secreted glycoprotein expressed, for example, in (blood) leukocytes, in epithelial cells treated with various inducers, but most strongly in interleukin-1 (IL-1)-treated fibroblasts. After finding it had antiviral and 2-5A synthetase-inducing activity, one group of authors called this protein IFN-β_2. However, recently the full-length 26-kd cDNA sequence was shown to be identical with that of a B-cell-differentiating lymphokine called BSF-2, and another report suggested that the 26-kd protein could support the growth of some transformed murine B cell lines. To define its biological activities, we expressed the recombinant 26-kd protein by translating in *Xenopus laevis* oocytes a pure, synthetic chimeric mRNA containing the 26-kd protein coding region surrounded by *Xenopus laevis* β-globin untranslated regions. A similar construction, but containing the HuIFN-β cDNA coding region, was used to produce HuIFN-β by the same procedure. Both recombinant glycoproteins were secreted, glycosylated, and their amounts were measured by [^{35}S]methionine incorporation by the oocyte. Here we show that the recombinant 26-kd protein exhibits a high growth factor activity when assayed on an IL-HP1-dependent murine B cell hybridoma (sp. act. $\sim 2 \times 10^8$ U/mg) as well as a potent differentiating activity on human CESS cells (sp. act. $\sim 5 \times 10^7$ U/mg). While rHuIFN-β was inactive in the latter two assays, it had the expected antiviral activity of $1-5 \times 10^8$ U/mg. The parallel recombinant 26-kd protein preparations had no detectable antiviral activity (i.e. a maximal specific activity of $1-3 \times 10^2$ U/mg, if any). The 26-kd protein is thus clearly an interleukin, and considering the confusing nomenclature now in use, this factor may better be renamed 'interleukin 6'.

Key words: antiviral activity/IgG secretion/interleukin 1/interleukin HP-1/interleukin 6

Introduction

When human fibroblastic cells are induced to produce IFN-β by treatment with poly(I)·poly(C) in the presence of cycloheximide, synthesis of a protein of 26-kd (Content *et al.*, 1982), previously described as IFN-β_2 (Weissenbach *et al.*, 1980), is stimulated. Induced epithelial cells (Poupart *et al.*, 1984), and peripheral blood lymphocytes (Vaquero *et al.*, 1986) also express the 26-kd protein mRNA. Furthermore, in fibroblasts, the '26-kd protein' gene is also induced by natural interleukin-1β (IL-1β) (Content *et al.*, 1985; Van Damme *et al.*, 1987;) and by TNF (Kohase *et al.*, 1986; Zilberstein *et al.*, 1986b; Defilippi *et al.*, 1987). Induction of the 26-kd protein by TNF seems to be correlated with the absence of susceptibility to the cytotoxicity of TNF (Defilippi *et al.*, 1987).

The biological function of the 26-kd protein has remained both elusive and controversial. One group of authors called the protein inteferon-β_2 because they found that it had antiviral and 2-5A synthetase-inducing activity (Weissenbach *et al.*, 1980). However, we and others have failed to detect such activities in several preparations of natural and cDNA-selected, *in vitro* synthesized 26-kd protein (Content *et al.*, 1982). Recently, two reports have raised the possibility that the 26-kd protein could be a new interleukin with multiple effects on cells of the B cell lineage. One report described a cDNA sequence identical with that previously described for the 26-kd protein (Haegeman *et al.*,

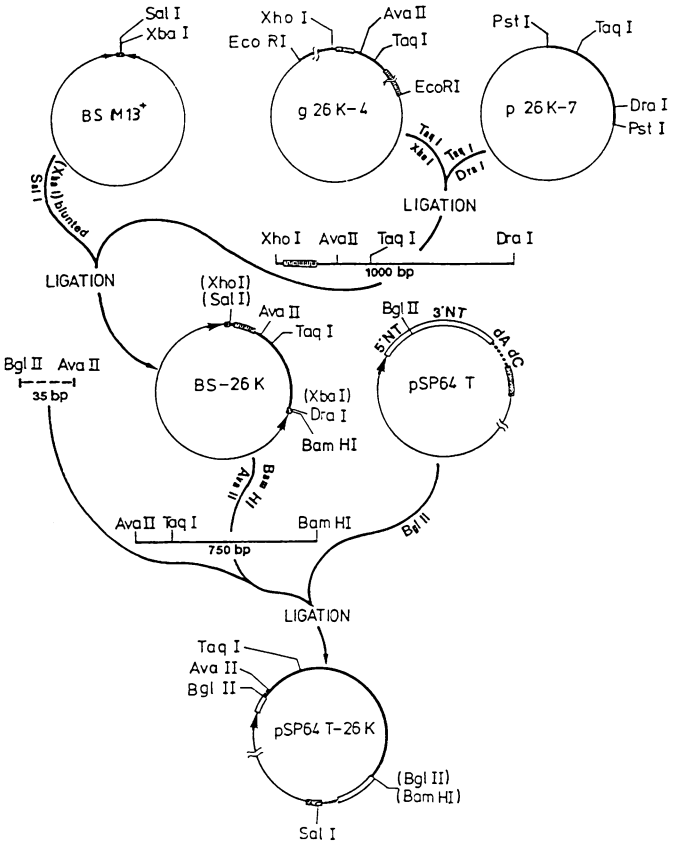

Fig. 1. Schematic outline of the construction of the different plasmids. The 26-kd protein DNA is indicated by a heavy line, the hatched areas being the introns. The heavy arrows indicate the T7, T3 and SP6 polymerases promoters. Polylinker sites are indicated by dotted boxes. Open areas noted 5′ NT and 3′ NT represent 5′ and 3′ untranslated regions of *Xenopus laevis* β globin gene. The poly(A) (23 nucleotides) and poly(C) (30 nucleotides) template tails are noted here dA dC (Krieg and Melton, 1982). The line of 35 bp representing the double-stranded synthetic oligonucleotide is not in scale.

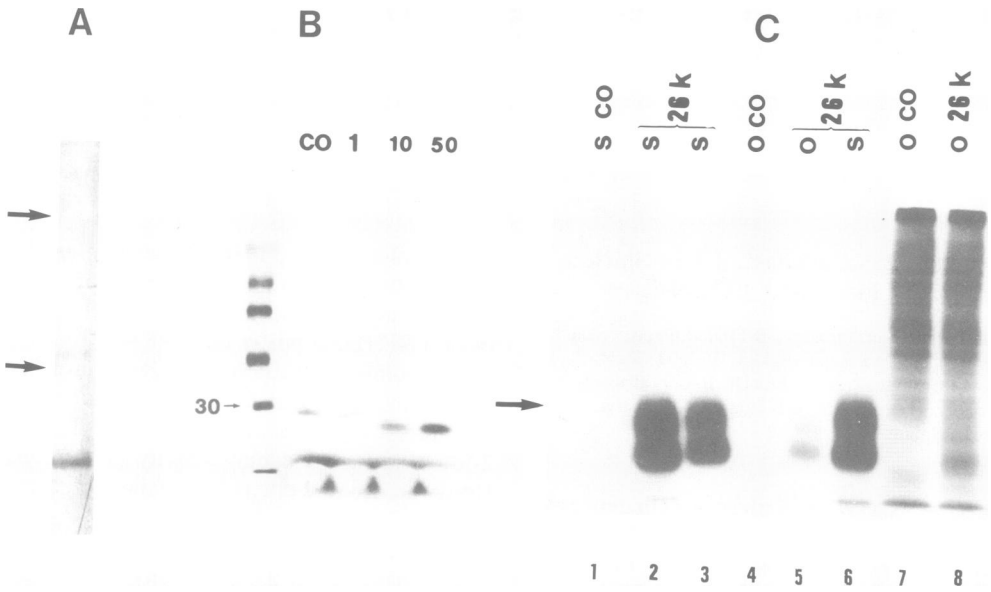

Fig. 2. (**A**) The 26-kd protein mRNA: 0.5 μg of 26-kd protein mRNA produced *in vivo* was denatured, run on a 1.1% agarose gel and stained (see Materials and methods). The arrows indicate the position of the 18S and 28S rRNA markers. (**B**) Translation of 26-kd protein mRNA in rabbit reticulocyte lysate. Different concentrations of 26-kd protein mRNA were incubated 30 min at 31°C in the presence of [^{35}S]methionine in reticulocyte lysate. The products of translation were run on a 13% SDS−polyacrylamide gel and autoradiographed. CO represents control lysate and the RNA concentrations are in ng/ml. (**C**) Production of 26-kd protein by *Xenopus laevis* oocytes. Groups of five oocytes were injected with 2 ng of 26-kd protein mRNA/oocyte (26K) and incubated for 20 h in Barth's medium containing 0.1 mg/ml BSA, 10 U/ml Trasylol and 900 μCi [^{35}S]methionine/ml. Non-injected oocytes (CO) were incubated in the same way. The oocytes (O) and their incubation medium (S) were treated as described in Materials and methods. Samples 1−5 were immunoprecipitated with an antiserum directed against partially purified IFN-β, also containing natural 26-kd protein (Content *et al.*, 1982). Samples 6−8 were not immunoprecipitated. Lane 2 and lane 3 contain the same material but 15 μl and 7.5 μl respectively.

1986) or IFN-β_2 (Zilberstein *et al.*, 1986b) for a lymphokine, called BSF-2, which stimulated immunoglobulin synthesis in an EBV-transformed cell line (Hirano *et al.*, 1986). The other report described the purification of a fibroblast-derived 24-kd protein (Van Damme *et al.*, 1987) with the same N-terminal amino acid sequence as the 26-kd protein. This protein supported the growth of a set of factor-dependent, transformed murine B cells (Van Snick *et al.*, 1986).

In this context, we decided to test whether recombinant 26-kd protein, which can be obtained in virtually pure form by high-level expression in *Xenopus* oocytes, displayed both differentiation and growth factor activities for B cells and whether or not it had any antiviral activity.

Results

Construction of the pSP64-T 26K plasmid

In order to study the biological properties of the 26-kd protein, we subcloned the corresponding gene in the SP64-T plasmid described by Krieg and Melton (1984). By this system, it is possible to produce a synthetic mRNA by cell-free transcription. This mRNA is capped, polyadenylated and contains 5′ and 3′ untranslated regions which will allow efficient translation in *Xenopus laevis* oocytes. This approach was complicated by the fact that we did not have a full-length cDNA clone at our disposal. We completed the gene using a genomic clone (g 26K-4, see Materials and methods). However, the latter segment contained a 162-bp intron located close behind the ATG codon, and this intron had to be deleted. The general procedure is explained in Materials and methods and is illustrated in Figure 1.

In vitro transcription of the SP64-T 26K gene

The plasmid could be linearized with *Sal*I and transcribed *in vitro* with SP6 RNA polymerase resulting in the 26-kd protein mRNA coding sequence flanked with 5′ and 3′ untranslated regions of *Xenopus laevis* β globin gene. We expected such a flanked construction to be efficiently translated in *Xenopus laevis* oocytes (Kruys *et al.*, 1987). This mRNA also possesses a poly(A)·poly(C) tail which does not interfere with its biological activity (Krieg and Melton, 1982). After denaturation, the synthetic RNA runs as a major band of 1000 nucleotides on agarose gel (some uncharacterized larger RNA of ~2000 bp can also be seen) (Figure 2a).

Translation of the 26-kd protein mRNA

The synthetic 26-kd protein mRNA was first tested in a rabbit reticulocyte lysate translation assay. The products of the translations were analysed on an SDS−polyacrylamide gel and autoradiographed. Figure 2b shows that three endogenous proteins are present in the control lysate. In addition, a 26-kd protein appeared in the lysates incubated with different concentrations of the RNA while the amount of the endogenous proteins progressively diminished. This preliminary assay suggests that the construction is correct and that the mRNA can be translated *in vitro* in a protein which could be a non-cleaved and non-glycosylated precursor of the two proteins of apparent mol. wt of 22 kd and 27 kd observed *in vivo* (Content *et al.*, 1982). We expected that such proteins if efficiently translated, would be secreted by *Xenopus laevis* oocytes after injection of the corresponding mRNA. To test this hypothesis, this mRNA was injected in oocytes and the incubation performed in the presence of [^{35}S]methionine as described in Materials and methods. Figure 2c shows secreted as well as internal proteins produced by the oocytes, analysed on SDS−acrylamide gel. As expected, most of the translated protein is secreted in the medium as two components of 22 kd and 27 kd. Moreover, the protein can be obtained in the incubation medium without any immunoprecipitation as the only newly synthesized component (Figure 2c). ^{35}S-

Table I. Antiviral activity of the 26-kd protein and IFN-β

Cell	Virus	Antiviral activity determined		Calculated sp. act. (U/mg)	
		26K	IFN-β	26K	IFN-β
FS4	VSV	< 1U/600 ng	300 U/ng	< 1500	3×10^8
FS4	EMC	< 1U/600 ng	500 U/ng	< 1500	5×10^8
FS4 (aged: 1 week confluent)	VSV	< 1U/600 ng	160 U/ng	< 1500	1.6×10^8
FS4 (aged: 1 week confluent)	EMC	< 1U/600 ng	300 U/ng	< 1500	3×10^8
T21	VSV	< 1U/600 ng	500 U/ng	< 1500	5×10^8
T21	EMC	< 1U/2000 ng	1600 U/ng	< 150	5.5×10^8
UAC	VSV	< 1U/1500 ng	300 U/ng	< 600	3×10^8

The assay was performed as described in Materials and methods. (The interferon activity is expressed in laboratory units per ml; in the standard assay, FS4 cells and EMC virus, 5×10^8 laboratory units/ml corresponds to 1.7×10^8 international U/ml.)

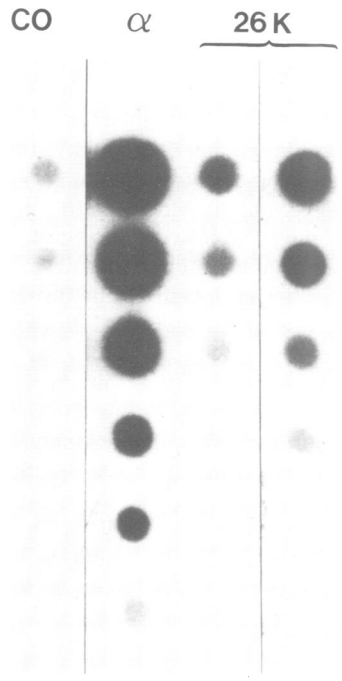

Fig. 3. Induction of 56-kd protein mRNA. Confluent UAC cells were treated with 500 U/ml IFN-α or with 1.5 μg/ml 26-kd protein for 6 h. They were then extracted as described in Materials and methods. The samples were diluted 1:3 several times, spotted on a nylon membrane, hybridized with a probe specific for the 56-kd protein mRNA (Wathelet *et al.*, 1986) and autoradiographed. CO untreated cells (20 μl); α, IFN-α treated cells (20 μl); 26K, 26-kd protein-treated cells 20 μl (left lane) and 40 μl (right lane).

monitoring of the protein allowed us to quantify the amount of protein secreted by the oocytes which amounted to 1−3 μg protein/ml after a 24-h incubation. This allowed us to calculate the specific activities of the proteins used in different bio-assays.

Antiviral activity of the 26-kd protein

Several studies were reported in the last few years on the antiviral activity of the 26-kd protein and the question remains controversial (Weissenbach *et al.*, 1979, 1980; Content *et al.*, 1982). Recently, Zilberstein *et al.* (1986a) showed that a 26-kd protein (which they called IFN-β_2) produced constitutively by stable transfection of the 26-kd protein gene in CHO cells, has an antiviral activity on different types of cells challenged with several types of viruses. They calculated that the sp. act. of the protein was 50−100 times less than that of IFN-β_1 in the same assays. In order to clarify this situation we performed antiviral assays on the 26-kd protein produced by microinjection in oocytes. To standardize accurately, IFN-β produced in the same way (see Materials and methods) was used as a positive control.

Human amniotic cell line (UAC cells), human fibroblasts trisomic for chromosome 21 (T21) as well as FS4 cells (confluent monolayer used either directly or after ageing) were used in this assay with VSV or EMC virus as challenging virus. The results presented in Table I show that although IFN-β has a strong antiviral activity on the different cell lines, 26-kd protein has no activity at all in the same conditions. The calculated sp. act. of IFN-β is $1-5 \times 10^8$ U/mg protein which fits very well with generally accepted data (Knight *et al.*, 1980).

If the 26-kd protein has any antiviral activity at all its sp. act should be < 150 U/mg. This means that it is at least 3×10^6 times lower than that of IFN-β, translated and secreted from oocytes in identical conditions.

Induction of the 56-kd protein mRNA

To complete the comparison between IFN-β and the 26-kd protein, we tested for induction of the 56-kd protein mRNA, a protein well known to be induced by different types of IFN (Chebath *et al.*, 1983; Wathelet *et al.*, 1986). Figure 3 shows that the 26-kd protein indeed induced weakly the 56-kd protein mRNA. This induction required 150 000 times higher concentration of the 26-kd protein than that of HuIFN-α.

B cell growth promoting activity

Supernatants of oocytes were first tested for their ability to support the growth of the murine IL-HP1-dependent B cell hybridoma 7TD1. As shown in Table II, oocyte supernatants that contained ~ 3 μg/ml of 26-kd protein supported the growth of these cells up to a dilution of $\sim 7 \times 10^{-6}$. The specificity of this effect was shown by the fact that control supernatants or supernatants containing a similar concentration of human IFN-β had no such growth-promoting effect on 7DT1 cells. Oocyte-derived 26-kd protein was also tested on the murine plasmacytoma TEPC2033C, another IL-HP1-dependent cell line. As described previously for murine IL-HP1, this cell line required ~200 times more 26-kd protein than did hybridoma 7DT1 (data not shown).

Stimulation of IgG production

26-kd protein made in *Xenopus laevis* oocytes induced IgG secretion in CESS cells; the sp. act was $\sim 5 \times 10^7$ U/mg of protein (Figure 4), which is in agreement with results obtained by Hirano *et al.* (1986) using purified natural BSF-2 (1.7×10^7 U/mg). Proliferation of the murine B cell hybridoma 7DT1 is ~ 4 times more sensitive to 26-kd protein (sp. act of $\sim 2 \times 10^8$ U/mg). 26-kd protein only induced a slight proliferation

Table II. Hybridoma growth factor activity of recombinant 26-kd protein

Recombinant material in oocyte supernatant	Hybridoma growth factor activity[a]	
	U/ml	U/mg
Control	<10	–
IFN-β	<10	$<10^4$
26-kd protein	1.5×10^5	2×10^8

[a]Tested as described in Materials and methods.

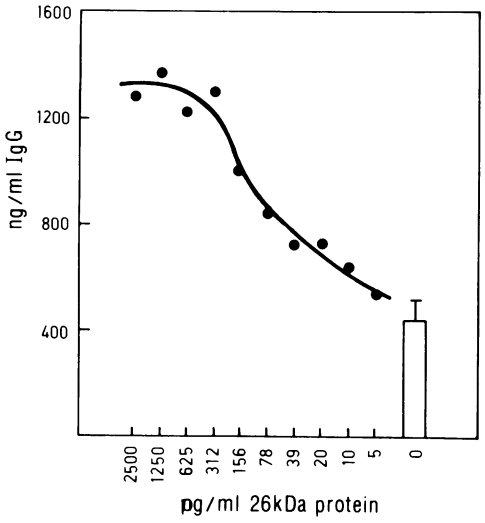

Fig. 4. Induction of IgG production by 26-kd protein in CESS cells. CESS cells (6×10^3/microwell) were incubated for 4 days in a 1:2 serial dilution of 26-kd protein containing oocyte supernatant (600 ng/ml) and the concentration of IgG was determined. One unit is defined as the amount of 26-kd protein required to induce an IgG concentration equal to 50% of the increase at plateau level and corrected for the control (open bar on the right; SD indicated on top).

enchancement of CESS cells (data not shown). Control by mock-injected oocyte supernatant did not reveal any induction of IgG secretion or proliferation-enhancing activity.

Discussion

The use of recombinant 26-kd protein in known amounts allows its biological activities to be defined accurately. The recombinant protein has been produced by translating in oocytes a pure synthetic chimeric mRNA containing the 26-kd protein coding region, flanked by *Xenopus* oocytes β-globin 5′ and 3′ untranslated regions. In parallel we have produced HuIFN-β by using exactly the same technique. Both molecules are processed, glycosylated and efficiently secreted by the oocytes and found radiochemically pure, in their medium. Therefore, by using oocyte incubation medium without any further purification we avoid the pitfalls inherent to such techniques. On the other hand, internal standardization of the antiviral assay is conveniently based on the antiviral activity of recombinant HuIFN-β produced in identical conditions. The exact extent of glycosylation in the oocyte system is not known and should be evaluated by using inhibitors of glycosylation and/or digesting the molecules with purified endoglycosidases. It is unlikely, however, that different extent of glycosylation would affect the biological activity of such a molecule. This is indeed neither the case for IFN-β (Derynck *et al.*, 1980) nor for HuIFN-γ (Rinderknecht *et al.*, 1984). Furthermore, natural BSF-2 has been purified in two forms of 20K and 27K with similar BSF activity (Hirano *et al.*, 1985).

In view of the data reported for natural 26-kd protein (named IFN-β_2 by others; Zilberstein *et al.*, 1986b) it was surprising to find no antiviral activity with this recombinant 26-kd protein (if anything, the sp. act should at least be 3×10^6 times lower than that reported for IFN-β or IFN-α_2). The fact that we failed to detect antiviral activity is unlikely to be due to denaturation of the protein or to the presence of inhibitors, as the same preparation has a very high activity on B cells (Table II, Figure 3).

Interferons have been shown to induce the production of several proteins, including 2-5A synthetase, 56-kd protein and HLA antigens. Zilberstein *et al.* (1986a) have reported previously that the 26-kd protein induced the production of these proteins. Using pure recombinant 26-kd protein we confirmed that it could induce the 56-kd protein mRNA; the efficiency per mg protein however, was at least 150 000 times lower than that of type I interferons. Furthermore, the induction of 56-kd protein is not a unique property of interferons since poly(I)·poly(C) and IL-1 can also induce this gene (M.Wathelet *et al.*, in preparation).

In contrast to its lack of antiviral activity, recombinant 26-kd protein translated in oocytes proved to be very active as a B cell differentiation and growth modulating factor. The B cell differentiation activity of the protein was demonstrated by its ability to stimulate immunoglobulin secretion by an EBV-transformed cell line, which confirmed its identity with BSF-2 (Hirano *et al.*, 1986). However, while Hirano *et al.* (1986) failed to detect any effect of BSF-2 on the proliferation of some types of B cells, our results indicate that the recombinant 26-kd protein was also endowed with growth factor activity on two factor-dependent, transformed mouse B cell lines. Interestingly, the sp. act of the 26-kd protein was similar in the murine and human cell assays, which implies that its B cell growth factor activity is at least as high as its B cell differentiation activity. In the absence of human cells equivalent to the transformed mouse cell lines used here, it is difficult, however, to draw definitive conclusions regarding the relative potency of these two activities. At any rate, one conclusion seems inescapable: the strong activity of 26-kd protein on mouse B cells cannot be attributed to its interaction with the human chromosome 21-encoded Type I interferon receptor, which, according to Zilberstein *et al.* (1985) also mediates the controversial antiviral effect of the 26-kd protein.

Recently Van Damme *et al.* (1987) identified a protein in the supernatant of IL-1-treated human fibroblasts, which supported the growth of the same transformed murine B cells as the ones used in the present report. N-terminal amino acid sequencing of this protein yielded a stretch of 12 amino acids that was identical with the deduced N-terminal sequence of the 26-kd protein. However, as this protein was devoid of any detectable antiviral activity, some doubts remained as to its complete identity with the 26-kd protein. The results obtained here with recombinant material now formally prove that the 26-kd protein is indeed a B cell growth factor and strongly suggest that the factor identified by Van Damme *et al.* (1987) corresponds to a natural form of the 26-kd protein. Defilippi *et al.* (1987) have reported that the 26-kd protein could be an inhibitor of the growth-promoting activity of tumor necrosis factor on certain fibroblast cell lines. Although this observation may seem surprising in view of the growth factor effect of the molecule on B cells, the possibility that the 26-kd protein may stimulate the growth of some cells while inhibiting that of others should be given careful consideration, by analogy with, for instance, the results reported for TGFβ (Roberts *et al.*, 1985).

As mentioned above, the mouse B cell lines that allowed us to detect the B cell growth activity of the 26-kd protein have previously led to the discovery of a new murine lymphokine termed IL-HP1. This raises the question of the relationship between murine IL-HP1 and the human 26-kd protein. Functionally, these factors are obviously closely related because they can be used interchangeably to support factor-dependent cell lines which fail to survive with any other lymphokine tested so far. It is not unreasonable, therefore, to speculate that IL-HP1 and the 26-kd protein may eventually prove to be homologous. However, it should be stressed that what is known so far from the amino acid sequence of IL-HP1 shows no significant homology with the sequence of the 26-kd protein (Van Snick *et al.*, 1986).

The B cell growth-promoting activity of the 26-kd protein reported here is not accurately described by the term BSF-2. For this reason, and also because we do not know whether the activity of the protein will be restricted to B cells, we suggest the protein to be renamed interleukin-6. This would end the confusion of the present nomenclature, which uses three different names for the same molecule (IFN-β_2, BSF-2 and 26-kd protein).

Materials and methods

pSP64-T 26K plasmid construction

DNA constructions and plasmids preparations were made using standard procedures (Maniatis *et al.*, 1982). The genomic 26K clone and the cDNA clone p26K-7 were described previously (Haegeman *et al.*, 1986). pSP64-T (Krieg and Melton, 1982) was kindly given to us by Dr D.Melton.

To construct the BS-26K plasmid, the Blue-scribe M13$^+$ vector (Vector Cloning Systems) (BS-M13$^+$) was first digested with *Xba*I, blunted by Klenow polymerase and then digested with *Sal*I. A subclone (g 26K-4) containing the 3800-bp *Eco*RI fragment of the 26-kd protein gene and coding for the promoter 5′ region of the gene was digested with *Xho*I and *Taq*I. Insert p26K-7 cDNA was digested with *Taq*I and *Dra*I. The resulting 400-bp and 600-bp fragments were first ligated together and then subcloned in the *Sal*I, *Xba*I digested BS M13$^+$ vector. The latter (BS-26K) was digested with *Ava*II and *Bam*HI to obtain a 750-bp fragment.

To produce the pSP-64-T 26K, two synthetic oligodeoxynucleotides (of 35 and 36 nucleotides respectively) were annealed to form a *Bgl*II−*Ava*II double-stranded oligodeoxynucleotide comprising the first nine amino acid codons of the 26K protein as well as the ATG codon. This oligonucleotide was ligated to the 5′ end of the 750-bp *Ava*II−*Bam*HI fragment and to a *Bgl*II digested pSP64-T. This general procedure is illustrated in Figure 1.

Capped transcripts of pSP64-T 26K

The pSP64-T 26K plasmid was digested with *Sal*I, phenol/chloroform and ether extracted, and ethanol precipitated. One microgram linearized DNA was transcribed in a 20-μl reaction mixture containing 40 mM Tris−HCl (pH 8.5), 6 mM $MgCl_2$, 10 mM dithiothreitol, 2 mM spermidine−HCl, 25 U human placental ribonuclease inhibitor (Amersham), 250μM GTP, 500 μM ATP, UPT and CTP, 10 U of SP6 polymerase (BRL). As 5′ terminal cap structure, 500 μM of the methylated m7G(5′)ppp(5′)G (Pharmacia) was added during the transcription in the reaction which was performed at 37°C for 1 h (Contreras *et al.*, 1982; Wathelet *et al.*, 1986). Following RNA synthesis, the DNA template was removed by the addition of 20 μg/ml RNase-free DNase (Worthington) and 10 mM vanadyl-ribonucleoside (BRL). After 10 min incubation the sample was extracted with phenol/chlorophorm, then with ether, and ethanol precipitated. The same procedure was followed to produce an IFN-β mRNA from a pSP64-T IFN-β plasmid (Kruys *et al.*, 1987). The synthetic 26-kd protein mRNA was denatured by glyoxal treatment, run on a 1.1% agarose gel, and transferred on a nylon membrane (Poupart *et al.*, 1984). The latter was stained following a method described by Saman (1986) using 'ferry dye' (Janssen Pharmaceutica).

Translation of the 26-kd protein mRNA in reticulocyte lysate

The preparation of the reticulocyte lysates was as described elsewhere (Content *et al.*, 1982). Different concentrations of mRNA were translated in 10 μl of reticulocyte lysate containing 45 mM fructose, 10 mM spermidine, 1.5 mg/ml tRNA and 15 μCi[^{35}S]methionine by incubation at 31°C for 30 min. Proteins were analysed by electrophoresis in 13% SDS−acrylamide gels with a 3% stacking gel. Gels were fixed with methanol (30%) and acetic acid (10%), treated for 20 min with 'Amplify' (Amersham), dryed and exposed to Kodak XR films with two intensifying screens at −70°C for 15 h.

Microinjection in Xenopus laevis oocytes

The RNA produced *in vitro* was dissolved in water at a final concentration of 100 μg/ml for injections into oocytes. Injected oocytes were incubated in Barth's medium (Gurdon, 1968) (10 μl/oocyte) containing 900 μCi/ml [^{35}S]methionine, 0.1 mg/ml BSA and 10 U/ml Trasylol, for 15 h at 18°C. To examine the newly synthetized intracellular proteins, oocytes were homogenized in 10 mM Tris−HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton-X100, 0.1% deoxycholate, 0.1% SDS and 1 U/ml Trasylol. The incubation medium or the homogenized oocytes were immunoprecipitated with a goat serum directed against partially purified IFN-β known to immunoprecipitate IFN-β as well as the 26-kd protein (Content *et al.*, 1982), and run on a 13% SDS−acrylamide gel. The non-immunoprecipitated proteins were also analysed on the same gel.

IFN-β mRNA obtained by *in vitro* transcription of the pSP-64T IFN-β plasmid was also microinjected in the same conditions (Kruys *et al.*, 1987).

Antiviral assay

Incubation fluid was tested by a cytopathic effect (CPE)-inhibition assay on human fibroblasts trisomic for chromosome 21, on human FS4 fibroblasts and on human amniotic UAC cells in microtiter trays. The cells were challenged with vesicular stomatitis virus or encephalomyocarditis virus and the CPE was recorded at 24 or 48 h (Derynck *et al.*, 1980). All assays included an internal IFN-β reference of *in vitro* synthesized IFN-β mRNA in *Xenopus laevis* oocytes as described above.

Induction of '56K protein' mRNA

Confluent UAC cell cultures were treated with 1.5 μg/ml of 26-kd protein or 500 U/ml IFN-α (~10^6 U/mg, H.Claeys, KUL) for 6 h. Cytoplasmic extracts were then prepared following the method of White and Bancroft (1982) and treated as described previously (Poupart *et al.*, 1984). The samples were hybridized with a specific 56K protein gene probe (Wathelet *et al.*, 1986) as described before (Defilippi *et al.*, 1987).

IL-HP1 assay

This assay was performed with mouse B cell hybridoma 7TD1 and plasmacytoma TEPC1033C, as described previously (Van Snick *et al.*, 1986, 1987). Briefly, cells were washed free from murine IL-HP1 and seeded at a density of 2×10^3 cells/microwell in a final volume of 0.2 ml. Cultures were incubated at 37°C in 8% CO_2 in the presence of serial dilutions of growth factors. After 3−4 days, the number of living cells was evaluated by measuring hexosaminidase levels as described by Landegren (1984).

Assay for BSF-2 activity (Hirano et al., 1985)

CESS-cells (ATCC TIB 190), an (EBV)-transformed B cell line, were cultured in 200 μl of RPMI 1640 medium with 10% fetal calf serum in the presence of 1:2 dilutions of recombinant 26-kd protein. After 4 days the concentration of secreted IgG was determined by an ELISA. One U/ml was defined as the amount 26-kd activity that induced 50% of the maximal response after correction for the background.

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