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Combined Effects of the Signal Sequence and the Major Chaperone Proteins on the Export of Human Cytokines in *Escherichia coli*

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We have studied the export of two human proteins in the course of their production in *Escherichia coli*. The coding sequences of the granulocyte-macrophage colony-stimulating factor and of interleukin 13 were fused to those of two synthetic signal sequences to direct the human proteins to the bacterial periplasm. We found that the total amount of protein varies with the signal peptide-cytokine combination, as does the fraction of it that is soluble in a periplasmic extract. The possibility that the major chaperone proteins such as SecB and the GroEL-GroES and DnaK-DnaJ pairs are limiting factors for the export was tested by overexpressing one or the other of these chaperones concomitantly with the heterologous protein. The GroEL-GroES chaperone pair had no effect on protein production. Overproduction of SecB or DnaK plus DnaJ resulted in a marked increase of the quantity of human proteins in the periplasmic fraction, but this increase depends on the signal peptide-heterologous protein-chaperone association involved.

The proteins of the cytokine family, essential mediators of cellular communication in eukaryotic organisms (2, 5), are the object of intensive research. The large amount of purified protein that is necessary to analyze their structure and function or to test their potential use in therapy can only be provided through recombinant DNA technology. Among the possible hosts, *Escherichia coli* offers the advantage of its well-known genetics as well as the availability of numerous efficient vectors. We therefore chose this bacterium for the production of two cytokines of human origin, the granulocyte-macrophage colony-stimulating factor (hGM-CSF) (2) and interleukin 13 (hIL-13) (20). High-level production of these proteins in a biologically active conformation in *E. coli* is hampered either by the formation of inclusion bodies (hIL-13) or by the poor stability of the protein expressed in the cytoplasm (hGM-CSF). We therefore decided to direct these polypeptides to the bacterial periplasmic space (23), with the expectation that they may find better conditions for proper folding at that site, especially for the formation of stabilizing disulfide bonds (3). An additional reason is that, after maturation by the signal peptidase (23), the exported proteins should have the correct N-terminal sequence.

In *E. coli*, most of the proteins destined for export are first synthesized in the cytoplasm as precursor protein with an amino-terminal signal sequence (for recent reviews, see references 11, 23, and 24). The preprotein is then taken up by the translocation machinery which, starting from the amino terminus, threads it through the membrane. To this end, it is maintained after its synthesis in a very partially folded conformation through the combined action of different factors which are the signal sequence itself and special proteins called molecular chaperones (10, 23). Among them, the most involved with exported polypeptides is SecB (8, 14, 23, 27), but some precursor proteins require other molecular chaperones such as GroEL and GroES (1, 15, 22) or DnaK and DnaJ (1, 22, 28).

In this analysis of the conditions leading to improved expression and solubility of the hGM-CSF and hIL-13 cytokines, we limited our scope to factors operating at an early stage of the export pathway, when the preproteins still are on the cytoplasmic side. A key point of the efficiency of the process being the adequation of the signal sequence and the protein, we first focused on the analysis of the influence of the signal peptide on the yield of exported hGM-CSF and hIL-13. We then tested whether overproduction of a given chaperone could improve cytokine export. To investigate this possibility, we overexpressed each of the two cytokine genes together with the genes of the major chaperone systems of *E. coli*, i.e., SecB, GroEL and GroES, or DnaK and DnaJ, and measured the effect of coexpression on cellular growth, protein production, and export.

MATERIALS AND METHODS

Bacterial strain and growth conditions. *E. coli* K-12 strain JS219 [MC1061 derivative (F^-) *araD139* Δ(*ara-leu*)*7696* Δ(*lac*)*X74 galU galK hsdR2 mcrB1 rpsL* (Str^r) *malP*::*lacI*^{q}] was used as the host for the various expression plasmids. Cultures were grown in Luria-Bertani (LB) medium (19) supplemented with ampicillin plus methicillin, chloramphenicol, or tetracycline at a concentration of 50 and 500, 25, or 12.5 $\mu g \cdot ml^{-1}$, respectively. Induction of the transcription at the tac promoter was accomplished by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) at a concentration of 1 mM.

Plasmid constructions. The hIL-13 and GM-CSF coding sequences were cloned by use of standard protocols in pVG388 (12), a derivative of pBR322. As shown in Fig. 1A, it carries the *bla* gene (Amp^r), the *lac*I gene, and an improved version of the tac promoter, followed by an efficient translation initiation region (with an ATG initiator codon included in an *Nde*I site) and a pair of transcription terminators (preceded by a *Bam*HI site). A sequence coding for the SP1 or SP3 signal peptide was also inserted in front of each gene (see Table 1). The four resulting plasmids are pEMR639 (SP1–hGM-CSF fusion), pEMR727 (SP3–hGM-CSF fusion), pLNA12 (SP3–hIL-13 fusion), and pLNA13 (SP1–hIL-13 fusion). A control plasmid, pLNA15, was constructed by deleting the *Xba*I-*Bam*HI fragment containing hGM-CSF from plasmid pEMR727 (see Table 1). The chaperone genes were taken from plasmids pOF39 (7), pDM38 (21), and pJW25 (27) and subsequently cloned in a p15A-derived plasmid to allow compatibility with the cytokine expression vector (Fig. 1B to D). The expression of the *groES groEL* operon in pEMR602 is driven by its natural promoters, as is that of the *dnaK dnaJ* operon carried by pLNA2. This ensures a high level of all of these proteins (about 5 to 20% of the total protein), especially at high temperature (37°C and above), since the promoters in question are controlled by the σ^{32} heat shock transcription factor. pEMR602 was constructed as follows. The *groE*-containing *Sca*I-*Hin*dIII fragment of pOF39 (7) was inserted into the pUC18

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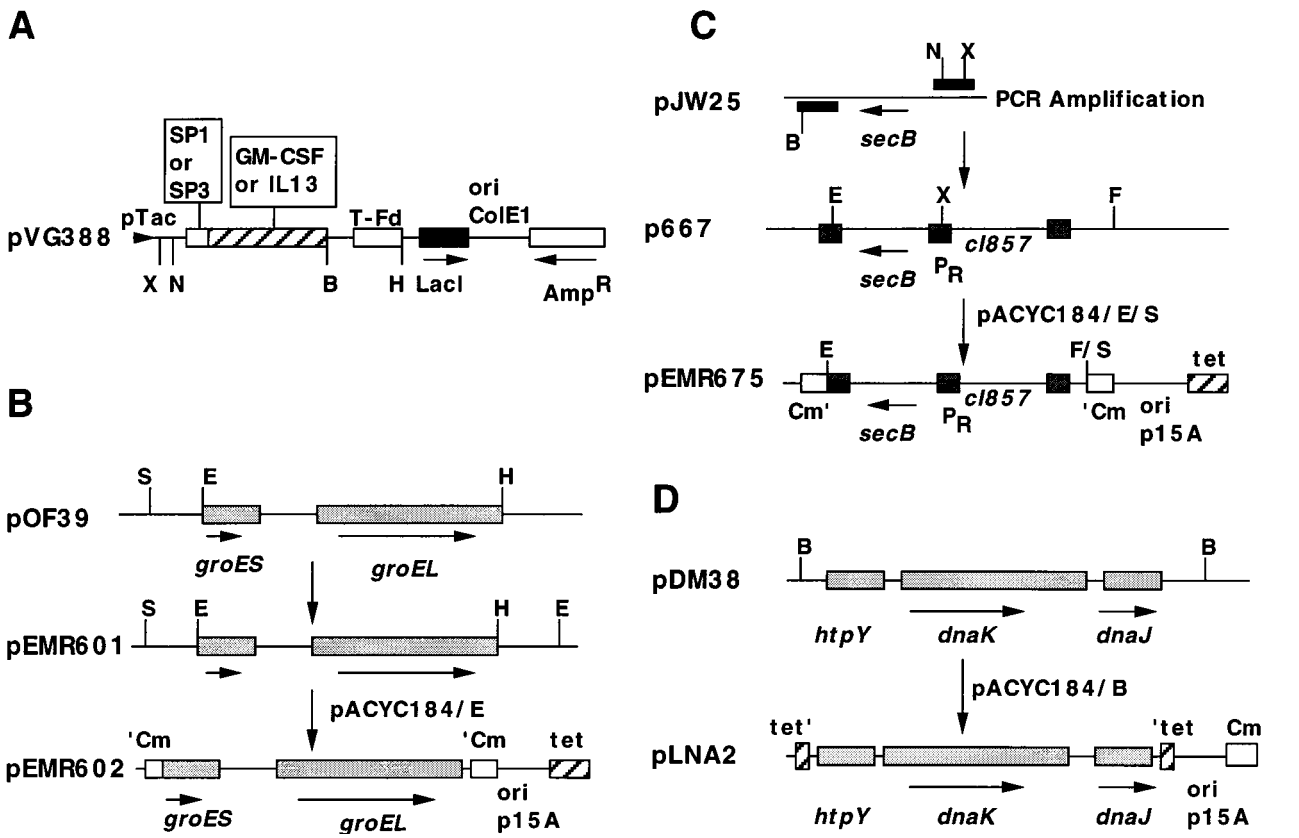

FIG. 1. Construction of the cytokine and chaperone expression vectors. (A) The hGM-CSF and hIL-13 plasmids were derived from the pVG338 vector (13) as described in Materials and Methods. Restriction sites for *Xba*I (X), *Nde*I (N), *Bam*HI (B), *Sca*I (S), *Eco*RI (E), *Fsp*I (F), and *Hin*dIII (H) are indicated. T-Fd, transcription terminator. (B) pEMR602 corresponds to the GroEL and GroES expression plasmid. (C) The SecB expression plasmid, pEMR675, is also a derivative of pACYC184. (D) pLNA2 corresponds to the DnaK and DnaJ expression vector.

vector to give pEMR601, from which an *Eco*RI fragment carrying the *groE* operon was then inserted into the *Eco*RI site of pACYC184. pLNA2 was constructed by recloning the *Bam*HI fragment of pDM38 (21), containing the *dnaK* and *dnaJ* operon, into the *Bam*HI site of pACYC184. In plasmid pEMR675, the *secB* gene is positioned behind the p_R promoter of bacteriophage λ. The presence on this plasmid of a gene coding for a thermosensitive repressor (*c*I857) allows derepression of expression by simply raising the temperature; at 37°C, SecB constitutes about 0.5% of the total protein. The *secB* gene was obtained from plasmid pJW25 (27) by PCR amplification; a ribosomal binding site and a properly positioned ATG initiator codon were provided by the *Xba*I-*Nde*I primer. The *Xba*I-*Bam*HI DNA fragment was inserted into a plasmid vector (plasmid p667). The *Eco*RI-*Fsp*I DNA fragment containing the *secB* expression cassette was inserted into pACYC184 digested with *Eco*RI and *Sca*I, to give pEMR675.

Analysis of protein production. (i) Fractionation. *E. coli* cells were transformed by electroporation (25) with either a plasmid directing synthesis of one of the heterologous proteins, a plasmid expressing one of the chaperones genes, or both. The resulting strains were grown in LB medium containing ampicillin and methicillin. When the optical density measured at 600 nm (OD_{600}) reached 0.5, expression was induced by adding IPTG at a final concentration of 1 mM, and the culture was incubated for an additional 150 min. For the analysis of the total protein content, the cells were harvested by centrifugation and resuspended

TABLE 1. Nucleotide and amino acid sequences corresponding to the amino-terminal regions of the hybrid proteins generated by fusion of the SP1 and SP3 signal peptides with the hGM-CSF and hIL-13 cytokines

Plasmid	Fusion	Sequence(s)[a]	
		Signal peptide	Heterologous protein
pEMR639	SP1–hGM-CSF	ATG GCT CCA TCT GGC AAA TCC ACG CTG CTT CTC TTA TTT CTG CTC CTG TGC CTG CCC TCT TGG AAC GCC GGC GCT	
		MAPSGKSYLLLLFLLLCLPSWNAGA	APARSPSPSTQ...
pEMR727	SP3–hGM-CSF	ATG AAT CAC TCA GGC AAA TCC ACG CTG CTT CTC TTA TTT CTG CTC CTG TGC CTG CCC TCT TGG AAC GCC GGC GCT	
		MNHSGKSYLLLLFLLLCLPSWNAGA	APARSPSPSTQ...
pLNA13	SP1–hIL-13	ATG GCT CCA TCT GGC AAA TCC ACG CTG CTT CTC TTA TTT CTG CTC CTG TGC CTG CCC TCT TGG AAC GCC GGC GCT	
		MAPSGKSYLLLLFLLLCLPSWNAGA	GPVPPSTALRE...
pLNA12	SP3–hIL-13	ATG AAT CAC TCA GGC AAA TCC ACG CTG CTT CTC TTA TTT CTG CTC CTG TGC CTG CCC TCT TGG AAC GCC GGC GCT	
		MNHSGKSYLLLLFLLLCLPSWNAGA	GPVPPSTALRE...

[a] The top sequence is the nucleotide sequence; the lower sequence is the amino acid sequence.

in a denaturing sample buffer (25 mM Tris-HCl [pH 6.8], 5% glycerol, 1.25% SDS, 2.5% β-mercaptoethanol, 0.02% bromophenol blue) at a concentration of 1 OD_{600} unit · ml^{-1}. For the estimation of the relative amounts of soluble and insoluble proteins, the cells were treated as described above except that the buffer was TNE buffer (10 mM Tris-HCl [pH 7.5], 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol). The proteins were lysed by sonication, and the soluble and insoluble fractions were separated by centrifugation at 12,000 rpm for 5 min in a Sigma model 201 M microcentrifuge. For the analysis of the soluble proteins present in the periplasm, osmotic shock was performed as described by Joseph-Liauzun et al. (12). The cells were resuspended as described above in a buffer containing 100 mM Tris-HCl [pH 8], 15% sucrose, 1 mM dithiothreitol, and 2 mg of lysozyme per ml. After a 1-h incubation at 4°C, soluble and insoluble components were separated by centrifugation at 12,000 rpm for 5 min (Sigma model 201 M microcentrifuge).

(ii) Gel electrophoresis of proteins and Western blot (immunoblot) analysis. Proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (17). Prestained low-molecular-weight markers (Bio-Rad) were used for calibration. Transfer of the resolved proteins onto nitrocellulose membranes and the subsequent immunodetection were carried out as described by Harlow and Lane (9). The primary antibody preparation used for probing the Western blot consisted of rabbit polyclonal antibodies raised against hGM-CSF (Genzyme Corporation) or against hIL-13 (Sanofi). The secondary antibody was goat anti-rabbit immunoglobulins G conjugated with horseradish peroxidase (Amersham). Binding of the second antibody was revealed with an enhanced chemiluminescence kit, as described in the manufacturer's instructions (Amersham), and the relative amounts of protein were estimated by densitometric scanning of the films. Absolute amounts were obtained by comparing the signals given by cell extracts with those given by known amounts of purified hGM-CSF or hIL-13 applied to a nitrocellulose membrane.

Mutagenesis of the signal sequence. The SP3 signal sequence differs from that of SP1 by a few nucleotides downstream of the initiation codon (the AUG-GCUCCAUCU stretch of SP1 is replaced by AUGAAUCACUCA in SP3 mRNA). This leads to amino acid changes at position −24 (Asp instead of Gly) and at position −25 (His instead of Pro; +1 being the first amino acid of hGM-CSF or hIL-13). Mutagenesis of SP1 to SP3 was carried out by PCR amplification of the SP1–hGM-CSF gene, present in pEMR639, with an oligonucleotide containing the desired mismatches as one of the primers. The amplified fragment was digested with *Nde*I and *Bam*HI and recloned into the same vector; the sequence of the resulting construct, pEMR727, was verified before use. The fusion of the hIL-13 gene to SP1 (pLNA13) or SP3 (pLNA12) was subsequently generated by substitution of the hIL-13 coding phase for that of hGM-CSF in the pEMR639 or pEMR727 plasmid (see Table 1).

RESULTS

Export of cytokines fused to bacterial signal peptides. The hGM-CSF gene was fused in vector pVG388 (13) (Fig. 1) to an artificial leader sequence, called SP1, which can promote the efficient export of human growth hormone in *E. coli* (13). This sequence codes for an optimized signal peptide (Table 1) (11, 23). A second leader sequence, called SP3, was generated by the introduction of a few point mutations into SP1 (see Materials and Methods) (Table 1). These changes were designed to obtain a more efficient initiation of translation by disrupting the potential secondary structure of the mRNA around the initiator codon and by increasing at the same time the complementarity with the anti-downstream box region of 16S rRNA (26). The substitution of SP3 for SP1 indeed has a marked effect on the total amount of protein: the production of hGM-CSF protein is stimulated threefold (Fig. 2A). The results in Fig. 2B, showing that most of the protein is in a single band and that this band corresponds to the mature form (data not shown), suggest that more than 95% of the accumulated protein can be translocated, at least to an extent sufficient to allow cleavage of the signal sequence (see Table 2). However, as exemplified in Fig. 2B, the soluble form of the cytokine does not predominate since about 67% of the protein remains in the pellets. In comparison, we found 80% of the mature form of β-lactamase, an indigenous and truly periplasmic protein (Fig. 2C), present in the supernatant obtained after the osmotic shock.

The coding sequence of hIL-13 was similarly fused, in vector pVG388, to the SP1 and SP3 signal sequences to give plasmids

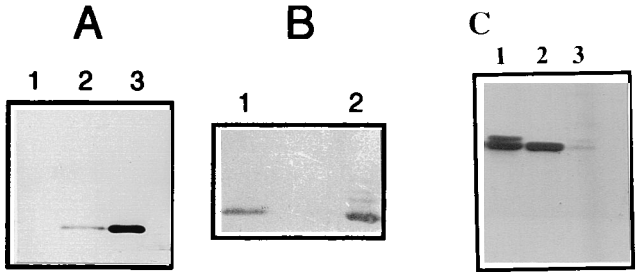

FIG. 2. Effect of signal sequences SP1 and SP3 on hGM-CSF production. (A) Total cell extracts were prepared by sonication of IPTG-induced cultures (150 min at 37°C) of strain JS219 with or without the hGM-CSF expression plasmids. Proteins were separated by SDS-PAGE (15% gel) and analyzed by the Western blot technique. Lanes: 1, strain JS219; 2, JS219 carrying plasmid pEMR639 (corresponding to the SP1–hGM-CSF fusion); 3, JS219 carrying plasmid pEMR727 (corresponding to the SP3–hGM-CSF fusion). (B) Quantity of hGM-CSF present in the soluble (lane 1) or insoluble (lane 2) fraction obtained after osmotic shock treatment of an induced culture of strain JS219(pEMR727). (C) Quantity of β-lactamase present in the total cellular extract (lane 1) or in the soluble fraction obtained after osmotic shock treatment of strain JS219 containing pEMR727 (lane 2) or of strain JS219 without plasmid (lane 3). Each lane was loaded with the proteins obtained from 0.1 OD_{600} unit of culture to allow direct comparison.

pLNA13 and pLNA12, respectively (Fig. 1 and Table 1). Unfractionated lysates from induced cultures were analyzed by the Western blot technique (Fig. 3A). The two constructs lead to a qualitatively very different outcome. With SP1, the mature form of the protein is by far predominant (more than 95%) (Fig. 3A, lane 2), whereas with SP3, the precursor form prevails slightly (60%) (Fig. 3A, lane 3) over the processed one. The overall quantity of protein is about the same in both situations (3 $\mu g \cdot ml^{-1} \cdot OD_{600}$ $unit^{-1}$). These observations suggest that the two types of protein are translated with the same efficiency but that the presence of the SP3 signal sequence severely impedes translocation through the membrane. When solubility was examined after lysis by sonication, 53% of the protein was present in the supernatant fraction for SP1 and 48% (of the mature form) was present in that for SP3. For the latter construct, the preprotein form was found in the pellet fraction only. Surprisingly, solubility in the periplasmic fraction is also influenced by the signal sequence: for the SP1 fusion, about 5% of the accumulated hIL-13 was found in the super-

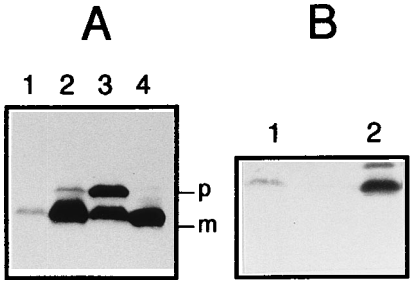

FIG. 3. Influence of the SP1 and SP3 signal peptides on hIL-13 production. (A) Total cell extracts were prepared by sonication, separated by SDS-PAGE on a 15% gel, and analyzed by the Western blot technique. The following strains were analyzed: JS219 (lane 1), JS219 carrying pLNA13 (SP1–hIL-13 fusion) (lane 2), and JS219 with plasmid pLNA12 (SP3–hIL-13 fusion) (lane 3). Purified hIL-13 (50 ng) was also included (lane 4). Positions of the precursor (p) and mature (m) forms of the protein are indicated. (B) Quantity of hIL-13 present in the soluble (lane 1) or insoluble (lane 2) fraction after an osmotic shock treatment of an induced culture of strain JS219(pLNA13) (SP1–hIL-13 fusion). Each lane was loaded with the proteins obtained from 0.1 OD_{600} unit of culture to allow direct comparison.

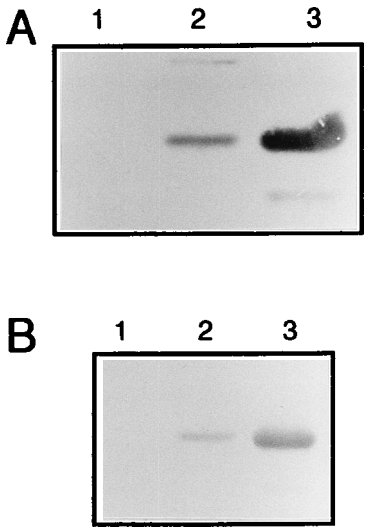

FIG. 4. Effect of chaperone overexpression on hGM-CSF production. Soluble fractions recovered after an osmotic shock treatment, as described in Materials and Methods, were analyzed by Western blotting. (A) Influence of the SecB chaperone. Lanes: 1, strain JS219 without plasmid; 2, strain JS219 carrying the pEMR727 plasmid (SP3–hGM-CSF fusion); 3, strain JS219 carrying the pEMR727 and pEMR675 (*secB* gene carrier) plasmids. (B) Influence of DnaK-DnaJ overexpression on hGM-CSF production. Lanes: 1, strain JS219 without plasmid; 2, strain JS219 carrying the pEMR727 plasmid (SP3–hGM-CSF fusion); 3, strain JS219 carrying the pEMR727 and pLNA2 (DnaK-DnaJ overproducer) plasmids.

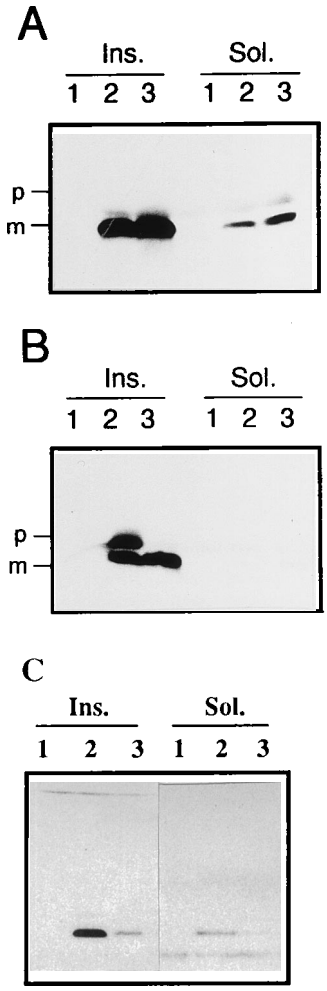

FIG. 5. Chaperone overproduction and hIL-13 expression. Insoluble (Ins.) and soluble (Sol.) fractions recovered after osmotic shock treatment of induced cultures as described in Materials and Methods were analyzed by Western blotting. To allow direct comparison, each lane was loaded with material from 0.1 OD_{600} unit of culture. (A) Effect of SecB overexpression. Lanes: 1, strain JS219 without plasmid; 2, JS219 with the pLNA13 plasmid (SP1–hIL-13 fusion); 3, JS219 with both pLNA13 and pEMR675 (SecB overproducer) plasmids. (B) Effect of DnaK-DnaJ overexpression. Lanes: 1, strain JS219 without plasmid; 2, JS219 with the pLNA12 plasmid (SP3–hIL-13 fusion); 3, JS219 with the pLNA12 and pLNA2 (DnaK-DnaJ overproducer) plasmids. (C) Effect of DnaK-DnaJ overexpression. Lanes: 1, strain JS219 without plasmid; 2, JS219 with the pLNA13 plasmid (SP1–hIL-13 fusion); 3, JS219 with the pLNA13 and pLNA2 (DnaK-DnaJ overproducer) plasmids.

natant after the osmotic shock, whereas none of it was detected for the SP3 construct (Fig. 4 and 5). Thus, even if the yield of soluble protein recovered in the periplasmic fraction is sixfold lower than in the case of hGM-CSF, the absolute quantity ($0.15\ \mu g \cdot ml^{-1} \cdot OD_{600}\ units^{-1}$) is less than threefold lower because of a more efficient expression from the SP1–hIL-13 construct than from the SP3–hGM-CSF construct. The soluble hIL-13 obtained by the osmotic shock method is in its biologically active conformation, since the supernatant fraction stimulated the in vitro proliferation of MB9 mouse cells, (15a, 16) and an hIL-13 concentration similar to the one indicated above resulted (Table 2).

We also analyzed the effect on cell growth of the expression of the different cytokine constructs. In two cases (SP3–hGM-CSF and SP1–hIL-13), the IPTG induction affects the growth curve at 37°C (Fig. 6). The strain which bears the pLNA13 construct (SP1–hIL-13) ceases to grow after 2.5 doublings. The pEMR727-containing cells behave in a strikingly different manner: growth stops after a doubling, and the cells eventually lyse. This induced growth defect may be related to the larger amount of periplasmic cytokine generated by the pLNA13 and pEMR727 plasmids. This would suggest that, with pEMR727 at least, the upper limit of tolerance of the host strain is reached.

Effect of the overproduction of the major molecular chaperones. An insufficient supply of the major cytoplasmic chaperones is one of the factors which might contribute to the limitation of the export of the hGM-CSF and hIL-13 cytokines. To test this possibility, we analyzed the effect of the overproduction of SecB, GroEL-GroES, or DnaK-DnaJ on cytokine expression and on cell growth and viability. Strains harboring one or the other of the four cytokine plasmids were transformed with a second, compatible plasmid containing either the *groES-groEL* operon (pEMR602), the *secB* gene (pEMR675), or the *dnaK-dnaJ* operon (pLNA2) (Fig. 1) (see Materials and Methods).

Strains overexpressing the two GroE proteins generate, in unfractionated or fractionated extracts, an amount of hGM-CSF or hIL-13 identical to that produced by the corresponding strains without the chaperone plasmid (Table 2). However, the pattern of growth is affected after induction of the cytokine genes: the chaperone effect is to retard growth in two cases (SP3–hIL-13 and SP1–hIL-13) and to improve it moderately for the pEMR727 (PS3–hGM-CSF) construct since it prevents the lysis that normally follows induction (Fig. 6A).

When SecB is overproduced, there is again no significant change in the total quantity of either cytokine accumulated during the induction period (Table 2) or in the partitioning between the soluble and insoluble fractions after lysis by sonication. In two cases, the amount of protein recovered in the soluble fraction after an osmotic shock is altered. A two- to

TABLE 2. Production of the hIL-13 and hGM-CSF cytokines in the absence or presence of chaperone overproducer plasmids

Chaperone plasmid	Amt of cytokine recovered ($\mu g \cdot ml^{-1} \cdot OD_{600}$ unit)[a]							
	pEMR639 (SP1–GM-CSF)		pEMR727 (SP3–GM-CSF)		pLNA13 (SP1–IL-13)		pLNA12 (SP3–IL-13)	
	T[b]	S[b]	T	S	T	S	T	S
None	0.4	0.13	1.2	0.4	3	0.15	3	<0.01
+pEMR602 (GroES-GroEL)	NC[c]	NC	NC	NC	NC	NC	NC	NC
+pEMR675 (SecB)	NC	NC	1.25	1.18	3.2	0.3	NC	NC
+pLNA2 (DnaK-DnaJ)	NC	NC	1.28	1.23	2.80	0.015	NC	NC

[a] Induced cultures were prepared and fractionated by osmotic shock as described in Materials and Methods. Values are the means of two or three independent determinations.
[b] T, total cellular extract; S, periplasmic soluble fraction.
[c] NC, no significant change observed in comparison with the strain without chaperone plasmid.

threefold increase in the amount of periplasmic hGM-CSF is obtained with plasmid pEMR727 (Fig. 4A); an effect of the same magnitude is observed with the pLNA13 construct (Fig. 5A). Thus, in terms of protein production via the chosen osmotic shock technique, the overproduction of SecB does provide a notable improvement (Table 2). The influence of the larger supply of SecB on growth, after induction of cytokine synthesis, is presented in Fig. 6C. The results suggest the existence of a correlation between the amount of soluble cytokine in the periplasm and the SecB-associated growth arrest.

When the DnaK-DnaJ overproducer plasmid (pLNA2) is present, the overall amounts of the two cytokines are similar to those made by the strains without it (Table 2). However, the partition between the soluble and insoluble fractions of the osmotic shock is different for two of the cytokine plasmids. The quantity of soluble hGM-CSF present in the periplasm is increased two- to threefold with plasmid pEMR727 (Fig. 4B). Thus, for this same cytokine plasmid, the excess of DnaK and DnaJ provides an amelioration of the same magnitude as does an excess of SecB. Surprisingly, when IL-13 is produced from the fusion with SP1 (plasmid pLNA13), there is a 10-fold reduction in the production of the amount of cytokine when DnaK and DnaJ are overproduced (Fig. 5C). A notable change also appears when production from the SP3–hIL-13 construct is examined: nearly all of the protein is now matured, but it remains insoluble (Fig. 5B). The growth after induction of the four strains is influenced by the presence of the pLNA2 plasmid (Fig. 6D) (i) in a positive way for strains carrying the pLNA13 (restoration of normal growth) and pEMR727 plasmids (suppression of lysis), (ii) in a mildly negative way for the pEMR639-containing cells (longer generation time), and (iii) in a severely negative way when pLNA12 is present (inhibition of growth).

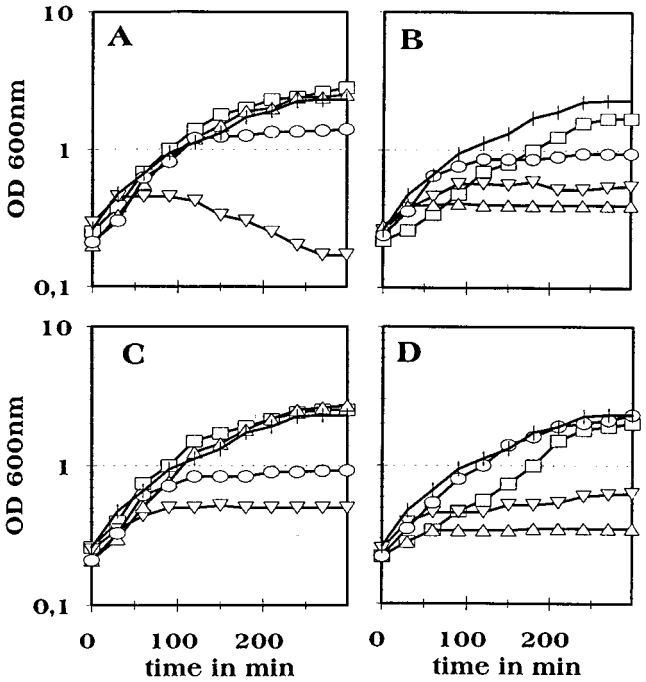

FIG. 6. Influence on bacterial growth of cytokine gene expression in the absence or presence of chaperone overproducer plasmids. The increase in the OD_{600} of the cultures was monitored after the addition of IPTG for derivatives of strain JS219 containing plasmid pEMR639 (SP1–hGM-CSF) (□), plasmid pEMR727 (SP3–hGM-CSF) (▽), plasmid pLNA12 (SP3–hIL-13) (△), plasmid pLNA13 (SP1–hIL-13) (○), or plasmid pLNA15 (vector without cytokine gene) (|) without (A) or with (B to D) the concomitant overproduction of GroES and GroEL (pEMR602) (B), SecB (pEMR675) (C), or DnaK and DnaJ (pLNA2) (D).

DISCUSSION

The aim of our study was to improve the production in *E. coli* of the hGM-CSF (2) and hIL-13 (20) cytokines of human origin. We decided to direct the proteins to the bacterial periplasmic space. We found that four types of problem render the efficiency of this process very variable. The first one is the limitation of mRNA translation. Of the two related signal sequences we used, i.e., SP1 and SP3, the second was designed to obtain a better rate of translation initiation. This expectation was fulfilled in the case of hGM-CSF but not in the case of hIL-13. Thus, the overall efficiency of translation of the mRNA depends not only on the sequence corresponding to the signal peptide but also on that of the protein coupled to it. In this respect, the SP1– and SP3–hIL-13 pairs are severalfold better than the SP1– and SP3–hGM-CSF associations. The second type of problem is the physiological consequence of inducing expression of the fusions. For example, with the SP3–hGM-CSF construct, the price paid for a better production is rapid growth arrest followed by slow lysis. The third factor is the efficiency of the maturation process, which is good in three cases but clearly deficient for the SP3–hIL-13 construct. The fourth one is the proportion of soluble protein eventually found in the periplasmic fraction. It varies from less than 1% to about 33% and is also very much influenced by the nature of the signal peptide-cytokine combination.

To try to overcome one or the other of these problems, we chose to increase the supply of possibly limiting cytoplasmic

factors. This approach has already been taken but in a less systematic way (4, 6, 18, 22). The most obvious candidate was the export-oriented SecB chaperone (23), although the other general chaperones, GroEL and DnaK, also had to be considered (1, 10, 15, 22, 28). Our results show that there is no general effect of any of the three chaperone plasmids when tested with the four cytokine constructs. However, in contrast to previously published studies (6, 18), the approach was justified by the finding that 3 combinations of 12 showed a significant increase (two- to threefold) in the amount of soluble protein in the periplasm (Table 2). Here again, the determinating factor is intrinsic to the signal peptide-cytokine association. Therefore, since it is still not possible to predict which combinations will be successful, any further improvement will require methodical testing of a larger number of different associations.

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