Specific high affinity binding of human interleukin 1β by Caf1A usher protein of *Yersinia pestis*

Vladimir P. Zav'yalov[a,*], Tatiana V. Chernovskaya[a], Elena V. Navolotskaya[a], Andrey V. Karlyshev[b], Sheila MacIntyre[b], Anatoly M. Vasiliev[a], Vyacheslav M. Abramov[a]

[a]*Institute of Immunology, 142380 Lyubuchany, Moscow Region, Russian Federation*
[b]*Department of Microbiology, University of Reading, Whiteknights, P.O. Box 228, Reading RG6 2AJ, UK*

Received 12 July 1995

Abstract **Understanding the interaction of *Yersinia pestis* with the key components of the immune system is important for elucidation of the pathogenesis of bubonic plague, one of the most severe and acute bacterial diseases. Here we report the specific, high affinity binding ($K_d = 1.40 \times 10^{-10}$ M ± 0.14×10^{-10}) of radiolabelled human interleukin 1β (hIL-1β) to *E. coli* cells carrying the capsular *f1* operon of *Y. pestis*. Caf1A outer membrane usher protein was isolated to greater than 98% purity. Competition studies with purified Caf1A, together with immunoblotting studies, identified Caf1A as the hIL-1β receptor. Competition between Caf1 subunit and hIL-1β for the same or an overlapping binding site on Caf1A was demonstrated. Relevance of these results to the pathogenesis of *Y. pestis* and other Gram negative bacterial pathogens with homologous outer membrane usher proteins is discussed.**

Key words: Interleukin receptor; Usher protein; *Yersinia*; Capsule

1. Introduction

As early as 1894, it was known that the causative agent of plague, *Yersinia pestis*, is capable of forming a large capsule [1]. A major component of the capsule is the F1 (fraction 1) antigen – a polypeptide of 15.6 kDa, encoded by the *f1* operon [2,3]. The F1 antigen is highly immunogenic and has attracted most attention as a diagnostic tool [4] and component of protective vaccine [5]. Although the capsule has been implicated as a virulence factor, possibly by conferring resistance to phagocytic cells, its importance and role in the pathogenesis of this organism is not clear and appears to depend on the animal model [6,7].

Recently, the *f1* operon was cloned from the 110 kb (Fra1) plasmid of *Y. pestis*. It consists of four genes responsible for the synthesis and surface assembly of F1 capsule – *caf1*, *caf1M*, *caf1A* and *caf1R* encoding, respectively, the capsular protein Caf1 [3], Caf1M (capsule antigen F1 mediator) [8], Caf1A (capsule antigen F1 assembly) [9] and a DNA-binding transcriptional regulator Caf1R [10]. The *f1* operon is one of the simplest members of a widespread family of surface assembly related operons the prototype model of which is the *pap* operon involved in Pap pili assembly in uropathogenic *E. coli* [11–14]. Thus, Caf1M shares 26% identity and 54% amino acid sequence similarity with the periplasmic chaperone, PapD and as with PapD stabilises the periplasmic intermediate – in this case of the Caf1 subunit [8]. Similarly, Caf1A shares 26% amino acid identity with the outer membrane usher protein PapC [12], is required for surface localisation of the Caf1 subunit [9] and belongs to the family of outer membrane usher (chaperone) proteins [14].

As a result of a computer search of the PIR protein sequence library, we have recently identified a statistically significant level of similarity between the Caf1 subunit and the interleukins (ILs)-1α, β, ra (receptor antagonist), central cytokines of the immune system [15]. Interestingly, it had previously been reported that IL-1 could bind specifically to virulent but not to avirulent strains of *Escherichia coli* and that this binding led to enhancement of bacterial growth [16]. Because of the apparent structural similarity of Caf1 and the interleukins we therefore investigated the ability of hIL-1β to bind to *E. coli* cells expressing the Caf1 operon. In this report we not only demonstrate specific binding of hIL-1β to these cells, but also identify for the first time a bacterial protein which exhibits high affinity binding for interleukin.

2. Materials and methods

2.1. Bacterial strains, plasmids and culture conditions

For all experiments *E. coli* HB101 or DH5α [17] carrying the appropriate plasmid was grown at 37°C in LB broth containing ampicillin (100 μg/ml). Plasmid p12R [8] has been described. For overexpression of *caf1M*, the *caf1M* gene was isolated on a 0.8 kb *Bsp*HI–*Sma*I DNA fragment from p12R and ligated into *Nco*I–*Hpa*I digested pTrc99a vector (Pharmacia) to give pTCA1. The *caf1* gene was amplified by PCR from p12R using primers 5′ CTGCAGGGTTATCCACAACCTGCATC and 5′ CTGCAGCGAGGGTTAGGCTCAAAGTA which are complementary to regions 112–93 bp upstream from the start codon and 8–37 bp downstream from the transcription terminator of *caf1*, respectively. The 720 bp amplified fragment was subcloned into pGEM-T vector (Promega) and the DNA sequence verified. Using the unique *Pst*I sites designed in the primers, the correct *caf1* gene was then isolated on a *Pst*I fragment and inserted into a unique *Pst*I site 79 bp downstream from the end of *caf1M* in pTCA1. Correct orientation of *caf1* in this final construct (pFM1) was verified by restriction analyses. All DNA manipulations were performed according to standard procedures [17]). Cultures of *E. coli* DH5α carrying either pTCA1 or pFM1 were maintained in the presence of 0.2% glucose.

2.2. Kinetics of IL-1β binding

Recombinant hIL-1β was purified as described by Mashko et al. [18] and iodinated using the chloramine-T method [19] to a specific activity of 80 Ci/mole. 16 to 18 h cultures of HB101/p12R were washed in LB and resuspended in 50 mM Tris-HCl pH 7.2, 150 mM NaCl containing 1 mM PMSF (TSP-buffer). Binding experiments were performed in triplicate at 0°C for 1 h with 5×10^5 cells in a final volume of 1 ml. For the analysis of specific binding, the cells were incubated with radiolabelled hIL-1β (concentration range from 10^{-12} to 10^{-8}M). Specific binding was defined as that inhibited by 1 μM unlabelled hIL-1β. The incubation was terminated by rapid filtration through GF/B glass fiber filters (Whatman) under vacuum pressure. Filters were rinsed twice

*Corresponding author. Fax: (7) (095) 5461574.

SSDI 0014-5793(95)00878-0

with 5 ml volumes of ice-cold TSP-buffer. Radioactivity was measured with a Mini Gamma Counter (LKB).

2.3. Immunoblotting

(i) Binding of hIL-1β. Cells of 16–18 h cultures of *E. coli* HB101 carrying p12R or pUC19 were harvested by centrifugation and solubilised in Laemmli's sample buffer at 100°C. Samples (containing 10 mg protein) were resolved by SDS-PAGE and electroblotted to nitrocellulose membrane for 18 h at 150 mA. Non-specific binding sites were blocked by incubation in 10 mM Tris-HCl pH 7.5, 150 mM NaCl, 2% bovine serum albumin, 0.05% Tween 20 (blocking buffer) for 18 h at 4°C. Blots were then incubated for 1 h at 37°C in blocking buffer containing hIL-1β (10 μg/ml), washed three times with blocking buffer, incubated 1 h with horseradish peroxidase-conjugated rabbit anti-hIL-1β immunoglobulin G in blocking buffer. Following three additional washes blots were developed using 1.3 M diaminobenzidine and 0.2% H_2O_2 in 10 mM Tris-HCl pH 7.4, 150 mM NaCl. Biotinylated markers were stained with peroxidase-conjugated avidin (Sigma).

(ii) Identification of Caf1M. A synthetic peptide corresponding to residues 135–150 of Caf1M [8] i.e. the additional loop in comparison with the known periplasmic molecular chaperones [8,11] was synthesized by the semi-automatic continuous-flow solid phase method [20]. Polyclonal antisera was obtained from mice immunised with the peptide coupled to keyhole limpet hemocyanin with glutaraldehyde. Samples were subjected to SDS-PAGE, transferred to nitrocellulose membrane and developed as described above using mouse anti-Caf1M peptide serum (1/500 dilution) and antimouse IgG peroxidase conjugate as the first and second antibodies, respectively.

2.4. Purification of Caf1A (p90)

Bacterial cells harvested from 300 ml of a 16 h culture of *E. coli* HB101/p12R were resuspended in 3 volumes TS buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl) containing 5 mM EDTA and 0.1 mM PMSF and disrupted on ice by four cycles of sonication (30 s with Labsonic 2000 homogenizer at 20 kHz with 60 s cooling). After centrifugation at 15,000 × *g* for 30 min, the pellet was resuspended in 1.5 volume TS buffer with 5 mM EDTA and 0.1 mM PMSF, homogenized and centrifuged as before. The pooled supernatants (4.5 ml, 4 mg total protein) were fractionated by gel-filtration chromatography on a TOYOPEARL HW-55 (1 × 70 cm) column in TS buffer, containing 5 mM EDTA. The column was calibrated with markers from Pharmacia (ribonuclease A, 13.7 kDa; chymotrypsinogen A, 25 kDa; ovalbumin, 43 kDa; bovine serum albumin, 66 kDa; aldolase-158 kDa and catalase, 232 kDa). Fractions containing IL-1β-binding activity (90–160 kDa region; 360 μg protein) were precipitated with ammonium sulphate (45 g/100 ml), and the resulting precipitate recovered by centrifugation (27,000 × *g*, 30 min) and dissolved in TS-buffer. This preparation (250 μg protein) was dialyzed against TS-buffer and further fractionated by chromatography on a MonoQ column with a Pharmacia FPLC system. Following elution with a 0.15–0.5 M NaCl gradient in 20 mM Tris-HCl buffer pH 7.2 (A-buffer), p90 was identified in fractions containing 150 mM NaCl. Pooled fractions (75 μg protein) were diluted sixfold with A buffer and re-fractionated by chromatography on a MonoQ column. Fractions containing p90 were pooled (37 μg protein).

2.5. Interleukin-1β plate binding assay

Samples of fractions from Gel-filtration or ion exchange chromatography were diluted to 5 μg per ml with TS-buffer. Fifty ml of the diluted samples were allowed to adhere to microtiter wells (Dyneteck) overnight at 4°C. After blocking the wells with 200 μl of blocking buffer, wells were sequentially incubated for 1 h each with 100 μl of blocking buffer containing 1 μg/ml hIL-1β, followed by rabbit antiserum (1/5000), and finally anti-rabbit IgG conjugated to peroxidase. Between successive incubations the wells were rinsed four times with TS-buffer containing 0.05% Tween 20. The assays were read on Titertek Multiskan (Flow) after incubation with 0.4 mM 3,3'5,5'-tetramethylbenzidine per ml in 10 ml 100 mM Na acetate buffer pH 5.0 containing 0.004% H_2O_2 (v/v).

2.6. Purification of Caf1M chaperone and chaperone-subunit complex

Following induction of exponentially growing cells (OD_{600} = 0.4–0.5) with 1 mM IPTG for 2 h, *E. coli* DH5α/pTCA1 and *E. coli* DH5α/pFM1 possess high levels of periplasmic chaperone and chaperone complex, respectively. The periplasmic fraction was isolated from such cultures by osmotic shock. Cells from 500 ml culture were suspended in 5 ml 20% (w/v) sucrose, 20 mM Tris-HCl pH 8.0, 5 mM EDTA at 20°C. After 5 min cells were removed by centrifugation and resuspended in cold 10 mM $MgCl_2$ and incubated on ice for 5 min. Caf1M or complex were recovered in the supernatant fraction (periplasmic fraction) following further centrifugation. To recover Caf1M, the periplasmic fraction was diluted tenfold with A buffer to 0.2–0.3 mg total protein per ml and fractionated on a MonoQ column with a Pharmacia FPLC system. Unbound fractions were precipitated with ammonium sulphate (37.5 g/100 ml), and the resulting precipitate containing Caf1M was recovered by centrifugation (27,000 × *g*, 30 min). The pellet containing Caf1M was dissolved in A-buffer and fractionated on a Superose 12 HR 10/30 column. Caf1M was eluted with a molecular weight of approximately 29 kDa.

To recover Caf1M–Caf1 complex, the periplasmic fraction was diluted with TS-buffer to 1 mg total protein per ml and fractionated on a MonoQ column. Following elution with 0.15–0.8 M NaCl in A buffer, the Caf1M-Caf1 complex was identified in a fraction containing 0.24 M NaCl.

For affinity chromatography of Caf1M-Caf1 complex, an anti-Caf1 IgG-sepharose affinity column was prepared as described [21]. A sample (20 μg protein in 0.5 ml A-buffer containing 0.24 M NaCl) of Caf1M–Caf1 complex eluted from the MonoQ column was applied to the affinity column (0.5 ml). The column was washed with 10 ml A-buffer containing 0.5 M NaCl and 5 ml A-buffer and bound proteins were eluted with 0.01 M acetic acid.

2.7. Analytical procedures

SDS-PAGE was performed according to Laemmli [22] using 12.5% acrylamide gels (except Fig. 3 which was a 7.5–12.5% gradient gel) under reducing conditions. Gels were stained with Coomassie brilliant blue or subjected to immunoblotting. Molecular weight markers were from Pharmacia (LMW Calibration kit) for stained gels and Sigma biotinylated markers for immunoblots, M_r as shown.

Protein concentrations were determined by the Bio-Rad protein-assay kit, using bovine serum albumin as standard.

3. Results and discussion

3.1. Specific binding of hIL-1β to E. coli*/p12R*

E. coli HB101 cells transformed by a recombinant plasmid (p12R), which carries the major fragment of the *f1* operon of *Y. pestis*, produce a functional periplasmic chaperone (Caf1M), outer membrane usher protein (Caf1A) and capsular polypeptide (Caf1) as indicated by the expression and assembly of the *Y. pestis* capsular polypeptide at the surface of *E. coli* [8]. Levels of capsular polypeptide are somewhat lower in this strain compared to levels of capsule in *E. coli* pFS2 due to the truncation of the gene for the transcriptional regulator, *caf1R* in p12R (Karlyshev, unpublished observations). Fig. 1a shows Scatchard analysis binding of ^{125}I-labelled recombinant hIL-1β to *E. coli*/p12R cells expressing the *f1* operon. The dissociation constant (K_d, mean ± S.E.) for [^{125}I]hIL-1β binding is equal to 1.40×10^{-10} M ± 0.14×10^{-10}. The number of [^{125}I]hIL-1β binding sites per *E. coli* HB101/p12R cell is a minimum of 10^4. No specific binding of [^{125}I]hIL-1β was observed for *E. coli* HB101 cells transformed by the plasmid pUC19.

3.2. Identification of Caf1A as hIL-1β receptor

Immunoblot analysis of *E. coli*/p12R cell lysates with hIL-1β and anti-IL-1β immunoglobulin G (IgG) consecutively, indicated that a protein of approximately 90 kDa was responsible for the specific binding of hIL-1β (Fig. 1b, lane 1). This protein was expressed by *E. coli* HB101/p12R, but no reaction was observed with *E. coli* HB101/pUC19 (Fig. 1b, lane 2). In comparison with plasmid pUC19, plasmid p12R contains only four

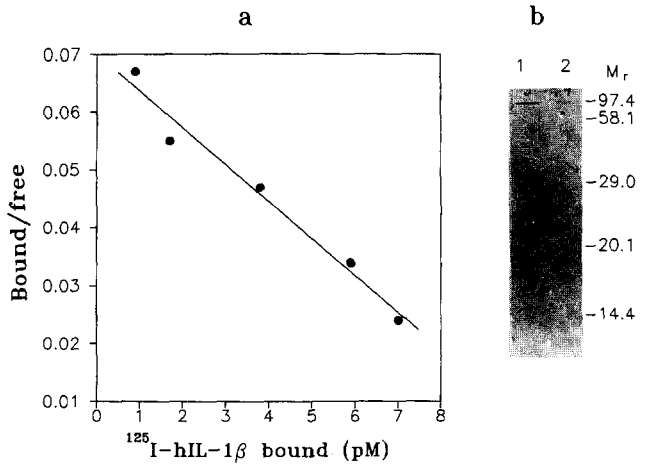

Fig. 1. (a) Scatchard analysis for [^{125}I]hIL-1β binding to *E. coli* HB101/p12R. (b) Immunoblot of binding of hIL-1β to a 90 kDa polypeptide in *E. coli* HB101/p12R. Lanes: (1) *E. coli* HB101/p12R; (2) *E. coli* HB101/pUC 19.

additional genes – those responsible for expression of the pre-Caf1 (17.6 kDa), preCaf1M (28.8 kDa), preCaf1A (93.6 kDa) and Caf1R (36 kDa) proteins [3,8–10]. Thus the outer membrane usher protein, Caf1A (mature polypeptide, putative M_r = 90.4) appeared to represent an obvious candidate as receptor for specific binding of hIL-1β to *E. coli* cells expressing the *f1* operon. The Caf1A protein (p90) was isolated from recombinant *E. coli* strain HB101/p12R by a protocol involving disruption of cells/release of Caf1A by sonication followed by gel-filtration and ion exchange chromatography of the supernatant fraction, as described in section 2. Throughout the purification procedure samples from different fractions were analysed for IL-1β binding activity as well as for protein concentration and by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Fractions containing IL-1β binding activity were eluted from the Toyopearl HW-55 column in the 90–160 kDa region (not shown). The final preparation of Caf1A was greater than 98% pure as judged by SDS-PAGE (Fig. 2a). In control experiments using similar fractions obtained from *E. coli* HB101/pUC19 no IL-1β activity could be detected. It can be seen from Fig. 2b that purified Caf1A effectively inhibited [^{125}I]IL-1β binding to HB101/p12R cells ($K_i = 2 \times 10^{-10}$ M).

Genetic structures, similar in organisation and function to the *f1* operon, have been found in many pathogenic Gram-negative bacteria (*Bordetella pertussis, Klebsiella pneumoniae, Haemophilis influenzae, Salmonella typhimurium, E. coli* [11–14]. Each of these operons encode an outer membrane usher protein which facilitates surface localisation and correct assembly of virulence-associated surface organelles (capsules, pili, fimbriae). Porat et al. [16] have found that IL-1 displays reactivity with virulent *E. coli* strains and increases the number of colony-forming entities. It was proposed that human IL-1 may recognize a functional IL-1-like receptor structure on virulent *E. coli* and may be a virulence factor for bacterial pathogenicity. In accordance with the data reported here a candidate for the receptor molecules for IL-1 on the surface of pathogenic strains of *E. coli* are the outer membrane molecular usher proteins involved in fimbriae/non-fimbrial adhesin assembly.

3.3. Competition between Caf1 and hIL-1β for binding to Caf1A

The Caf1A protein acts as an anchor to attach the Caf1 capsule to the outer membrane as well as an usher during export across the outer membrane [9]. It was, therefore, also important to ascertain the ability of Caf1 to compete for the IL-1β binding sites. Purified Caf1 protein is in a polymerized form (MW more than 10^6). Thus, it was not possible to test this form of Caf1 in competition experiments. The molecular chaperone, Caf1M, however, protects Caf1 from polymerisation. The capsular subunit-chaperone complex was, therefore, purified from *E. coli* HB101/pFM1 by osmotic shock and ion exchange chromatography (Experimental procedures) and tested in competition experiments. To confirm that the test fraction actually contained a Caf1–Caf1M complex rather than the free proteins, a sample of the test fraction was passed through an anti-Caf1 IgG-Sepharose affinity column. Fig. 3a and b show the results of SDS-PAGE and immunoblot analyses, respectively, of the bound and subsequently eluted protein complex. It can be seen from Fig. 3c that the Caf1–Caf1M complex effectively inhibited [^{125}I]hIL-1β binding to *E. coli* HB101/p12R cells ($K_i = 5 \times 10^{-10}$ M). No inhibition of [^{125}I]IL-1β binding by free Caf1M was observed ($K_i > 10^{-6}$). To exclude the possibility that Caf1 and hIL-1β might copolymerise as a consequence of the homology of their structures [15], we investigated the interaction of hIL-1β with immobilised Caf1 by ELISA. No specific binding of hIL-1β to Caf1 was observed. These data suggest that hIL-1β and Caf1 compete for a common binding site on the outer membrane usher protein, Caf1A.

It has been reported that some Gram-negative bacteria produce a protein with IL-1-like activities [16]. We found that in a search of the PIR protein sequence library, Caf1 shared the highest level of homology with hIL-1ra (28% identity between aa 62–89 of Caf1 and aa 57–85 of hIL-1ra) [15]. Subsequent alignment with other members of the IL-1-like superfamily of proteins revealed further significant homologies [15]. Thus, of 18 hydrophobic positions forming a hydrophobic core in all the previously studied proteins with the IL-1-like conformation [22], 15 positions are also occupied by hydrophobic residues in the Caf1 protein. All these residues are located in β-pleated

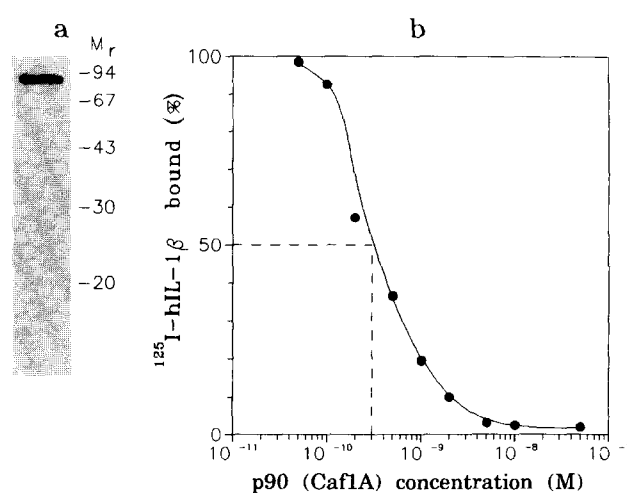

Fig. 2. (a) SDS-PAGE of purified p90 protein (Caf1A) isolated from recombinant *E. coli* strain HB101/p12R. (b) Concentration-dependent inhibition of [^{125}I]hIL-1β binding to *E. coli* HB101/p12R by p90 protein (Caf1A).

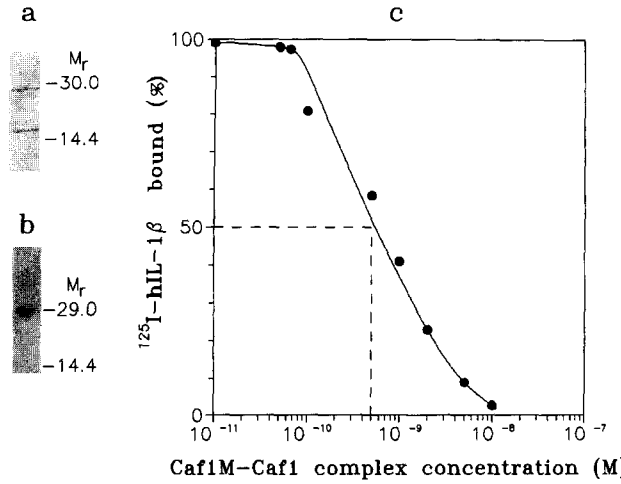

Fig. 3. (a) SDS-PAGE of the purified Caf1–Caf1M complex isolated from recombinant *E. coli* strain DH5/pFMI. (b) Immunoblot analysis of proteins eluted from affinity anti-Caf1 IgG-Sepharose column. (c) Concentration-dependent inhibition of [^{125}I]hIL-1β binding to *E. coli* HB101/p12R by the purified Caf1–Caf1M complex.

sheets forming the hydrophobic core in the IL-1-like proteins [22]. Moreover, of 10 homologous positions occupied by hydrophilic residues located on the surface of hIL-1α,β, ra molecules, 7 positions are conserved in the Caf1 protein. Arg-18, Glu-25, Asp-78, Asp-97, Glu-105, Ser-125 and Thr-145 in the *Caf1* sequence correspond, respectively, to Arg-4, Asp-12, Asp-75, Glu-96, Glu-105, Thr-124 and Thr-144 in the hIL-1β sequence. Importance of some of these conservative residues to the function of hIL-1β has been demonstrated by site-directed mutagenesis [23]. Mutation of Arg-4, Asp-12 or Glu-105 had a dramatic effect on the activity of hIL-1β, including its receptor binding capacity; mutations of Asp-75, Glu-96, Thr-124 and Thr-144 had only a limited effect. A similar dramatic effect was induced by replacement of Lys-93 with methionine in hIL-1β; whereas substitution with arginine did not reduce the receptor binding activity of hIL-1β [23]. Arg-94 of the Caf1 sequence corresponds to Lys-93 in the primary structure of hIL-1β [15]. These data are in accord with the competition studies and support the hypothesis that the Caf1 protein and hIL-1β are competing for the identical or overlapping binding sites of p90 (Caf1A) molecule.

The biological significance of these results to *Y. pestis* infection have yet to be clarified, however, possible ramifications to the pathogenesis of this organism are numerous. Competition of Caf1 with hILs-1α, β, ra for binding to the hIL-1 receptor on human lymphoid cells is one implication of the above data. The ability of viruses to elude the host's defense system by releasing soluble receptors for a number of key regulatory components of the immune system, including IL-1β receptor [24], has recently attracted much attention [25]. While viruses appear to use genes 'stolen' from their host to achieve this, we have shown here that *Y. pestis* expresses its own hIL-1 receptor in the outer membrane. This membrane bound bacterial receptor may like the soluble viral receptors function to intercept hIL-1 – a key macrophage signal for inflammation and the adaptive immune reponse. In addition, Caf1A may also have the ability to mediate attachment of *Y. pestis* to human macrophage via the membrane anchored hproIL-1 [26] at some crucial stage during the infection process. These proposed mechanisms together with previously identified virulence determinants of Yersiniae [27,28] are likely to represent multiple independent mechanisms of *Y. pestis* pathogenesis and only in their orchestration can they ensure the extreme virulence of *Y. pestis* for man.

Acknowledgements: This work was supported by grants from the International Science Foundation, International Center of Science and Technology (funded by USA and EC) and The Royal Society, UK.

References

[1] Kitazato, S. (1894) Lancet 2, 428–430.
[2] Amies, S. (1951) Br. J. Exp. Pathol. 32, 259–262.
[3] Galyov, E.E., Smirnov, O.Yu., Karlyshev, A.V., Volkovoy, K.I., Denesyuk, A.I., Nazimov, I.V., Rubtsov, K.S., Abramov, V.M., Dalvadyanz, S.M. and Zav'yalov, V.P. (1990) FEBS Lett. 277, 230–232.
[4] Williams, J.E., Arntzen,L., Tyndal, G.L. and Isaacson, M. (1986) Bull. W.H.O. 64, 745–752.
[5] Oyston, P.C.F., Williamson, E.D., Leary, S.E.C., Eley, S.M., Griffin, K.F. and Titball, R.W. (1995) Infect. Immun. 63, 563–568.
[6] Brubaker, R.R. (1991) Clin. Microbiol. Rev. 4, 309–324.
[7] Drozdov, I.G., Anisimov, A.P., Samoilova, S.V., Yezhov, I.N., Yeremin, S.A., Karlyshev, A.V., Krasilnikova, V.M. and Kravchenki, V.I. (1995) J. Med. Microbiol. 42, 264–268.
[8] Galyov, E.E., Karlyshev, A.V., Chernovskaya, T.V., Dolgikh, D.A., Smirnov, O.Yu., Volkovoy, K.I., Abramov, V.M. and Zav'yalov, V.P. (1991) FEBS Lett. 286, 79–82.
[9] Karlyshev, A.V., Galyov, E.E., Smirnov, O.Yu., Guzaev, A.P., Abramov, V.M. and Zav'yalov, V.P. (1992) FEBS Lett. 297, 77–80.
[10] Karlyshev, A.V., Galyov, E.E., Abramov, V.M. and Zav'yalov, V.P. (1992) FEBS Lett. 305, 37–40.
[11] Holmgren, A., Kuehn, M., Branden, C. and Hultgren, S. (1992) EMBO J. 11, 1617–1622.
[12] Hultgren, S.J., Abraham, S., Caparon, M., Falk, P., Geme III, J.W. and Normark, S. (1993) Cell 73, 887–901.
[13] Lindler, L.E. and Tall, B.D. (1993) Mol. Microbiol. 8, 311–324.
[14] Rosmalen, M. and Saier, M.H. (1993) Res. Microbiol. 144, 507–527.
[15] Zav'yalov, V.P., Denesyuk, A., Zav'yalov, G. and Korpela, T. (1995) Immunol. Lett. 45, 19–22.
[16] Porat, R., Clark, B.D., Wolff, S.M. and Dinarello, C.A. (1991) Science 254, 430–432.
[17] Maniatis, T., Fritsch, E.F., Sambrook, J. (1989) Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[18] Mashko, S.V., Mochulsky, A.V., Kobenko, S.Y., Lebedeva, M.I., Lapidus, A.L., Mochulskaya, N.A., Izotova, L.S., Veiko, V.P., Vinetsky Yu., P., Ketlinsky, S.A. and Debabov, V.G. (1991) Gene 97, 259–266.
[19] Ling, N., Leppaluoto, I. and Vale, W. (1976). Anal. Biochem. 76, 125–133.
[20] Atherton, E., Cameron, L.R. and Sheppard, R.C. (1988) Tetrahedron 44, 843–857.
[21] Dean, P.D.G., Johnson, W.S. and Middle, F.A. (1988) Affinity Chromatography, IRL Press, Oxford Washington DC, pp. 48–83.
[22] Auron, P.E., Quigley, G.J., Rosenwasser, L.J. and Gehrke, L. (1992) Biochemistry 31, 6632–6638.
[22] Laemmli, U.K. (1970) Nature 227, 680–685.
[23] Simon, P.L., Kumar, V., Lillguist, I.S., Bhatnagar, P., Einstein, R., Lee, J., Porter, T., Green, D., Sathe, G. and Young, P.R. (1993) J. Biol. Chem. 268, 9771–9779.
[24] Alcami, A. and Smith, G.L. (1992) Cell 71, 153–167.
[25] Baringa, M. (1992) Science 258, 1730–1731.
[26] Fuhlbridgge, R.C., Fine, S.M., Unanue, E.R. and Chaplin, D.D. (1988) Proc. Natl. Acad. Sci. USA 85, 5649–5653.
[27] Galyov, E.E., Hakansson, S., Forsberg, A. and Wolf-Watz, H. (1993) Nature 361, 730–732.
[28] Bliska, J.B. and Falkow, S. (1994) Curr. Opin. Infect. Diseases 7, 323–328.