

Table S1. Cleaved peptides from microarray screen, related to Figure 1.

Number	Peptide Sequence	Cleavage ratio	Number	Peptide Sequence	Cleavage ratio
1	DQASGSVL	0.051	46	EPSRLQES	0.331
2	ANDSWDRTYWKQWMS	0.082	47	GYPFHMKTQTVKTYG	0.340
3	PQDLDHSSRMTMDW	0.089	48	GAAFRPFY	0.340
4	EDKRHSQG	0.098	49	YPPGPIIP	0.345
5	YTAGNKVD	0.103	50	GAVHLPQP	0.345
6	QPHWSMQSGEGTQGG	0.111	51	PTMINMQYVSGLFNK	0.345
7	LAQFVRSS	0.113	52	MVWMVYTYHPHVTEP	0.347
8	PNCAYKTT	0.114	53	QEVYGMMP	0.353
9	METKVHWSGHINPMW	0.127	54	TYTDANKN	0.353
10	TPDNRMQYWLQNTTEW	0.138	55	SKAWYFKTASLARYY	0.356
11	QEWGRFSSWYPIHAN	0.149	56	DKAAMRKTMMSKHS	0.357
12	SPQTGDNS	0.173	57	YHAKATEH	0.357
13	EEMHRFFSHMDRYPD	0.174	58	GKENDKEE	0.359
14	MDHPQRDSRIMQFST	0.192	59	ARIGGRLN	0.361
15	VAASSLRN	0.206	60	MEKRYGGF	0.364
16	LHWHARTSFSVMIHL	0.208	61	ELGTYNVI	0.366
17	YPEEDTEG	0.214	62	FLLTKLGTHKMYMMN	0.369
18	NNGASTATNGKQLNI	0.225	63	VSKRYGGF	0.369
19	DVTDYKGE	0.231	64	NGQYNRETMWSVMLW	0.371
20	HFRWGGKPV	0.239	65	LQKRGIVE	0.374
21	MEFPMMSWQRRVTL	0.245	66	HMWRKHETVGNASKM	0.376
22	VIEFRVMV	0.251	67	MTDVFNPSPGSVYNTH	0.376
23	WKIWRVLTSAQQTVL	0.255	68	LAQNVRSS	0.376
24	GTPDGRISRRLIWP	0.265	69	PGTKMIFA	0.380
25	YADNDNDS	0.266	70	DAGDVGAA	0.380
26	LRTNNMKE	0.267	71	TEESEHRYMQNNANV	0.380
27	MANEHTWYRATGYER	0.268	72	LQGFSFETMYDTLGH	0.380
28	TYDQYQEN	0.280	73	VYGEGERN	0.383
29	GIHTDRITNARSTKL	0.281	74	MKIRRMKTNSLNHRW	0.384
30	DETDSGAG	0.282	75	PQFRIKGG	0.387
31	DVAQFVLY	0.286	76	RGQLKAWTEKDTSEE	0.391
32	PHQNRNSTYVVHHPM	0.289	77	ISVDRPVK	0.393
33	DEEDDSGK	0.290	78	MNVVPHRSMWWVAPF	0.394
34	RHIINDWTGDRNWFT	0.297	79	AAFFDTAS	0.394
35	MNGGAFNW	0.299	80	PGLYYFAY	0.395
36	YPIKAQTEGKLKEE	0.302	81	YKYELRFYPGYFGTM	0.398
37	PNLMAMTYDVIQSYP	0.309	82	NLPGAAYTSKVKDVM	0.399
38	VVTRSQEN	0.312	83	AGIKKKTE	0.400
39	FPCAGKKV	0.315	84	DRHELMSDYVGFH	0.400
40	RERKAGCK	0.319	85	LNGKQIKSVNTSGVN	0.404
41	HAWSDTQSEHRADPG	0.325	86	SVMFMIPA	0.406
42	DLGRFQTF	0.328	87	ENKPRRPY	0.407
43	RGFFYTPK	0.329	88	GKKRDAEN	0.412
44	HTQQMGMYDVTQGGK	0.330	89	GRWTNRWYIEIDPM	0.412
45	IPVSLRSG	0.331	90	EFKRELEG	0.415

Table S1. Cleaved peptides from microarray screen (continued).

Number	Peptide Sequence	Cleavage ratio	Number	Peptide Sequence	Cleavage ratio
91	EGEDDRDS	0.416	131	RISSSSGL	0.471
92	DEEDLQRA	0.419	132	HVIHHQESYMLEMVN	0.472
93	SKMLFVEP	0.420	133	DSGDLGPL	0.472
94	DYGRSSAL	0.421	134	SIAMSRMS (b)	0.474
95	DHTVNDLTMKTHLAP	0.421	135	EDWNTIST	0.474
96	IEGRIVEG	0.424	136	KGTIYALSNQPLNLK	0.474
97	TEEDGVPS	0.424	137	DIGAALVE	0.475
98	LVIYETGTPLSSAMH	0.425	138	KLRVDPVN	0.475
99	TGWKYALYHENIMAG	0.426	139	LTEDHLDL	0.476
100	IHRARQHSYLRKELG	0.430	140	DMSTKGKTQAAVRGR	0.476
101	NKPPSKRSPYTDPAI	0.431	141	KYIMIWRVFTADSW	0.476
102	TQFDAAHP	0.436	142	WYKDELQSSGMVYEQ	0.479
103	ALHDQSESVFTTEQR	0.437	143	HQTRDDKK	0.479
104	HMWWETLYLARNDMT	0.437	144	WIMWVGKSIQPHSAP	0.479
105	AVTKWTEK	0.441	145	RYMGADHTNYEMLMP	0.481
106	RKLDNTKF	0.443	146	TVYMKDVK	0.482
107	VMEKRMVTFPHHGYS	0.444	147	MIPNPVRSHHTERNF	0.482
108	WKEETLME	0.445	148	MNRFPKYMNSIRME	0.483
109	RLYKWSVSFAWFYNT	0.449	149	DYMGWMDF	0.483
110	ARAHVDAL	0.452	150	KALHVTNI	0.483
111	NKTDPKSM	0.452	151	LFKDKAM	0.485
112	SLEKQIGH (a)	0.452	152	EILRGSDG	0.485
113	GSSKYPNC	0.455	153	KSGGCFPG	0.485
114	VKKRSVSE	0.456	154	HEKWKDGTYRFFW	0.486
115	CMNFMVYAL	0.457	155	LRGGMVGS	0.489
116	PPAASSLR	0.458	156	VANHAAYTMDGENMS	0.489
117	KKGGDEEK	0.460	157	EIMREGSA	0.490
118	HHQKLFFF	0.461	158	FLKRFAEA	0.492
119	NQKRYGGF	0.462	159	MYDHLNTSIYHNHHP	0.492
120	MMHEHKHSVDRKTQD	0.462	160	VSNEMSKK	0.494
121	MRGGFLPF	0.464	161	LMEYLENP	0.494
122	LNGGAFSW	0.465	162	PEIPVTSTVFVAAAG	0.494
123	DISTEILTVRDKVMA	0.465	163	VQSTMTVTDSPGDTE	0.494
124	RTEYLTGV	0.466	164	CAAPSFDC (c)	0.495
125	RQMHNPMNQNPVYL	0.467	165	FNGGGDKN	0.496
126	EETDGIAY	0.468	166	EAMNWPMYHVYEQS	0.496
127	SDTQIRMSTQYKTEQ	0.468	167	AEALERMF	0.498
128	PMEAPHSTETATTQH	0.468	168	IYPWSRSAAQAGGT	0.498
129	IVLDGTDN	0.469	169	HLAPYSDE	0.498
130	EIMRTIPE	0.469	170	LNSAVKRQ	0.499

(a) Fas ligand, (b) alpha₂-antiplasmin, (c) plasminogen

Table S2. Strains and plasmids used in this study, related to Experimental Procedures.

Strain or plasmid	Strain designation or plasmid marker	Genotype and/or characteristics	Source
<i>Y. pestis</i> strains			
CO92	SAN1	pCD1 ⁺ pPCP1 ⁺ pMT1 ⁺ pgm ⁺	Lab stock
CO92 Δ <i>pla</i>	SAN6	Δ <i>pla</i>	Lab stock
CO92 Δ <i>yopJ</i>	SAN28	Δ <i>yopJ</i>	Lab stock
CO92 Δ <i>pla</i> Δ <i>yopJ</i>	SAN163	Δ <i>pla</i> Δ <i>yopJ</i>	This study
CO92 pgm ⁻	PAN260	pCD1 ⁺ pPCP1 ⁺ pMT1 ⁺ pgm ⁻	Lab stock
CO92 pCD1 ⁻	PAN259	pCD1 ⁻ pPCP1 ⁺ pMT1 ⁺ pgm ⁺	Lab stock
CO92 pCD1 ⁻ Δ <i>pla</i>	PAN314	Δ <i>pla</i>	Lab stock
CO92 pCD1 ⁻ Δ <i>pla</i> + D206A <i>pla</i>	PAN163	Δ <i>pla</i> + D206A <i>pla</i>	Lab stock
<i>E. coli</i> strains			
BL21	LAN211	pSE380	This study
BL21	LAN212	pMRK I	This study
BL21	LAN9	pWL223, pREP4	Lab stock
DH5 α	LAN413	pWL204	Lab stock
DH5 α	LAN29	pSkippy	Lab stock
Plasmids			
pSE380	amp ^R	Empty expression vector	T. Korhonen
pMRK I	amp ^R	<i>pla</i> in pSE380	T. Korhonen
pWL223	amp ^R	pQE30 (Qiagen) with mature <i>pla</i> and 5' His-tag	Lab stock
pREP4	kan ^R	Constitutively expresses <i>lac</i> repressor protein	Qiagen
pWL204	amp ^R	Carries <i>red</i> recombinase genes and <i>sacB</i> for sucrose counterselection	Lab stock
pSkippy	amp ^R	Carries FLP recombinase genes	Lab stock

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasminogen Activation Assay

E. coli strains were grown at 37°C and induced to express *pla* as described above. *Y. pestis* cultures were grown at 26°C overnight, diluted to an OD₆₂₀ of 0.2 and grown for an additional 5 h at 37°C. Bacteria were washed with 20 mM HEPES buffer and 8 x 10⁶ CFU were collected for the assay. In 100 µl total reaction volume, bacteria were incubated at 37°C with 4 µg plasminogen (Haematologic Technologies) and 50 µM D-AFK-ANSNH-iC₄H₉2HBr (SN-5, Haematologic Technologies), a fluorescent substrate of plasmin. Fluorescence (excitation: 360 nm, emission: 460 nm) was measured kinetically every 10 min for 2 - 3 h.

FasL qRT-PCR

At various times post-inoculation with *Y. pestis* or *Y. pestis* Δ *pla*, lungs were removed and immediately submerged in an excess of *RNAlater* RNA stabilization solution (Ambion), followed by total RNA isolation with the RiboPure RNA extraction kit (Ambion) as previously described (Lathem *et al.*, 2007). RNA was DNase-treated and reverse-transcribed in triplicate using random primers and the SuperScript II polymerase (Invitrogen). cDNAs were used as templates for the amplification of the genes for FasL (5' TGAATTACCCATGTCCCCAG and 3' AAAGTACCCTGGAGGAGCC) and β -actin (5' TTCGTTGCCGGTCCACA and 3' ACCAGCGCAGCGATATCG) with the SYBR Green dye (Bio-Rad) in an iCycler thermocycler (Bio-Rad). For each time point, the calculated threshold cycle (C_t) for FasL was normalized to the C_t of β -actin for each sample to calculate relative abundance using the $\Delta\Delta$ C_t method (Applied Biosystems, 1997).

Innate immune cell quantification

At 48 h post-infection with *Y. pestis*, mice were sacrificed and bronchoalveolar lavage (BAL) was performed. BAL fluid was collected by pooling 5 total lavages per animal. Cells were washed once each in PBS and FACS buffer (2% fetal bovine serum, 0.1% sodium azide in PBS). For *ex vivo* cell surface marker detection, cells were stained with antibodies for CD45 (Biolegend, clone 30-F11), CD11b (Biolegend, clone M1/70), CD11c (BD Biosciences, clone HL3), Ly6G (Biolegend, clone IA8), and aqua Live/Dead fixable stain (Invitrogen) for one h at 4°C. All antibodies were used at 1:100 dilution in FACS buffer while Live/Dead cell stain was used at 1:1000. An anti-CD16/32 FcBlock antibody was included to minimize non-specific staining (eBioscience). Cells were washed with FACS buffer and fixed with 2% paraformaldehyde. Samples were analyzed using a BD FACSCanto II flow cytometer and FlowJo software. The gating scheme is detailed in Figure S4B.

Albumin quantification from BAL fluid

BAL fluid was collected from infected mice as described above. The experiment was performed twice with 6-10 mice per group. Cells were pelleted and mouse albumin in the supernatant was quantified by ELISA following the manufacturer's instructions (Immunology Consultants Laboratory, Inc.).

SUPPLEMENTAL REFERENCES

Applied Biosystems. (1997). ABI Prism 7700 Sequence Detection System User Bulletin #2.