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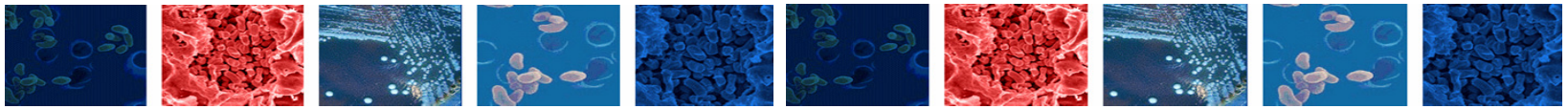
OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Phrama scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

2nd International Congress on
Bacteriology and Infectious Diseases
November 17-19, 2014, Chicago, USA

The cellular basis of protective immunity against experimental infection caused by *Francisella tularensis*

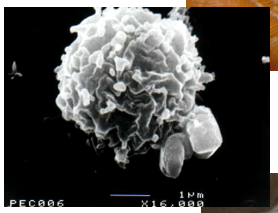


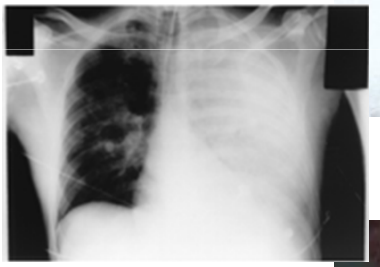
Kubelkova K., Orlikova A., Krocova Z., Pejchal J., Macela A., Stulik J.

Faculty of Military Health Sciences, University of Defense, 500 01 Hradec Kralove, Czech Republic

Francisella tularensis

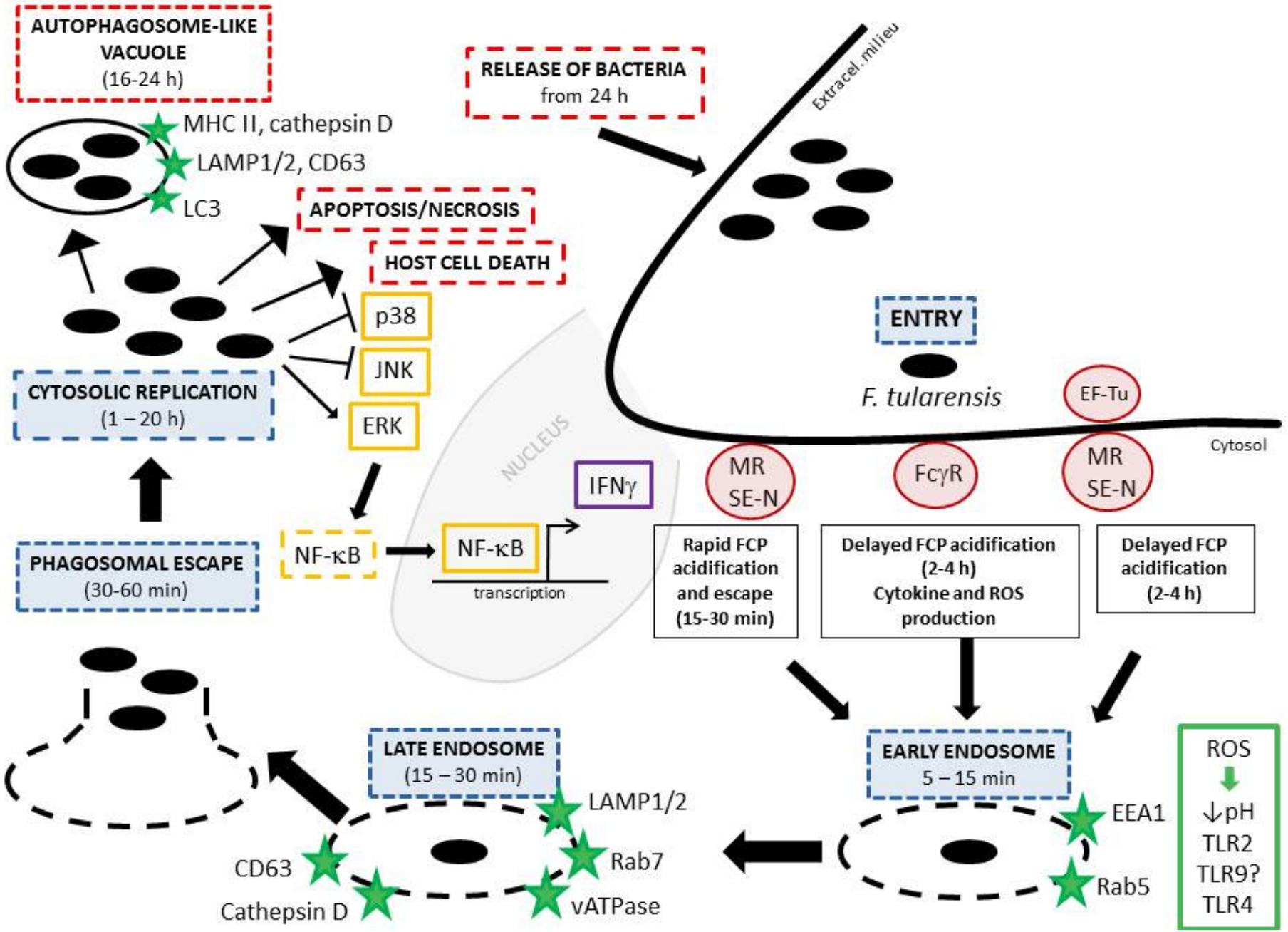
- ✓ *Francisellae* – facultative intracellular bacterial pathogens
- ✓ Small, nonmotile, obligate anaerobe
- ✓ One of the most infectious bacterial agens (10 CFU)
- ✓ *Francisella* proliferates inside macrophages, neutrophils, dendritic cells and hepatocytes
- ✓ Geographic distribution of four existing *Francisella tularensis* subtypes (*holarctica* – Type B, *tularensis* – Type A1 and A2, *mediasiatica, novicida*)





Tularemia

- ✓ Zoonotic infection
- ✓ Vectors – mainly ticks and mosquitoes
- ✓ Broad spectrum of clinical manifestations with dominant symptoms – granulomas and secondary atypical pneumonia
- ✓ Treatment – ATB (Gen, Tet)

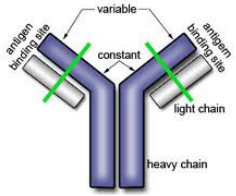


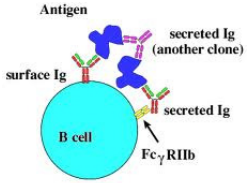
Kubelkova K. and Macela A.: Putting Jigsaw Together - A Brief Insight Into the Tularemia, Open Life Sciences, 2015, Ready to publish.

B cell involvement

- ✓ B cells and antibodies are necessary for mice to develop their natural resistance to primary and secondary LVS infections

….The role of antibodies in the protection against intracellular pathogen *F. tularensis* still remains poorly understood !





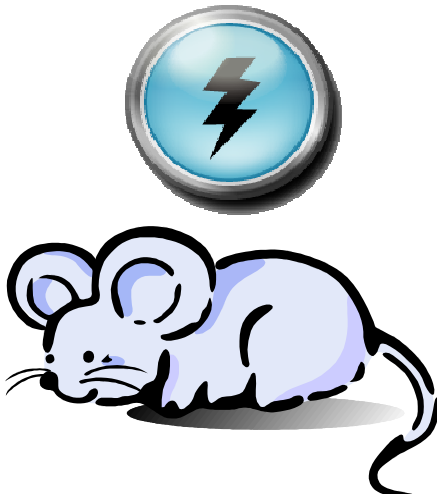
- ✓ Extracellular phase in the host, which makes it accessible to humoral immune responses
- ✓ Ab responses containing both Th-1 and Th-2 antibody isotypes are detectable as early as 3 days following *i.d.* infection
- ✓ Confer early as well as long term immunity
- ✓ Immune response against LPS (as a major protective antigen)
- ✓ No naturally B cell-deficient murine strain has been identified yet
- ✓ Serum Ab against bacterial proteins – FopA, OmpA, DsbA, GroEL, KatG etc.

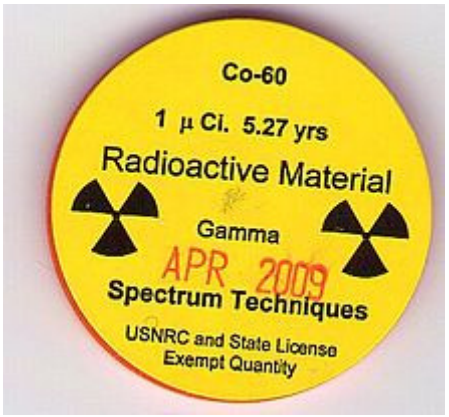
The role of antibodies in protective immune response

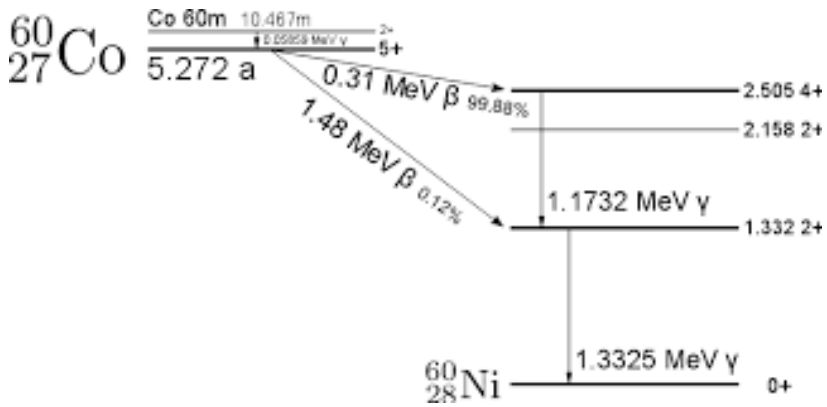
- ✓ Traditional view
  - Antibodies have little (if any) protective role against tularemia
- ✓ Late 1970ies
  - Antibodies can confer protection against attenuated *Francisella tularensis* strains and can confer some degree of protection against virulent strains of *holarctica* subtype /Macela A.: Thesis, 1980.
- ✓ Late 1990ies
  - B-cells but not circulating antibodies are indispensable in protective immunity against tularemia /Elkins K.et al: Infect Immun.,1999.
- ✓ New millennium:
  - Passive transfer of immunity protects against the same subtype
  - Passive transfer of immunity against tularemia is possible
  - Antibody-dependent cell-mediated cytotoxicity (ADCC)

/ Fulop M. et al: Vaccine, 2001. , Stenmark S. et al: Microb Pathog., 2003., Sanapala et al. 2012, Kubelkova

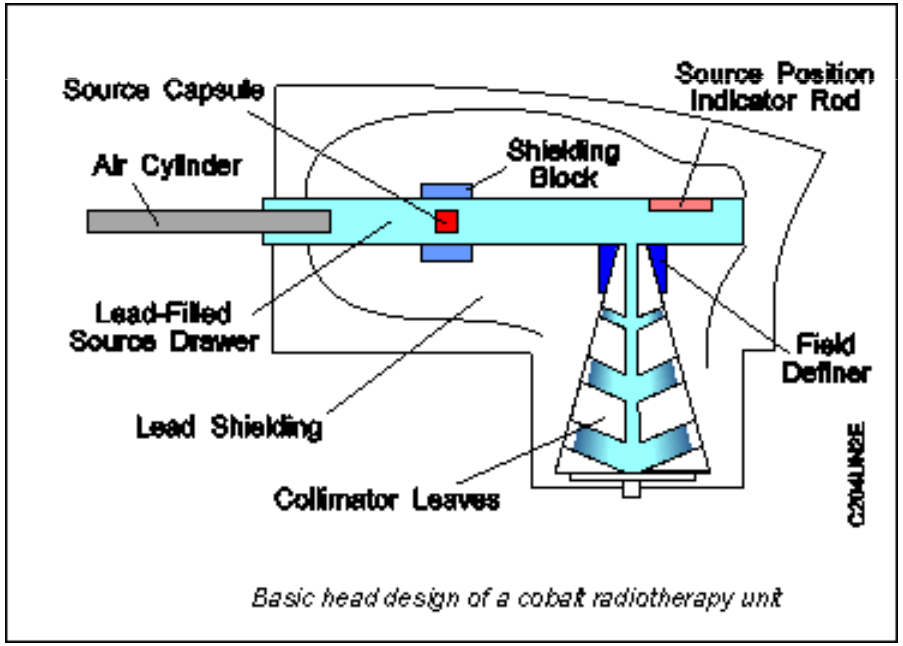
The role of antibodies in protective immune response





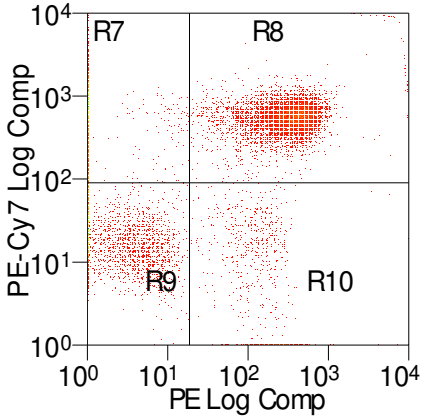


- ✓ **Balb/c mice**
- ✓**Cobalt 60**
- ✓**Dose of 4 Gy**

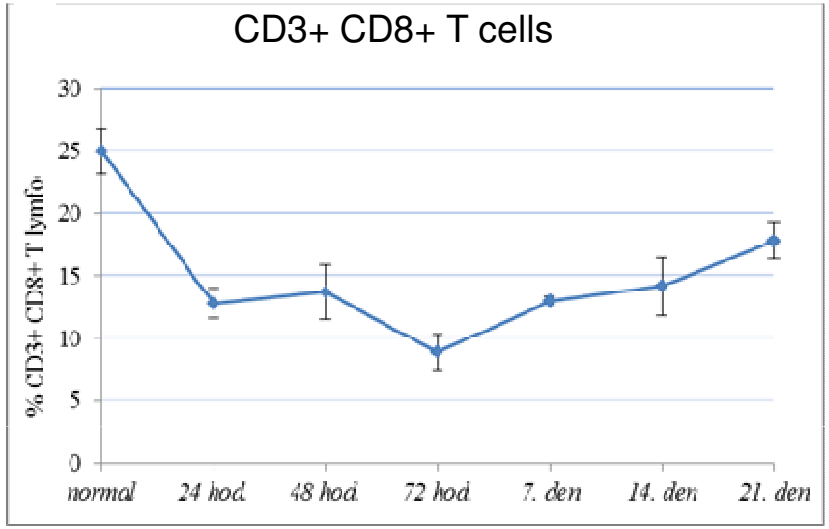


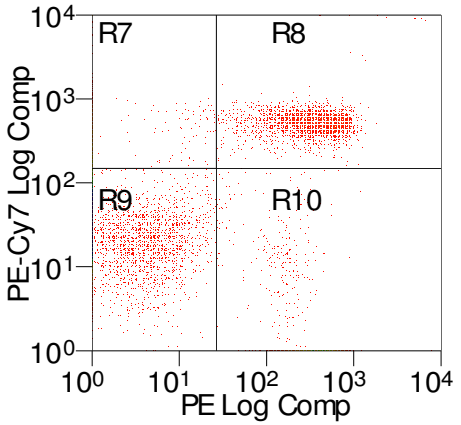
Basic head design of a cobalt radiotherapy unit

Fenotypization of spleen cells of immunosuprimmised Balb/c mice



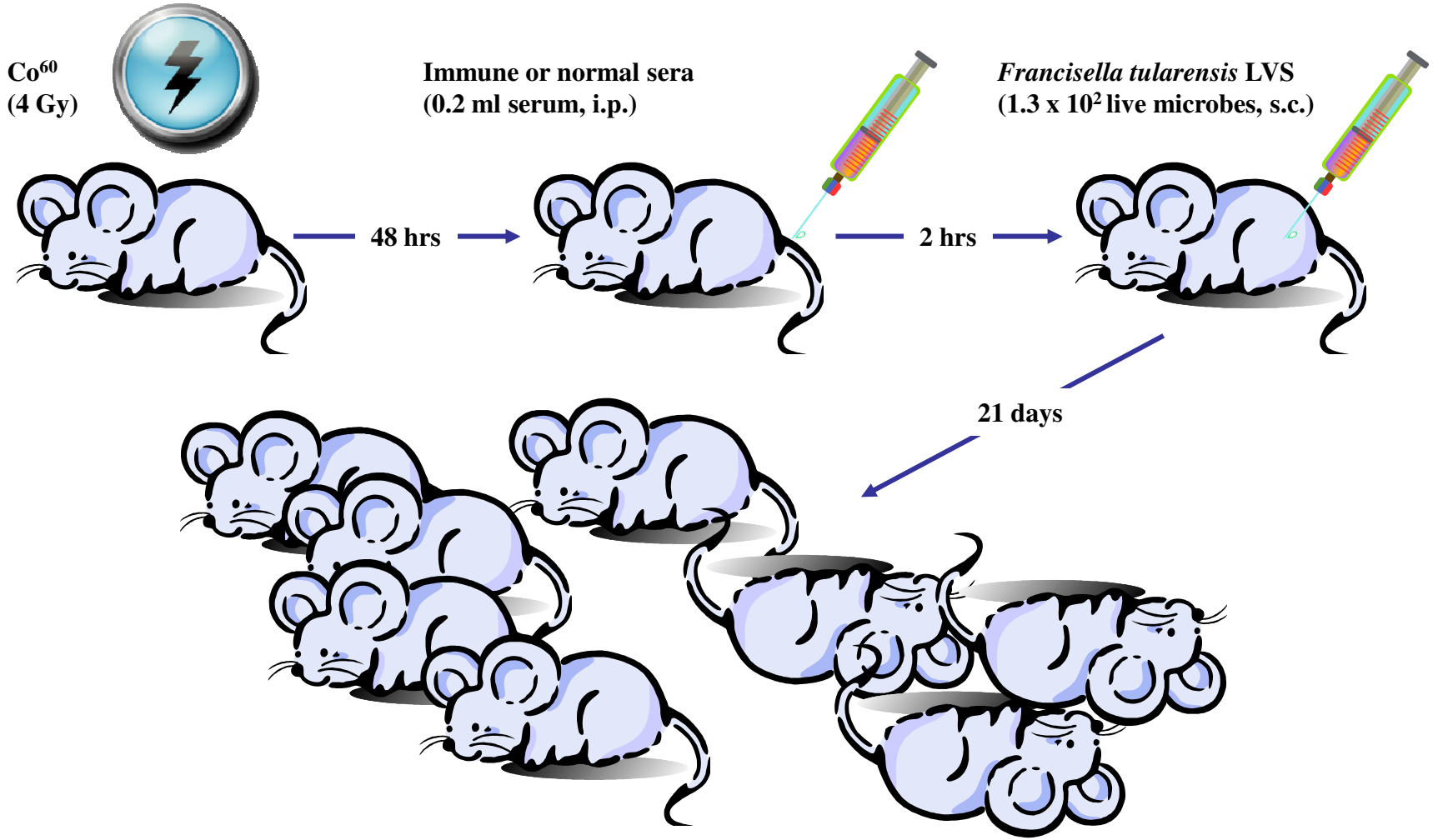
Region	Count	% Hist	% All
Total	10185	100.00	100.00
R7	1311	12.87	12.87
R8	5601	54.99	54.99
R9	2539	24.93	24.93
R10	734	7.21	7.21



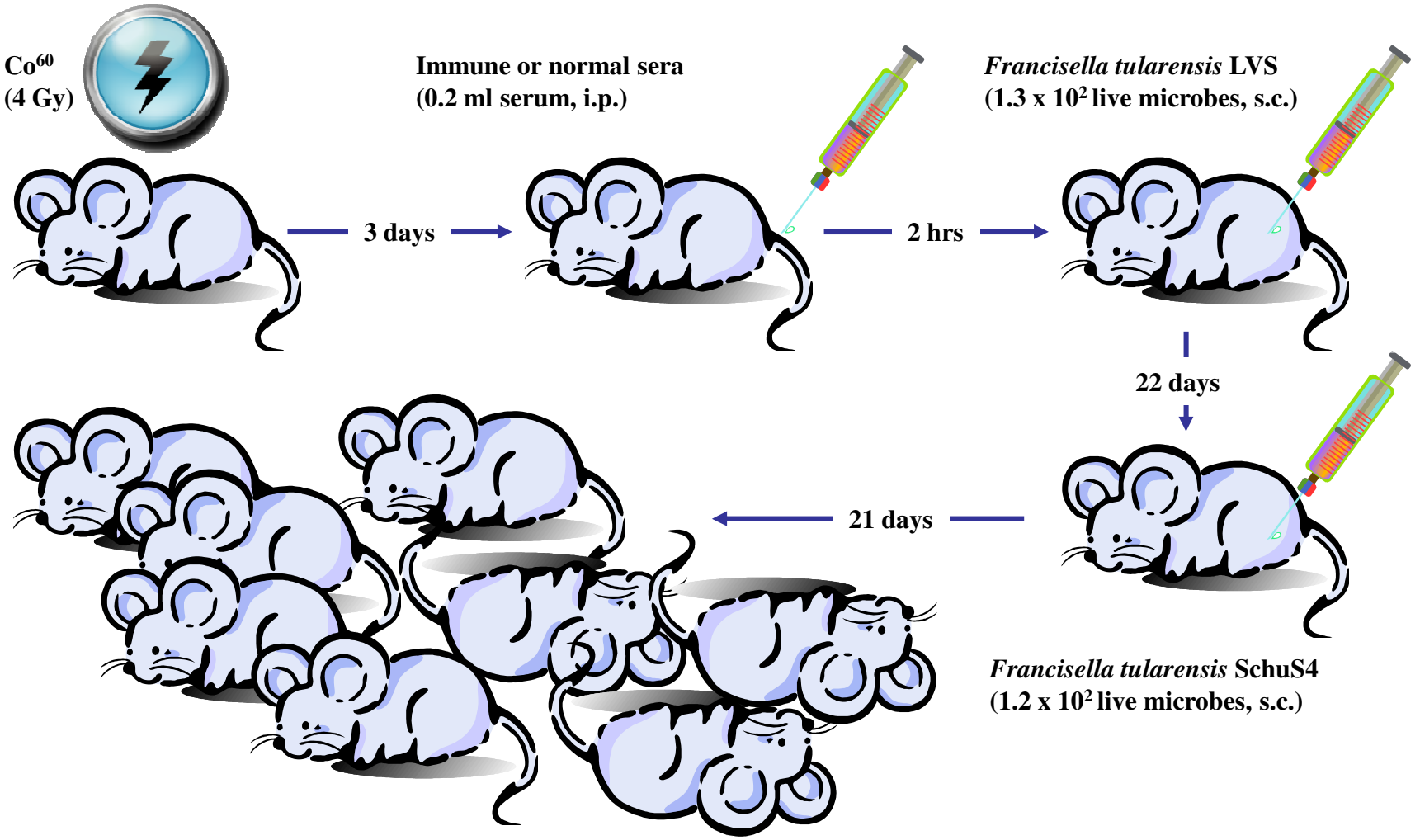


Region	Count	% Hist	% All
Total	9978	100.00	100.00
R7	2807	28.13	28.13
R8	3201	32.08	32.08
R9	3289	32.96	32.96
R10	681	6.83	6.83

Passive transfer of antibodies protects irradiated mice against *F. tularensis* holarctica LVS infection

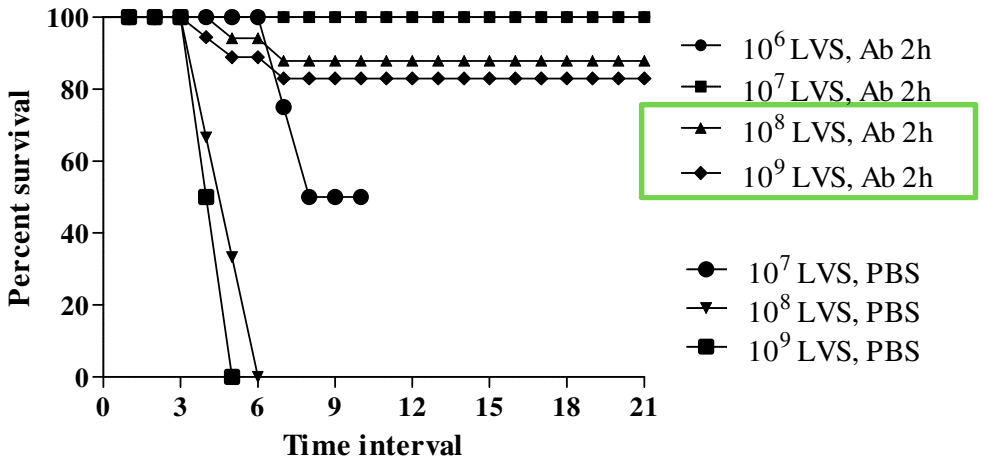


Passive transfer of immunity protects irradiated mice against primary as well as secondary *F. tularensis* infection



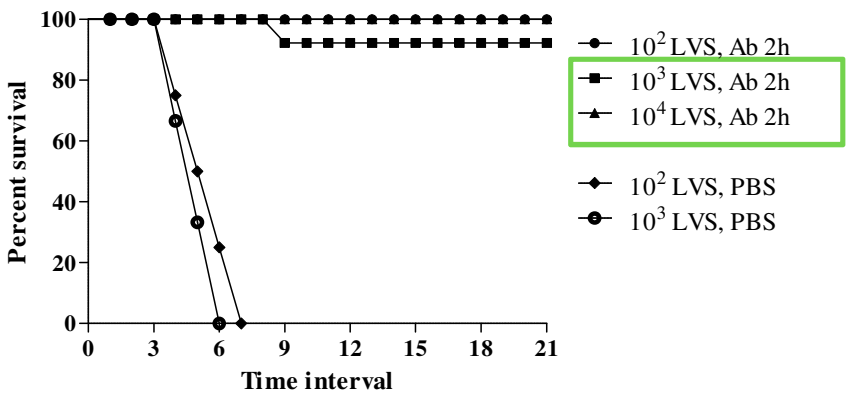
Live microbes induce protective Abs in immunosuprised individuals

	Number of mice	Irradiation	Interval of immunization	Route immun.	Immunization [200ul]	Infection	Dose LVS [200ul]	Route infect.
1KL	10	-	2 hod. before infection	i.p.	Ab 4Gy+LVS	*F. tularensis* LVS	10^6 bb/mouse	s.c.
2KL	10	-	2 hod. before infection	i.p.	Ab 4Gy+LVS	*F. tularensis* LVS	10^7 bb/mouse	s.c.
3KL	10	-	2 hod. before infection	i.p.	Ab 4Gy+LVS	*F. tularensis* LVS	10^8 bb/mouse	s.c.
4KL	10	-	2 hod. before infection	i.p.	Ab 4Gy+LVS	*F. tularensis* LVS	10^9 bb/mouse	s.c.
5KL	10	-	2 hod. before infection	i.p.	PBS	*F. tularensis* LVS	10^7 bb/mouse	s.c.
6KL	10	-	2 hod. before infection	i.p.	PBS	*F. tularensis* LVS	10^8 bb/mouse	s.c.
7KL	10	-	2 hod. before infection	i.p.	PBS	*F. tularensis* LVS	10^9 bb/mouse	s.c.



Live microbes induce protective Abs in immunosuprised individuals

	Number of mice	Irradiation	Immunization [200ul]	Interval of immunization	Route/ immun.	Infection	Dose LVS [200ul]	Route/infect.
1B	10	**4 Gy**	Ab 4Gy+LVS	2 hod. before infection	i.p.	*F. tularensis* LVS	10^2 CFU/mouse	s.c.
2B	10	**4 Gy**	Ab 4Gy+LVS	2 hod. before infection	i.p.	*F. tularensis* LVS	10^3CFU/mouse	s.c.
3B	10	**4 Gy**	Ab 4Gy+LVS	2 hod. before infection	i.p.	*F. tularensis* LVS	10^4CFU/mouse	s.c.
4B	10	**4 Gy**	PBS	2 hod. before infection	i.p.	*F. tularensis* LVS	10^2 CFU/mouse	s.c.
5B	10	**4 Gy**	PBS	2 hod. before infection	i.p.	*F. tularensis* LVS	10^2 CFU/mouse	s.c.



More efficient protection - using Ab 4Gy+LVS in irradiated animals

Live microbes induce protective Abs in immunosuprised individuals

	Number of mice	Gy	Immunization	Time	Route/immun.	Infection	Route/inf.	Dose LVS [200ul]
1A	5	-	Ab 4Gy+LVS	2 hod. before infection	*i.p.*	*F. tularensis* LVS	*s.c.*	10^8 CFU/mouse
2A	5	-	Ab 4Gy+LVS	2 hod. before infection	*i.p.*	*F. tularensis* LVS	*s.c.*	10^9CFU/mouse
3A	5	4 Gy	Ab 4Gy+LVS	2 hod. before infection	*i.p.*	*F. tularensis* LVS	*s.c.*	10^3 CFU/mouse
4A	5	4 Gy	Ab 4Gy+LVS	2 hod. before infection	*i.p.*	*F. tularensis* LVS	*s.c.*	10^4 CFU/mouse
5A	5	4 Gy	PBS	2 hod. before infection	*i.p.*	*F. tularensis* LVS	*s.c.*	10^1 CFU/mouse

Secondary infection with hypervirulent *F. tularensis* SchuS4 strain

	Number of mice	Infection	Route of infection	Dose SchuS4 [200ul]	Protection
1A	5	*F. tularensis* SchuS4	s.c.	10^2 CFU/myš	100%
2A	5	*F. tularensis* SchuS4	s.c.	10^2 CFU/myš	100%
3A	5	*F. tularens*is SchuS4	s.c.	10^2 CFU/myš	100 %
4A	5	*F. tularensis* SchuS4	s.c.	10^2 CFU/myš	100 %
5A	5	*F. tularensis* SchuS4	s.c.	10^2 CFU/myš	0 %

Cytokine profile of immunocompromised mice

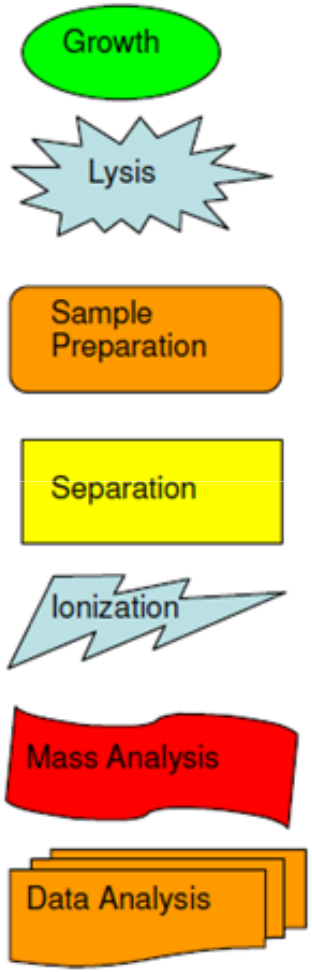
- ✓ The description of cytokine changes as a factor of importance during *Francisella* infection in naïve and immunocompromised mice
- ✓ ELISA kits (Invitrogen) – IL-1β, IL-4, IL-6, TNF-α, IFN-γ

	Serum level	Spleen	Liver	Lung
	Immunized irradiated mice	Immunized irradiated mice	Immunized irradiated mice	Immunized irradiated mice
IL-1β	Under the limit of detection	↓	↓	↓
IL-4	↓	↓	↓	↓
IL-6	↓	↓	↓	↓
TNF-α	↑	↑	↑	↑
IFN-γ	Not detectable	↑	↑	↑

Passive transfer of immunity rather normalize the cytokine levels

Dominant disproportion exists in the levels of IFN-γ in blood and tissue homogenates, which suggests the high consumption of this cytokine in the sites of production

Identification of immunoreactive *Francisella* proteins



Isolation, Amount, Conditions, Replicates, Timing

Small sample preparation, Lysis Buffers

PAGE, 2D-ELFO, Protease digestion, Cleanup

Off-line, Online

MALDI

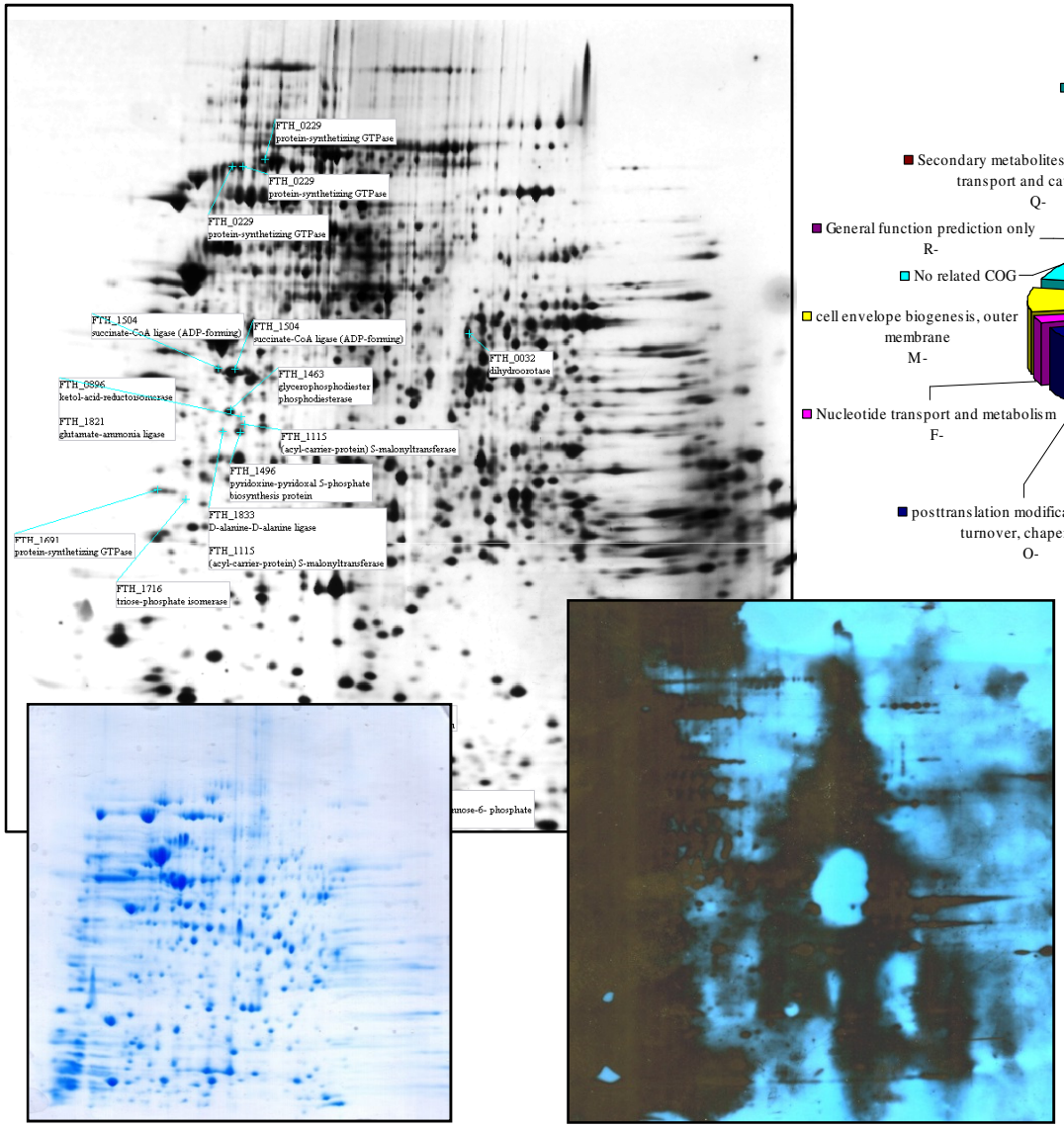
Time of Flight (TOF)

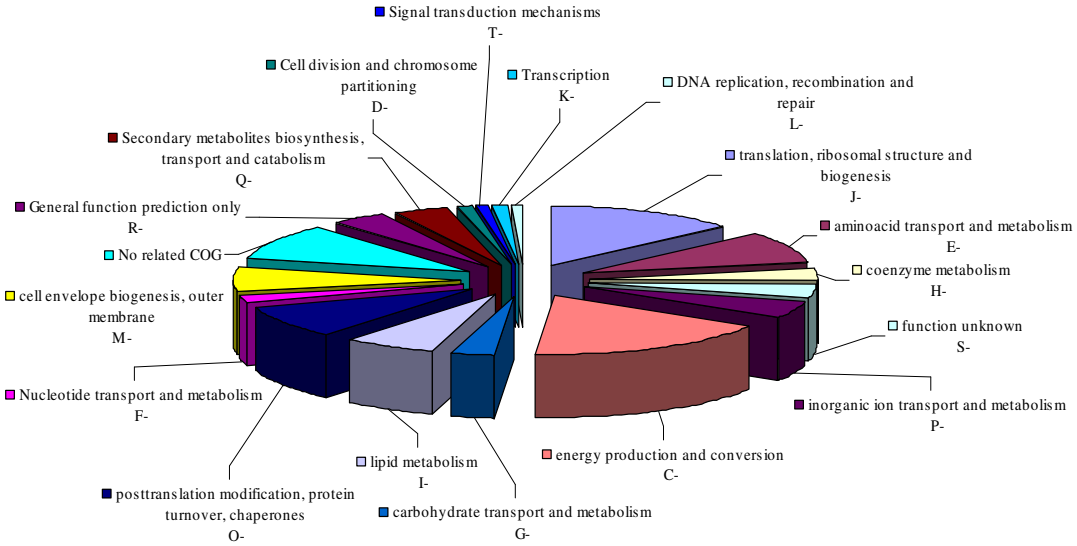
Protein fingerprinting, Database search, Filter and sort results

GPS Explorer™ Software v. 3.6 (Applied Biosystems), which integrates the *Mascot* search algorithm against *F. tularensis* LVS genome databases

- ✓ http://www.ncbi.nlm.nih.gov/COG/old/xognitor.html
- ✓ http://www.cbs.dtu.dk/services/SignalP
- ✓ http://www.psort.org/psortb/

Identification of immunoreactive *Francisella* proteins





http://www.ncbi.nlm.nih.gov/COG/old/xognitor.html

Spot No.	Gene locus	Name of protein	Accesion No.[a]	Gene	Mr/pI (theor.)[b]	Mr/pI (measur.)	SignalP[c]	PSORTb[d]	LipoP[e]	Ref.
3	**FTS_0084**/ FTH_0089/ FTL_0094/ FTT_1769c	Chaperone ClpB	K0E4P4	clpB	96.0/5.38	96.1/ 5.38	No	cyt	cyt	Janovska 2007 Twine 2010
4	**FTS_1471**/ FTH_1458/ FTL_1504/ FTT_0721c	Catalase-peroxidase	K7X4Y7	katG	82.6/5.43	82.5/5.43	Yes	Un	SpI	**Hubalek 2004** Havlasova 2005 Twine 2006 Janovska 2007
5	**FTS_1471**/ FTH_1458/ FTL_1504/ FTT_0721c	Catalase-peroxidase	K7X4Y7	katG	82.6/5.43	82.5/5.43	Yes	Un	SpI	**Hubalek 2004** Havlasova 2005 Twine 2006 Janovska 2007
6	**FTS_1471**/ FTH_1458/ FTL_1504/ FTT_0721c	Catalase-peroxidase	K7X4Y7	katG	82.6/5.43	82.5/5.43	Yes	Un	SpI	**Hubalek 2004** Havlasova 2005 Twine 2006 Janovska 2007
7	**FTS_0527**/ FTH_0524/ FTL_0525/ FTT_1600c	Fumerate hydratase	K0E9I8	fumA	55.1/5.27	55.3/5.27	No	cyt	cyt	
8	**FTS_1471**/ FTH_1458/ FTL_1504/ FTT_0721c	Catalase-peroxidase	K7X4Y7	katG	82.6/5.43	82.5/5.43	Yes	Un	SpI	**Hubalek 2004** Havlasova 2005 Twine 2006 Janovska 2007
9	**FTS_1116**/ FTH_1120/ FTL_1145/ FTT_1369c	Transketolase	K0E451	tktA	73.3/5.85	73.6/5.85	No	cyt	cyt	Havlasova 2005 Kilmury 2011
10	**FTS_0882**/ FTH_0876/ FTL_0891/ FTT_0623	Trigger factor	K0E8F9	tig	49.6/5.0	49.6/5.00	No	cyt	cyt	
11	**FTS_1457**/ FTH_1444/ FTL_1490/ FTT_1329	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	K0E4U3	gpmI	57.6/5.83	58.6/5.90	No	cyt	cyt	

Conserved hypothetical proteins:

- ✓ FTT0086
- ✓ FTT0848
- ✓ FTT0655

12	**FTS_1753**/ FTH_1734/ FTL_1797/ FTT_0062	ATP synthase subunit alpha	K0E5D9	aptA	55.5/4.94	55.7/4.94	No	cyt	cyt	Twine 2006 Twine 2010
13	**FTS_1709**/ FTH_1691/ FTL_1751/ FTT_0137	Elongation factor Tu	K0EAH4	tufA	43.6/5.12	43.4/5.11	No	cyt	cyt	Hubalek 2003 Havlasova 2005 Janovska 2007 Twine 2010
14	**FTS_0088**/ FTH_0093/ FTL_0099/ FTT_1773c	Tryptophan synthase beta chain	K0E6G9	trpB	43.3/6.9	43.1/6.90	No	cyt	cyt	
15	**FTS_1709**/ FTH_1691/ FTL_1751/ FTT_0137	Elongation factor Tu	K0EAH4	tufA	43.6/5.12	43.4/5.11	No	cyt	cyt	Hubalek 2003 Havlasova 2005 Janovska 2007 Twine 2010
16	**FTS_1295**/ FTH_1293 FTL_1328/ FTT_0583	OmpA family protein	K0E4G8	fopA	41.9/5.58	41.9/5.58	Yes	OM	SpI, TMH	Havlasova 2002 Havlasova 2005
17	**FTS_1517**/ FTH_1504/ FTL_1553/ FTT_0504c	Succinyl-CoA ligase [ADP-forming] subunit beta	K0E828	sucC	41.5/5.24	41.7/5.24	No	cyt	cyt	
18	**FTS_0935**/ FTH_0933/ FTL_0955/ FTT_0679	Ribosome-binding ATPase YchF	K0EAU8	ychF	40.2/4.97	40.5/4.97	Yes	Un	SpI	
19	**FTS_1295**/ FTH_1293 FTL_1328/ FTT_0583	OmpA family protein	K0E4G8	fopA	41.9/5.58	41.9/5.58	Yes	OM	SpI, TMH	Havlasova 2002 Havlasova 2005

The result applications

✓ Changing the vaccine strategy

- Important role of circulating antibodies during the interaction of *F. tularensis* with host immunoregulatory system
- Passively protected mice were also able to survive primary LVS and the subsequent challenge with the hypervirulent strain *F. tularensis* SchuS4
- Functional passive immunization protocol for naïve, as well as immunocompromised animals
- Cytokine production of immunocompromised mice has been characterized as a part the host response to *Francisella* infection
- Combination of passive transfer of antibodies and subsequent active immunization represents the safety way to protective immunity against tularemia
- New immunoreactive proteins – monoclonal Abs

With thanks to colleagues and collaborators

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Kubelkova K., Krocova Z., Balonova L., Stulik J., Macela A.: Specific antibodies protect gamma-irradiated mice against *Francisella tularensis* strain 15 and live vaccine strain (LVS) infection. Microbial Pathogenesis, 2012, 53, 259-268.

Kubelkova K., Krocova Z., Plzakova L., Macela A.: The Role of B cells in Intracellular Bacterial Pathogen Infection, B cells: Molecular Biology, Developmental Origin and Impact on the Immune Systém. 2013, ISBN: 978-1-62808-541-9, p.1-44.

Plzakova L., Kubelkova K., Krocova Z., Zarybnicka L., Sinkorova Z., Macela A.: B cell subsets are activated and produce cytokines in the course of early phases of *Francisella tularensis* LVS infection. Microbial Pathogenesis, 2014, http://dx.doi.org/10.1016/j.micpath.2014.08.009.

Kubelkova K. and Macela A.: Putting Jigsaw Together - A Brief Insight Into the Tularemia, Open Life Sciences, 2015, Ready to publish.

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