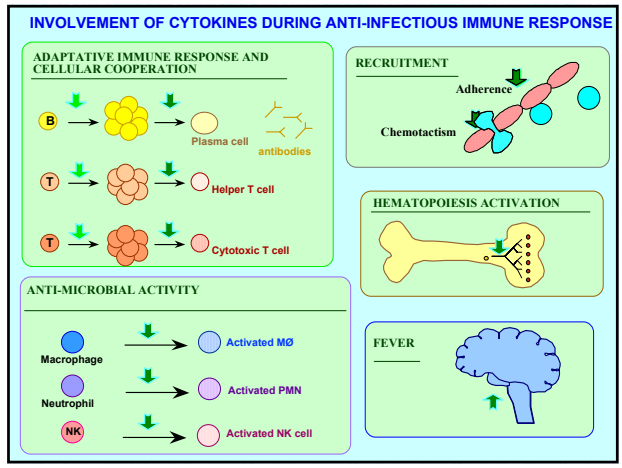
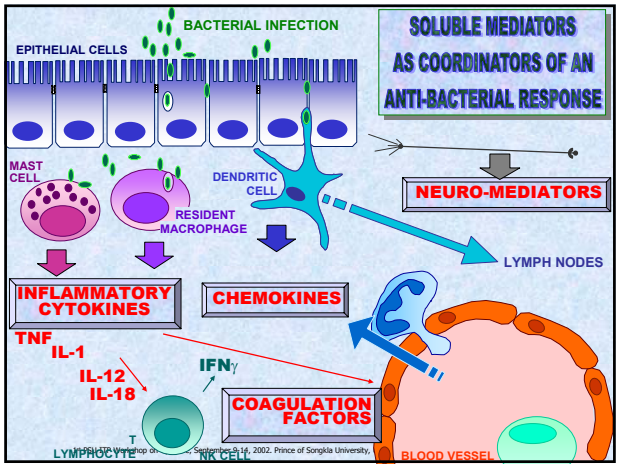
PRINCE OF SONGKLA UNIVERSITY

CYTOKINES AND INFECTIONS

Jean-Marc Cavaillon
Cytokines & inflammation
INSTITUT PASTEUR





CYTOKINES AND INFECTIONS

1/ CYTOKINE INDUCTION BY MICROBIAL PRODUCTS

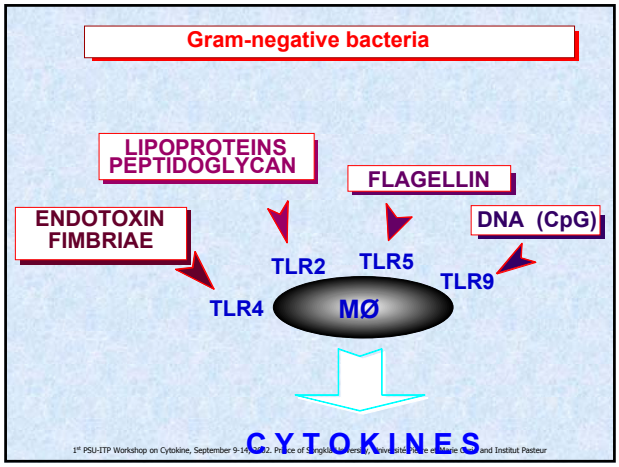
2/ CO-FACTORS & SYNERGY

3/ INFECTION AND Th1 / Th2 PROFILE

4/ ANTI-INFECTIOUS ROLE OF CYTOKINES

5/ EXACERBATED PRODUCTION AND DELETERIOUS EFFECTS

6/ STRATEGIES ELABORATED BY PATHOGENS AGAINST CYTOKINES



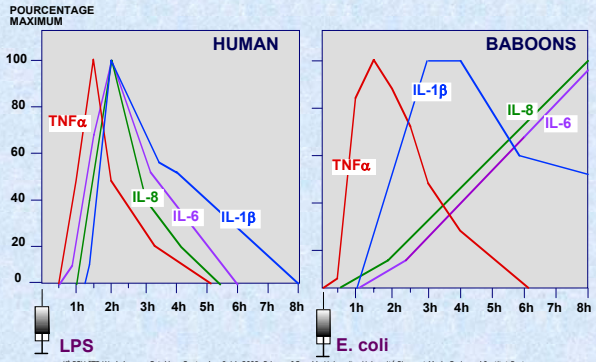
SHOCK AND MULTIPLE ORGAN DYSFUNCTION AFTER SELF-ADMINISTRATION OF SALMONELLA ENDOTOXIN

Taveira da Silva et al. N. Engl. J. Med. 1993, 328, 1457

1 mg LPS (15 µg/k, i.e. 3750 X dose given to human volunteers

	Serum concentration (pg/ml)				
Hours after LPS injection	LPS	TNF ELISA	TNF Bioassay	IL-6	IL-8
3.6	nd	14 630	9157	nd	nd
6.8	38	147	17	263 510	16 410
11.5	< 5	nd	nd	51 910	3 190

KINETICS OF CYTOKINES FOLLOWING LPS OR BACTERIA INJECTIONS

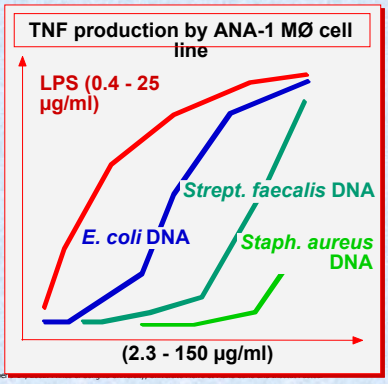


BACTERIAL DNA CAUSES SEPTIC SHOCK

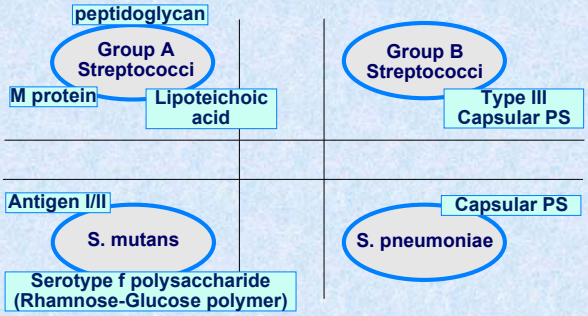
Sparwasser et al. Nature 1997, 386, 336

Gram positive and Gram negative DNA causes lethal toxic shock in galactosamine sensitized mice

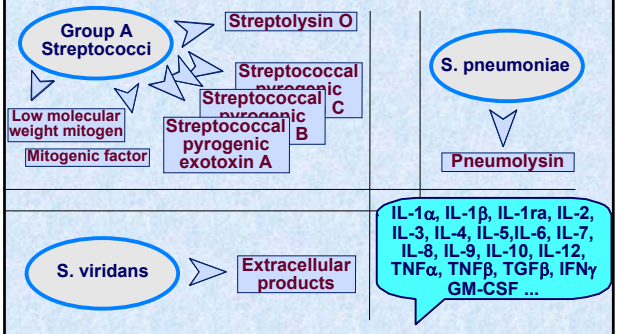
in contrats to bacterial DNA, in eukaryotic DNA, the dinucleotide motif 5'-CpG-3' is mostly methylated



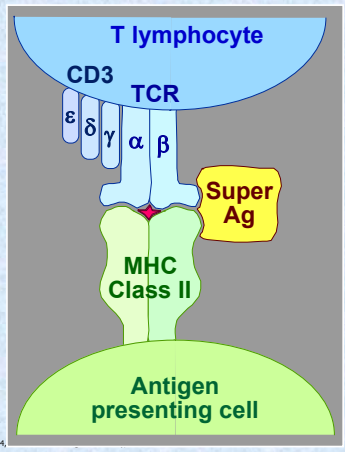
SURFACE COMPOUNDS CAPABLE TO INDUCE CYTOKINE PRODUCTION



STREPTOCOCCAL RELEASED PRODUCTS CAPABLE TO INDUCE CYTOKINE PRODUCTION



SUPERANTIGENS ACTIVATE T- LYMPHOCYTES



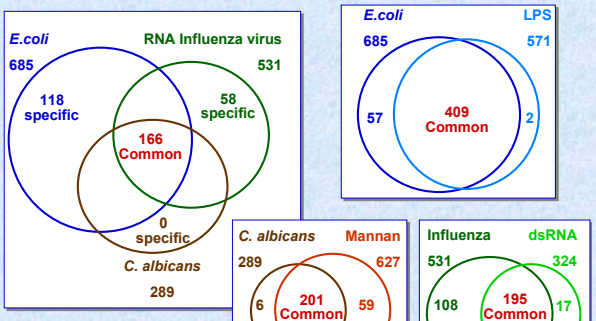
LPS *versus* Staphylococcus aureus ENTEROTOXIN B -INDUCED SHOCK

Gonzalo et al. Eur. J. Immunol. 1993, 23, 3272

			LETHALITY	
GalNH2	Dexamethasone	Cyclosporine	LPS	SEB
X			100	100
X	X		0	0
X		X	100	15

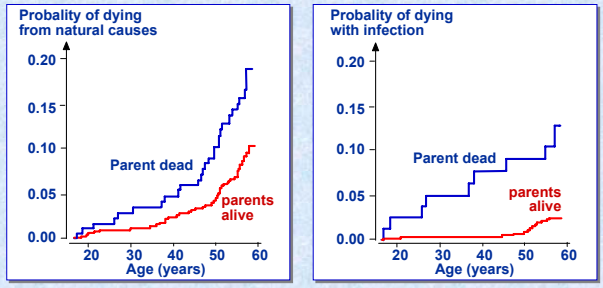
THE PLASTICITY OF DENDRITIC CELL RESPONSE TO PATHOGENS AND THEIR COMPONENTS

Huang et al. Science 2001, 294, 870,



GENETIC AND ENVIRONMENTAL INFLUENCES ON PREMATURE DEATH IN ADULT ADOPTEES

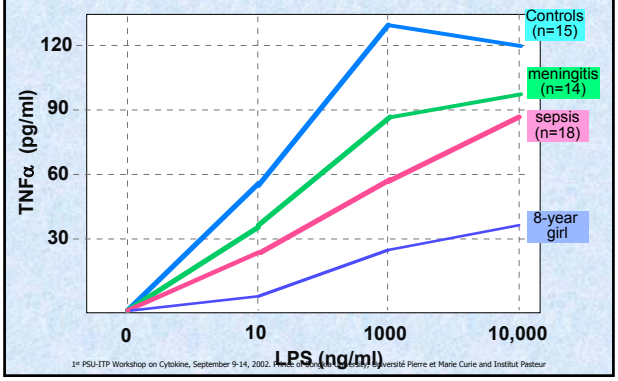
Sorensen et al. N. Engl. J. Med. 1988, 318, 727



Heritability of premature death due to infection (relative risk = 5.8) is higher than death due to cardio- & cerebro-vascular (4.5) causes or to cancer (1.1)

RELEASE OF TNF : AN INNATE HOST CHARACTERISTIC THAT MAY CONTRIBUTE TO THE OUTCOME OF MENIGOCOCCAL DISEASE

Westendorp et al. J. Infect. Dis. 1995, 171, 1057



HYPORESPONSIVENESS IN A PATIENT WITH RECURRENT BACTERIAL INFECTION

Kuhns et al. J. Immunol. 1997, 158,3959

15 years old girl

13 life-threatening infections

- *S. pneumoniae* meningitis (2 x)
- *N. meningitidis* endophtalmitis
- *S. aureus* cellulitis (several episodes)
- Gram positive abdominal abcess
- *Clostridium septicum* infection of the leg (amputation) + septic shock + ARDS

LPS in vivo :
- subnormal febrile response
- low TNF, G-CSF, IL-6, IL-8
- normal IL1ra, s TNF R I

Monocytes + LPS, Staph. IL-1, glucan
- No TNF, G-CSF

PMN activation by LPS :
- No increase of CD20, CD18, CD11b, CD67, CD45
- no priming to O2- induction by FM
- normal response to FMLP, TNF & PAF

A GENOMIC POLYMORPHISM WITHIN THE TNF LOCUS INFLUENCES PLASMA TNFα CONCENTRATIONS AND OUTCOME OF PATIENTS WITH SEVERE SEPSIS

Stüber et al. Crit. Care Med. 1996, 24, 381

polymorphic site of the restriction enzyme *Nco* I	TNFB1 homozygotes	TNFB1/B2 heterozygotes	TNFB2 homozygotes
frequeny	10 %	48 %	42 %
APACHE II	21	22	22
mean TNFα (pg/ml)[1]	150	250	600
Survivors	3 / 4	12 / 19	2 / 17

[1] every 6h during the first 48h, every 12h thereafter untill 96h

TLR MUTATIONS AND MENINGOCOCCAL SEPSIS

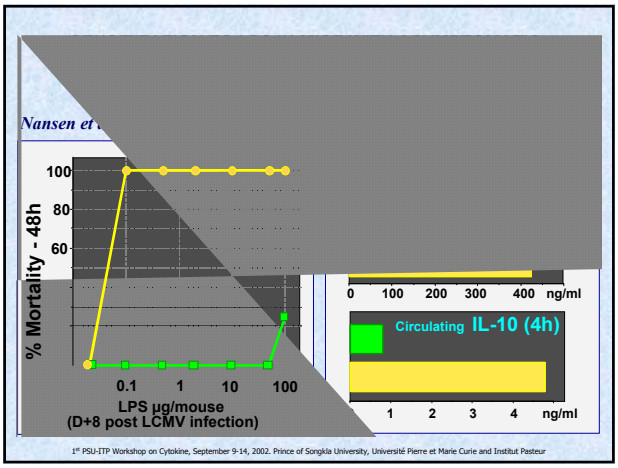
B. Beutler et al. 2001

TLR4

An excess of rare mutations are found in meningococcal sepsis as compared to healthy controls - $p = 0.0065$

TLR2

The mutation P631H found in around 5% of healthy controls is rarely found in meningococcal sepsis (<1%) - $p = 0.0086$



Th1 → Resistance to infection

Th2 → **IL-4**
- ↓ IL-2R on T-cells
- ↓ MØ anti-microbial activation by IFNγ
- ↓ IL-1 & TNF production by MØ
- ↓ NO generation necessary for killing intracellular parasite

Th2 → progressive forms of infection

IL-10
- ↓ Ag presentation by MØ
- ↓ MØ anti-microbial activation by IFNγ
- ↓ IL-1 & TNF production by MØ
- ↓ Reactive oxygen intermediates

Parasitic antigen (Leishmania)

IFNγ only

LYMPH NODES (day + 3)

IL-4, IL-5 and IFNγ

Leishmania

cutaneous lesion → RECOVERY

INFγ mRNA

+ anti- IFNγ => No more resistance

+ IL-4 : 0

CD4+

systemic infection → DEATH

IL-4 + IL-10 mRNA

+ anti-IL-4 => resistance Th2 response

Leishmania major

RESISTANT C57Bl/6

SUSCEPTIBLE BALB/c

+ anti-IL-12 → SUSCEPTIBLE

+ IL-12 → PROTECTION

Leishmaniasis

Pirmez et al. J.Clin.Invest.1993,91,1390
Melby et al. Infect.Immun.1994,62,837

Localized cutaneous leishmaniasis

few skin lesions

mRNA in lesions
- IL-2 +
- IFNγ ++
- LTα ++

Diffuse cutaneous leishmaniasis

widely disseminated lesions

mRNA in lesions
- IL-4 ++
- IFNγ ±

Visceral leishmaniasis

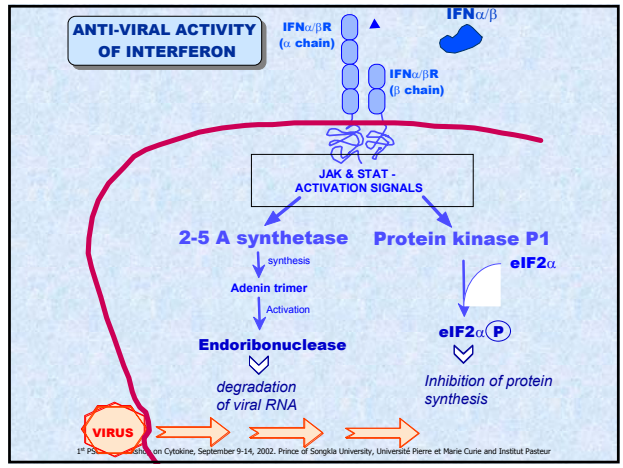
mRNA in bone marrow and lymph nodes

IL-10 ++

+ Pro-inflammatory cytokines : IFNγ , IL-1α, IL-6, TGFβ

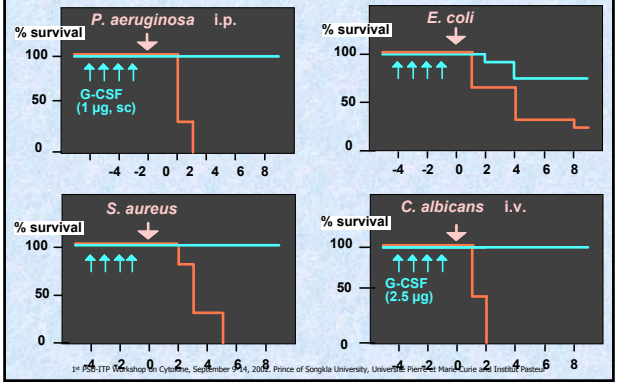
CD8+ : IL-4
MØ : IL-10

ANTI-VIRAL ACTIVITY OF INTERFERON



PROTECTIVE EFFECT OF HUMAN G-CSF ON MICROBIAL INFECTION IN NEUTROPENIC MICE

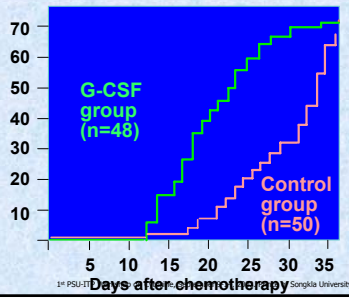
Matsumoto et al. Infect. Immun. 1987, 55, 2715



EFFECT OF G-CSF AFTER INTENSIVE INDUCTION THERAPY IN RELAPSED OR REFRACTORY ACUTE LEUKEMIA

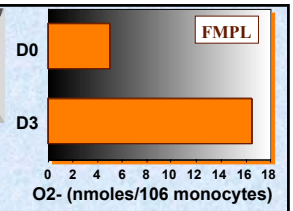
Ohno et al. New Engl. J. Med. 1990,323,871

Neutrophil count > 1000 mm3
(cumulative % of patients)

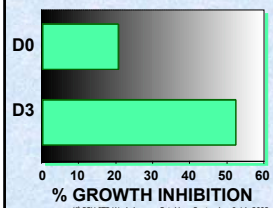


Documented infections	septicemia, pneumonia, perianal abscess, cellulitis, gingivitis and pharyngitis, cystitis
G-CSF group	5/27
Control group	15/33
	p=0.03

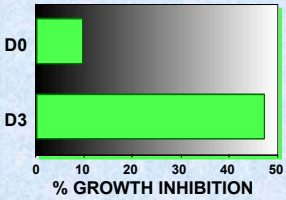
Roilides et al. Cytokine 1996, 8, 42



Candida blastoconidia



Staphylococcus aureus

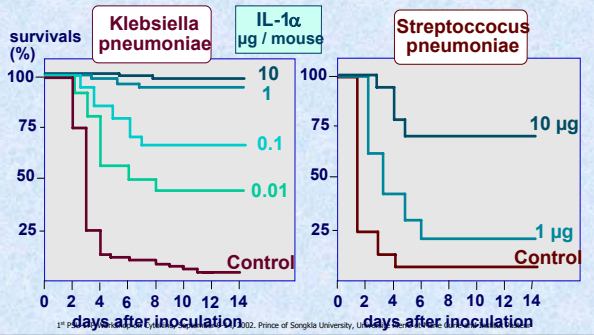


Beneficial effects of pro-inflammatory cytokines

IL-1

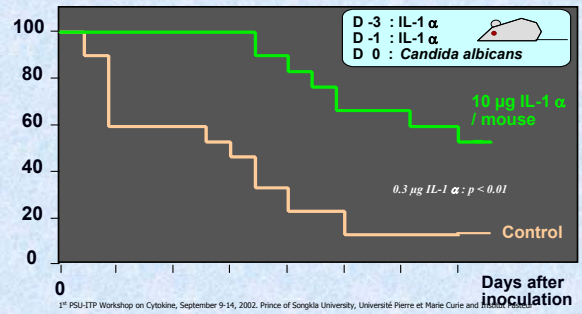
AUGMENTATION OF HOST RESISTANCE TO MICROBIAL INFECTION BY RECOMBINANT IL-1

Minami et al. Infect. Immun. 1988, 56, 3116



IL-1 INDUCES PROTECTION IN FUNGAL INFECTION

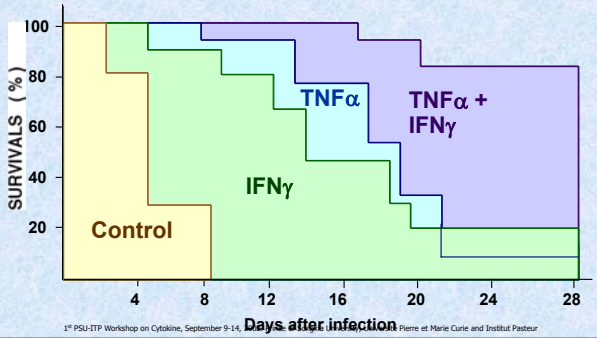
Minami et al. Infect. Immun.1988,56,3116



PROTECTIVE EFFECT OF rTNFα IN MURINE SALMONELLOSIS

T -6h : Cytokine (i.p.)
T 0 : *S. Typhimurium* (i.p.)

Nakano et al. J. Immunol. 1990, 144, 1935



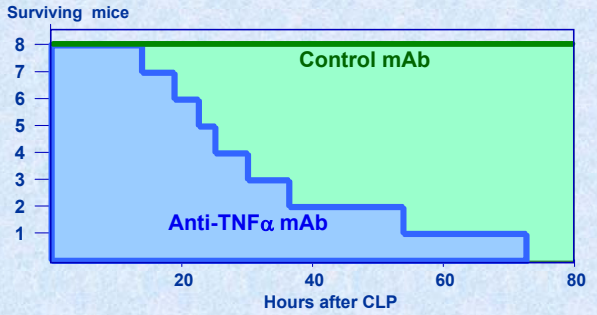
TNFα PLAYS A PROTECTIVE ROLE IN EXPERIMENTAL MURINE CUTANEOUS LEISHMANIASIS

Titus et al. J. Ep. Med. 1989, 170, 2097

Challenge (L. major)	Treatment (started on day7)	Number of Leishmania major in the lesion on day 21
20×10^6	none	1200
	TNFα (every 3 days)	100
0.5×10^6	Control Ig (every 2 days)	12
	anti-TNFα	190

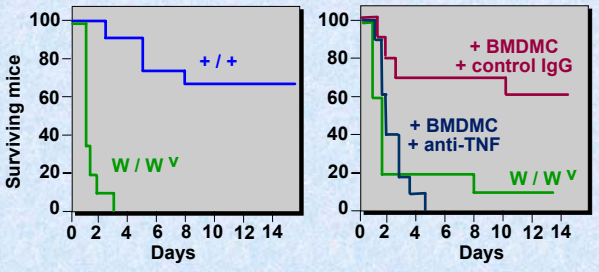
EFFECT OF AN ANTI-TNFα INJECTION ON EXPERIMENTAL PERITONITIS

Echtenacher et al. J. Immunol. 1990,145,3762

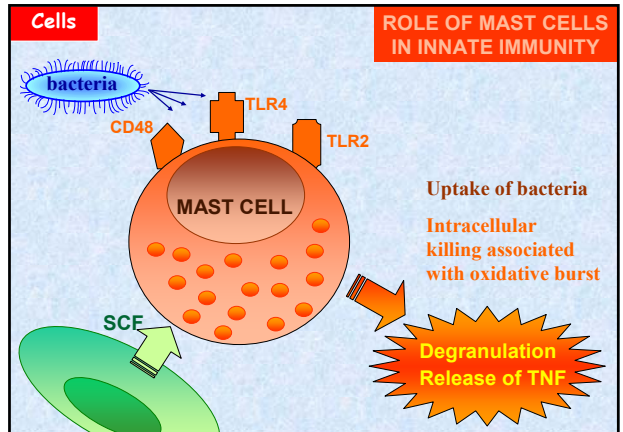


CRITICAL PROTECTIVE ROLE OF MAST CELL IN A MODEL OF ACUTE SEPTIC PERITONITIS

Echtenacher, Männel & Hültner, Nature 1996, 381, 75



ROLE OF MAST CELLS IN INNATE IMMUNITY



IL-12 INCREASES RESISTANCE OF MICE TO *Mycobacterium tuberculosis* INFECTION

Flynn et al. 1996
J. Immunol. 155, 2515

Treatment	mean survival (days)		viable bacilli in the organs
PBS	58 ± 8		
+ IFNγ (osmotic pump)	66 ± 2	ns	no significant changes
+ IL-12 (d-1 to d+5)	112 ± 3	p<0.001	10-50 fold reduction

BALB/c

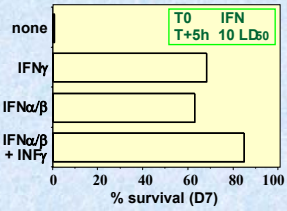
M. tuberculosis

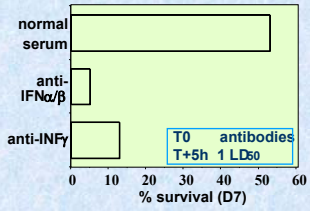
PBS	14 ± 1	the anti-tuberculous effect of IL-12 was not observed in the absence of INFγ
IL-12	8 ± 1	

BALB/c IFNγ $^{-/-}$

ROLE OF INTERFERON IN STREPTOCOCCAL INFECTION IN THE MOUSE

Weigent et al. 1986
Microbial. Pathogenesis 1, 399





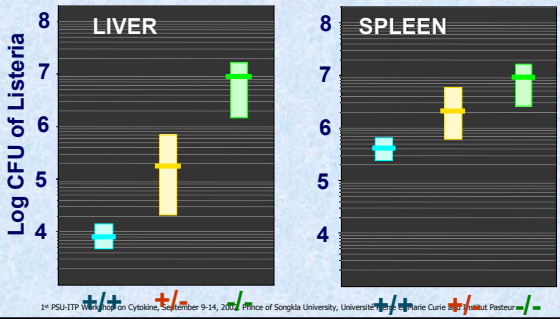
Beneficial effects of pro-inflammatory cytokines

TNF

TNFα / Lt α deficient mice

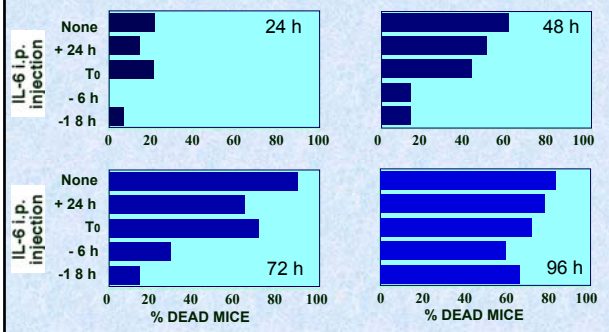
Amiot et al. Eur. J. Immunol. 1997, 27, 1035

Listeria monocytogenes **(8x10^3 i.v.)**
load 48h after infection



Beneficial effects of IL-6 in neonatal mouse models of Group B streptococcal disease

Mancuso et al. Infect. Immun. 1994,62,4997



IL-1ra CAN EITHER REDUCE OR ENHANCE LETHALITY

Mancilla et al. Infect. Immun. 1993,61,926

New Born Rat

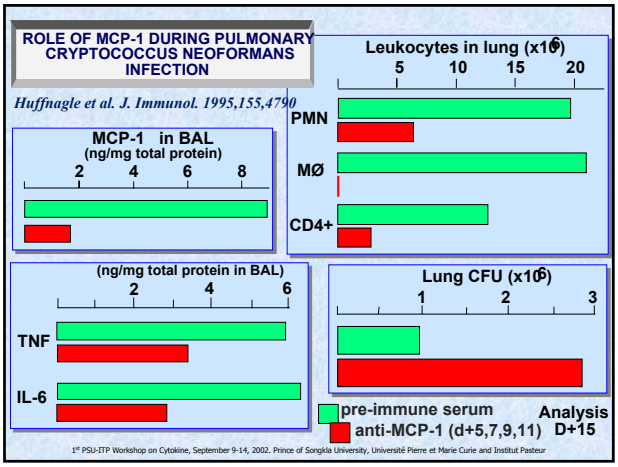
5x10^7 CFU s.c.
Klebsiella pneumoniae

SURVIVAL 120 H

Albumine		25 %	p<0.01
IL-1ra	5 mg/kg	45 %	p<0.01
	40 mg/kg	7 %	

ROLE OF MCP-1 DURING PULMONARY CRYPTOCOCCUS NEOFORMANS INFECTION

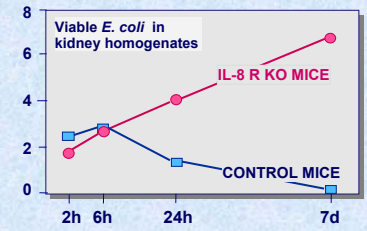
Huffnagle et al. J. Immunol. 1995,155,4790



Beneficial effects of chemokines

IL-8 RECEPTOR DEFICIENCY CONFERS SUSCEPTIBILITY TO ACUTE EXPERIMENTAL PYELONEPHRITIS

Frendéus et al. J. Exp. Med. 2000, 192, 881



SIMILAR OBSERVATION IN THE BLADDER

ASSOCIATED WITH AN IMPAIRED MIGRATION OF NEUTROPHIL FROM THE TISSUES TO THE EPITHELIAL BARRIER

PATIENTS WITH A HISTORY OF ACUTE PYELONEPHRITIS

DECREASED PMN CXCR1 EXPRESSION (P < 0.03 VS CONTROLS)

DECREASED PMN mRNA CXCR1 EXPRESSION (P < 0.001 *vs* CONTROLS)

NORMAL CXCR2 EXPRESSION

HALF ANGEL / HALF DEVIL

1st PSU-ITI ... tut Pasteur

MICE DEFICIENT FOR THE 55 kDa TNF RECEPTOR ARE RESISTANT TO ENDOTOXIC SHOCK YET SUCCOMB TO Listeria monocytogenes INFECTON

Pfeffer et al. Cell 1993, 73, 457

	Survival	
	55 kDa TNF R	
	+/+	-/-
5 x 10^4 live L. monocytogenes	6/6	1/7 Death Within 6 days
100 µg LPS *	0/6 Necrosis of almost all hepatocytes	6/6
200 µg Staphylococcal Enterotoxin B *	0/3	7/8

* D-GalNH2 treated mice

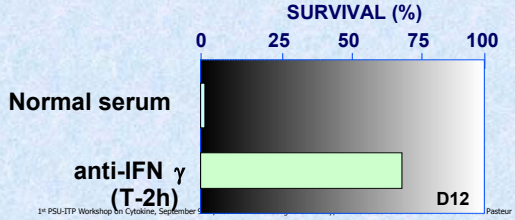
1st PSU-ITP Workshop on Cytokine, September 9-14, 2002. Prince of Songkla University, Université Pierre et Marie Curie and Institut Pasteur

Is IFNγ responsible of the deleterious effects observed during sepsis ?

ENDOGENOUS IFN γ IN *Staphylococcus aureus* INFECTION IN MICE

Nakane et al. Infect. Immun. 1995, 63, 1165

lethal infection (10^8 CFU, i.v)



1st PSU-ITP Workshop on Cytokine, September ... Pasteur

Experimental infectious models

Beneficial effect of IFNγ in murine salmonellosis

Nakano et al. J. Immunol. 1990, 144, 1935

	Mean day survival	% survivals
Control	5 d	0 %
IFNγ (T-6h)	14 d	20%

Deleterious effects of IFNγ in lethal *S. aureus* infection

Nakane et al Infect.Immun. 1995, 63, 1165

	% survivals
Control serum	0 %
+ anti-IFNγ (T-2h)	60%

Beneficial effect of IL-12 in murine *M. tuberculosis* infection

Flynn et al. J. Immunol. 1996, 155, 2515

	Mean day survival	Viable bacilli in organs
Control	58 ± 8	
IL-12 (d-1 to d+5)	112 ± 3	a 10 - 50 X reduction

Deleterious effects of IFNγ & IL-12 in murine polymicrobial septic peritonitis

Echtenacher et al. Infect. Immun. 2001, 69, 7271

	% survivals
Control	91 %
IFNγ (1 µg, T0)	40 %
IL-12 (100 ng; d-1)	20 %

1st PSU-ITP Workshop on Cytokine, September 9-14, 2002. Prince of Songkla

HOMODIMERIC IL-12 p40 PROTECTS MICE FROM IL-12 DEPENDENT SHOCK BUT NOT FROM TNFα DEPENDENT SHOCK

Mattner et al. Infect. Immun. 1997, 65, 4734

		% SURVIVAL (350 µg LPS i.p.)	% SURVIVAL (LPS 0.1µg /GalNH 10 mg)
CONTROLS		12	0
ANTI-IL-12	24, 12 & 1h before LPS challenge	90	0
(IL-12 p40)2	24, 12 & 1h before LPS challenge	93	20
TNF R - IgG	1h before LPS challenge	70	90

1st PSU-ITP Workshop on Cytokine, September 9-14, 2002. Prince of Songkla University, Université Pierre et Marie Curie and Institut Pasteur

Experimental infectious models

IL-10 and bacterial infections

injection of IL-10
use of anti-IL-10
use of IL-10 transgenic mice
(over-expression and KO)

BENEFICIAL

Pseudomonas aeruginosa	Sawa et al. *J.I.* 1997;Kurahashi et al. *J.C.I.* 1999 Maitsumoto et al. *Antimicr Agent & Chem* 1998; Chmiel et al. *A.J.R.C.C.M.* 1999

DELETERIOUS

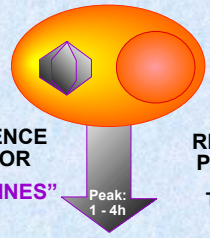
Mycobacterium bovis & avium	Denis et al. *J.I.* 1993
Klebsiella pneumonia	Murray et al. *J.I.* 1997; Jacobs et al. *Immunol.* 2000
Salmonella cholerasuis	Wang et al. *Immunol.* 2000
Brucella abortus	Arai et al. *Immunol.* 1995
Chlamydia trachomatis	Fernandes et al. *Infect Immun.* 1995
	Yang et al. *J. Immunol.* 1996

DEPENDING ON THE EXPERIMENTAL MODEL OR ON THE STUDIED PARAMETERS

Listeria monocytogenes	Kelly et al. *Eur.J.Imm.* 1994; Wagner et al. *I&I* 1994 Silva et al. *I&I.* 2001
Streptococcus pneumonia	van der Poll et al. *J.I.D.* 1996; Koedel et al. *J.I.* 1996

ENCODING OF A HOMOLOG OF THE IFN-γ RECEPTOR BY MYXOMA VIRUS

Upton et al. Science 1992, 258, 1369



VIRULENCE FACTOR
"VIROKINES"

Peak: 1 - 4h

MAJOR RELEASED PROTEIN :
T7 (37 kDa)

T7 protein neutralize the potency of rabbit interferon-γ to induce anti-viral status

homology with s IFNγ R
(≈ 26%; 8 cystein maintained)
"VIROCEPTOR"

GENE HIJACKING BY VIRUS

	Identity TNF R I	TNFR II
MYXOMA VIRUS T2	≈ 40% identity in the cystein-rich domains	
76%		
SHOPE FIBROMA VIRUS T2	38 %	29%

TNF
Soluble TNF Receptor

The viral disease in rabbits infected with Myxoma virus T2- is significantly attenuated

Upton et al. Virology 1991, 184, 370
Smith et al. Bioch. Biophys. Res. Comm. 1991, 176, 335

VACCINIA VIRUS

B15R GENE

GLYCOPROTÉINE
≡
SOLUBLE IL-1 RECEPTOR

IL-1α	0
IL-1β	+
IL-1 ra	0

Spriggs et al. Cell 1992,71,145
Alcami et al. Cell 1992,71,153

USE OF CHEMOKINE RECEPTORS TO ENTER THE CELL

CD4

ex.: VIH
(via CCR5 & CXCR4)

SYNTHESIS OF SOLUBLE LIGANDS FOR CHEMOKINES

CHEMOKINE

ex.: Myxoma virus
orthopoxvirus
pox virus β–chEmokines)
[cowpox; variola; vaccinia;
shope fibroma]

SYNTHESIS OF VIRAL CHEMOKINES

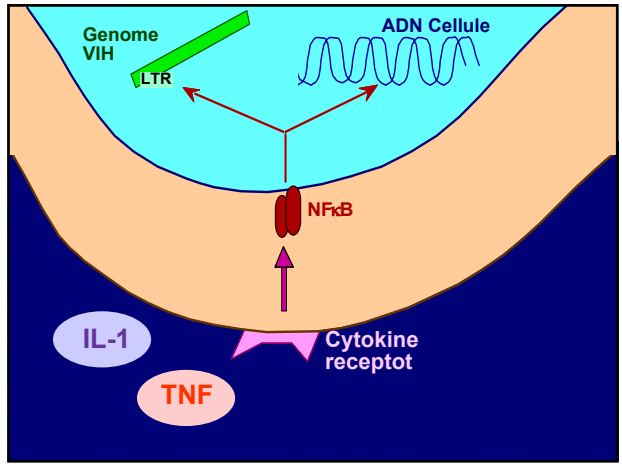
CHEMOKINE VIRALE

ex.: Stealth virus
(56% homol. GRCα)
Marek disease virus (40% homol. IL-8)
Kaposi's sarcoma associated virus
vMIP-I (43% homol. MIPα)
vMIP-II (51% homol. MIPα)
BCK (25% homol. MIPβ)
Molluscum contagium virus
MC148 (27% homol. MCP-1)
CC& CXC CHEMOKINE ANTAGONISTS

SYNTHESIS OF VIRAL CHEMOKINE RECEPTORS

CHEMOKINE

ex.: Cytomégalovirus
(β but not α-chemokines)
Herpes virus saimiri
(α but not β-chemokines)
Virus Herpes humain
(U12 : β but not α-chemokines
poxvirus



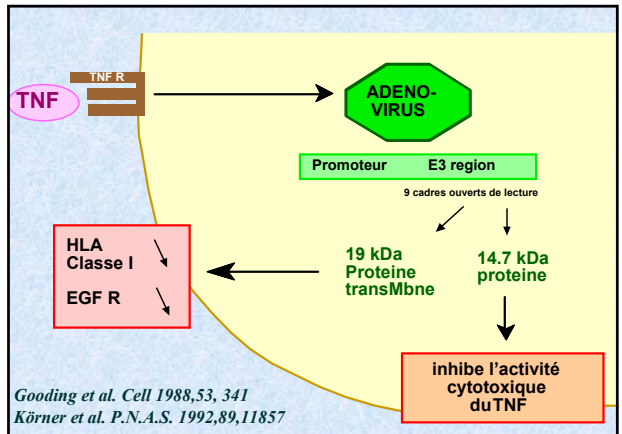
Haraguchi et al.
J.Leuk.Biol.
1992,52,469

Feline leukemia virus
Murine leukemia virus
human retrovirus HTLV-I
& HTLV-II

envelope protein p15E

→ IMMUNOSUPPRESSION

CKS-17, A SYNTHETIC PEPTIDE HOMOLOGOUS TO RETROVIRAL ENVELOPE PROTEIN DOWN-REGULATES TNFα AND IFNγ mRNA EXPRESSION



Gooding et al. Cell 1988,53, 341
Körner et al. P.N.A.S. 1992,89,11857

Strategies used by bacteria to counteract cytokines

IL-1α/β & IL-6 production by human monocytes

Mycobacterium avium-intracellulare

Michelini-Norris et al. J.Infect.Dis. 1992, 165, 702

Virulent	+
Avirulent	+++

Enteropathogenic *Escherichia coli*

Klapproth et al. Infect. Immun. 1995, 63, 2248

Encodes proteins that inhibit IL-2, IL-4, IL-5, & IFNγ production by activated T cells (but does not affect IL-1β, IL-6, IL-10, IL-12, IL-8 & RANTES)

LPS & IFNγ → Per MØ → TNF +++

+ *Y.enterocolotica* sup. ↘
+ YopB ↘

Yersinia enterocolitica

Peyer 's patches (d+6)

Beuscher et al. Infect. Immun. 1995, 63, 1270

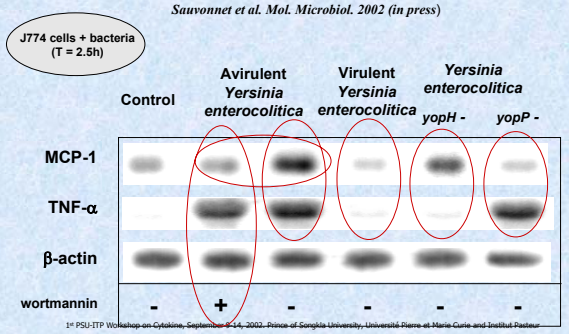
Oral infection → No TNF mRNA 5 x 10^5 CFU

Oral infection + anti-YopB → TNF mRNA : ++ 2 x 10^3 CFU

Strategies used by bacteria to counteract cytokines

YopH PREVENTS MCP-1 EXPRESSION IN MACROPHAGES THROUGH THE PHOSPHATIDYLINOSITOL 3-KINASE PATHWAY

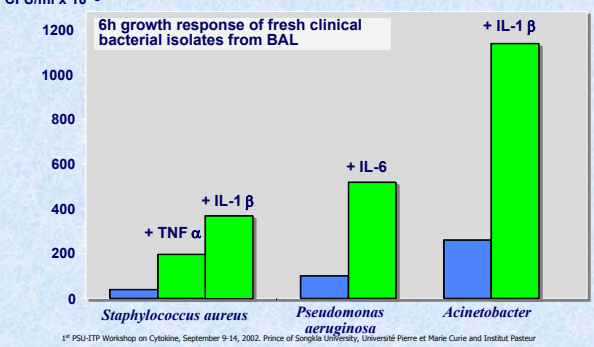
Sauvonnet et al. Mol. Microbiol. 2002 (in press)

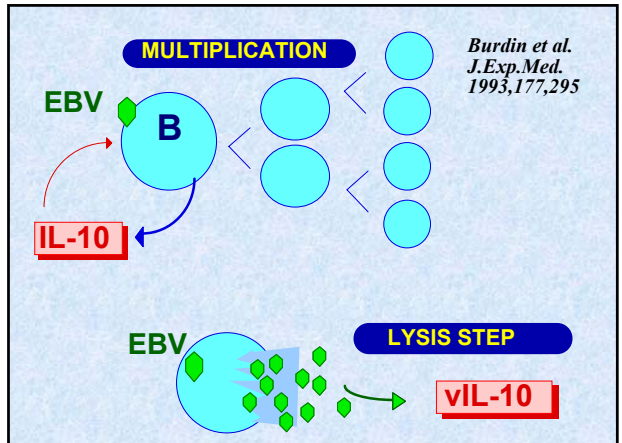


Strategies used by bacteria to counteract cytokines

CYTOKINES ENHANCE GROWTH OF BACTERIA

Meduri et al. Am.J.Respir.Crit.Care Med. 1999, 160, 961





Burdin et al.
J.Exp.Med.
1993,177,295