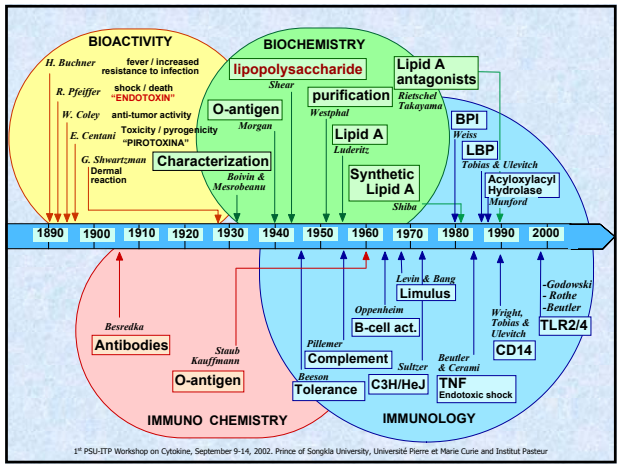
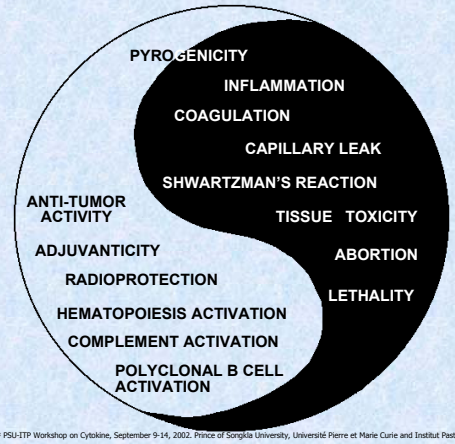
PATHOPHYSIOLOGY OF THE RESPONSE AGAINST ENDOTOXIN

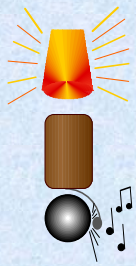
1/ BIOCHEMICAL CHARACTERISTICS
2/ MEASUREMENTS
3/ TOXICITY AND PYROGENICITY
4/ COMPLEMENT AND COAGULATION ACTIVATION
5/ INTERACTION WITH PROTEINS & LIPOPROTEINS
6/ THE LPS RECEPTOR
7/ B CELL ACTIVATION
8/ MACROPHAGE ACTIVATION
9/ ENDOTOXIN TOLERANCE



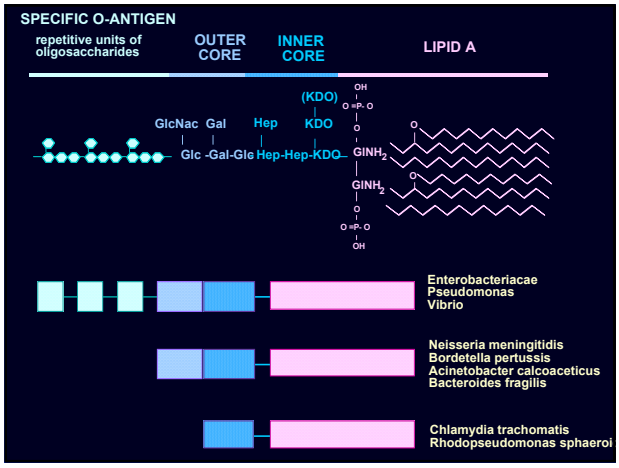


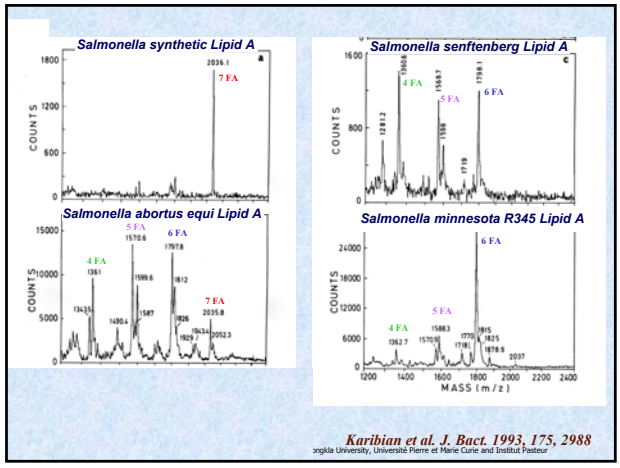
ENDOTOXIN AS AN ALARM SIGNAL OF BACTERIAL INVASION

adapted from E.K. Legrand



- Many of the body's defense system are triggered by LPS (coagulation, inflammation, immune response, acute phase response)
- Key defense cells have receptors for LPS (macrophages, neutrophils, platelets, lymphocytes)
- Some LPS-induced molecules are protective against infection (TNF, IL-1)
- Evolution has not favor LPS-non-responding animals
- LPS-binding molecules are found in all species (plants, insects, crustaceans, mammals)





Karibian et al. J. Bact. 1993, 175, 2988

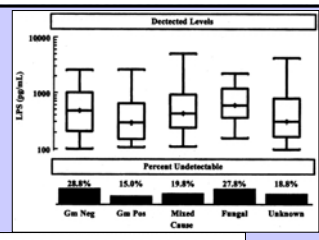
ENDOTOXIN CONTAMINATION OF ALLERGEN EXTRACTS

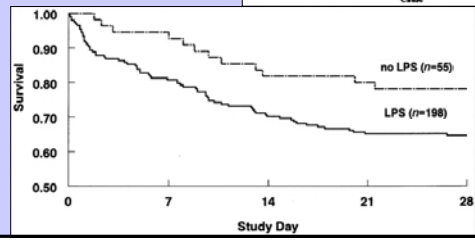
HUNT et al. Am J Respir Crit Care Med 1994, 149, 1471.

Segmental bronchoprovocation	Broncho-Alveolar Lavages t = 24 h	
	PMN	Eosino
Endotoxin-containing ALLERGEN (i.e. ⩾ 1,25 ng/ml)	68 x 10^6	0.8 x 10^6
LPS-free ALLERGEN	3.1 x 10^6	8.2 x 10^6

Circulating LPS in patients with severe sepsis

Opal et al. J. Infect. Dis. 1999, 180, 1584





ENDOTOXIN TOXICITY

	LD50	
CHICKEN EMBRYO	1 ng	
RABBIT	1 - 10 µg	[minimum pyrogenic dose : 2 - 6 ng/kg]
MOUSE	270 µg	surrenalectomized or hypophysectomized : DL50 10 000 x lower
RAT	5 mg	

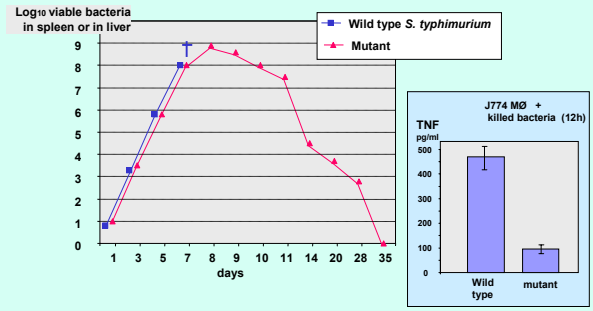
HUMAN	SEPTICEMIA (plasma LPS)	MORTALITY
maximum Tolerance: 4 ng/kg	< 1 pg/ml	0%
	1 - 4.9 pg/ml	38%
	5 - 9.9 pg/ml	67%
	10 - 14.9 pg/ml	80%
	> 15 pg/ml	100%

A LETHAL ROLE FOR LIPID A IN SALMONELLA INFECTIONS

Khan et al. Mol. Microbiol. 1998, 29, 571

Generation of a *Salmonella typhimurium* mutant lacking the waaN gene which encodes the enzyme that catalyses one of the acylation reaction --> The mutant Lipid A lacks one myristic acid.

Hexa versus penta-acyl Lipid A



ENDOTOXIN

COMPLEMENT ACTIVATION

CHEMOTAXIS
PMN ACTIVATION
MAST CELL DEGRANULATION

Via LIPID A moiety

Via POLYSACCHARIDE Moiety

Classical pathway

C1q

Alternative pathway

C3

Anaphylatoxins C5a / C3a

ENDOTOXIN

COAGULATION

DISSEMINATED INTRAVASCULAR COAGULATION

Endothelial cell

Monocyte / MØ

TISSUE FACTOR expression

Catalytic cofactor for Factor VIIa

PAF release

Platelets aggregation

PLASMINOGEN ACTIVATOR INHIBITOR

Fibrinolysis inhibition

INDUCTION OF TISSUE FACTOR

LPS — SYNERGIE → TF expression

anti-CD11/CD18	Human monocytes & mouse macrophages		*Fan et al. J.Clin.Invest.1991,87,50*
aggregated IgG	Human endothelial cell / monocyte coculture	IL-1	*Wharram et al. J. Immunol. 1991,146,1437*
Oxidized LDL	Human monocytes		*Brand et al. Arteriosc. Thromb. 1994,14,790*
Hypoxia -> PAF	Human endothelial cell and monocyte		*Herbert et al. J Cell. Physiol. 1996,169,290*
Activated Protein C	HUVEC		*Gray et al. Haemost. 1996,6,92*

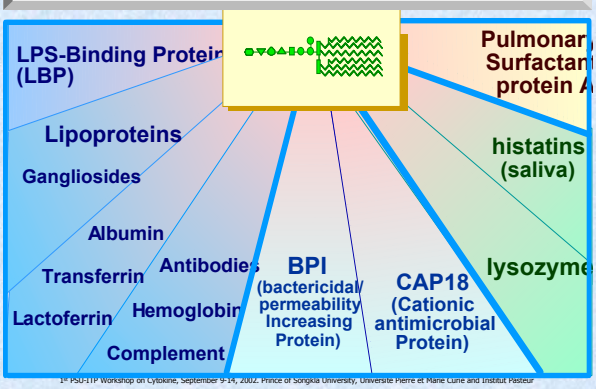
LPS-BINDING COMPOUNDS ARE FOUND NOT ONLY IN MAMMALS BUT ALSO IN INVERTEBRATES

Constitutive

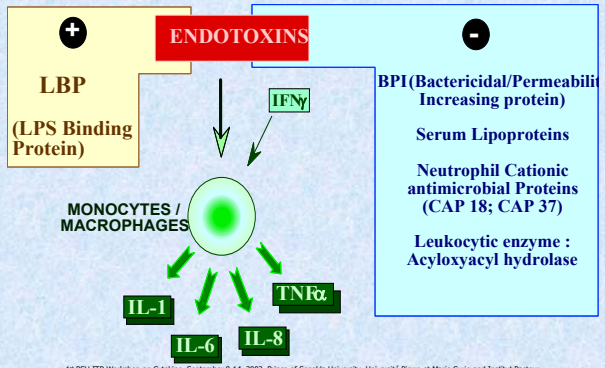
Inducible

- SILKWORM (Bombyx mori)
- COCKROACH (Periplaneta americana)
- HORSESHOE CRAB (Tachypleus tridentatus, Limulus polyphemus)
- HERMIT CRAB (Diogenes affinis)
- BROWN SHRIMP (Penaus californiensis)
- CRAYFISH (Pacifastacus leninsculus)

NATURAL LPS-BINDING COMPOUNDS

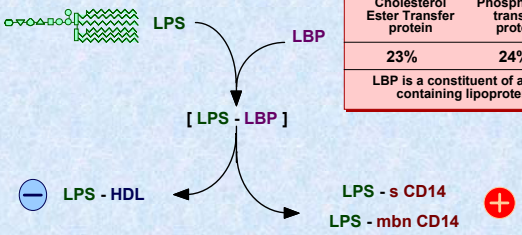


NATURAL FACTORS WHICH INTERACT WITH LPS



LPS-BINDING PROTEIN IS CARRIED ON LIPOPROTEINS AND ACTS AS A CO-FACTOR IN THE NEUTRALIZATION OF LPS

Wurfel et al. J. Exp. Med. 1994,180,1025



HOMOLOGY OF LBP with HDL-associated Protein	
Cholesterol Ester Transfer protein	Phospholipid transfer protein
23%	24%
LBP is a constituent of apoA-I containing lipoprotein	

LBP IS REQUIRED TO COMBAT A MURINE GRAM NEGATIVE BACTERIAL INFECTION

Jack et al. Nature 1997, 389, 742

(12 h)	SURVIVAL	(2 weeks)
LPS (200 ng) + GalNH (50 mg) i.p.		100 - 300 CFU *S. typhimurium* i.p.
1 / 12	Wild type	11 / 12
12 / 12	LBP -/-	2 / 12

LBP in sera (ng/ml)	
LBP + / +	114
LBP + / -	95
LBP - / -	0

Bacterial load on day 3 after *S. typhimurium* i.p.

	Peritoneum	liver	spleen
LBP + / -	10	14	8
LBP - / -	2 127	729	894

B.P.I.

55 kDa Glycoprotein

Chromosome : 20 q11.23 - q12

homology LBP : 44%

Granules and membranes of PMN

ng/ml plasma	serum
0.2 - 2.0	5 - 70
	LBP : 1 - 24 µg/ml

Kd Lipid A : $rBPI_{23}$ = 2.6 nM; rLBP = 58 nM

	Bactericidal activity $nBPI_{55}$	Bactericidal activity $rBPI_{23}$	TNFα induction $nBPI_{55}$ & $rBPI_{23}$
E. coli	++	++	↓
P. aeruginosa	-	++	↓
K. pneumoniae	-	-	↓
S. aureus	-	-	-

RELATIVE CONCENTRATIONS OF LBP AND BPI IN BODY FLUIDS

Opal et al. Lancet 1994, 344, 429

		Abscess fluids	Peritoneal fluids from peritonitis	Non infected fluids(pleural, joint, peritoneal)
BPI / LBP	median	7.6	0.24	0.01
	95% CI	2.3 - 22.1	0.18 - 0.47	0.001 - 0.04
BPI	Gram -	2040 ng/ml		
	Gram +	260 ng/ml		

BPI concentration was proportional to the quantity of white blood cells within body fluids (r=0.8)

PROTECTIVE EFFECTS OF AN N-TERMINAL FRAGMENT O BACTERICIDAL/PERMEABILITY INCREASING PROTEIN

Ammons et al. J.Infect.Dis. 1994,170,1473

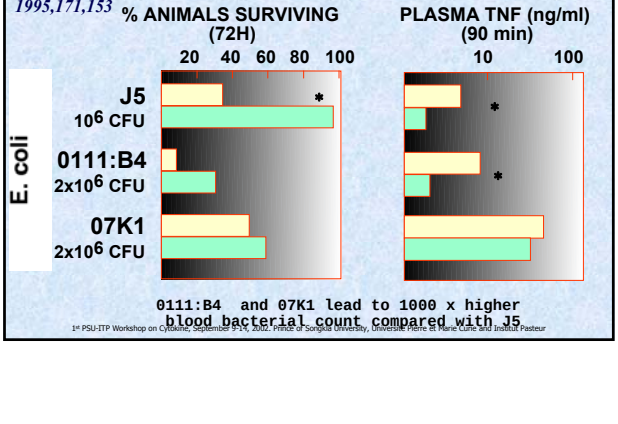
Species	*E. coli*	Timing	$rBPI_{21}$	Survival (D7)
RAT	$3x10^8$ CFU i.v. 07:K1	T_0	none	0 / 15
			10 mg/kg	5 / 15
			20 mg/kg	11 / 15
MICE	10^7 CFU i.p. 07:K1	T_0	none	0 / 15
		T_0	200 µg	15 / 15
		T+1h	"	9 / 15
		T+2h	"	2 / 15
MICE	$2.5x10^9$ CFU i.p. 0111:B4	T_0	none	4/20
			500 µg	1/20
			Cefamandole (100 mg/kg)	8/20
			BOTH	17/20

BPI CAN BE PROTECTIVE ALTHOUGH ITS EFFICIENCY DEPENDS ON THE ORGANISM

Evans et al. J.Infect.Dis. 1995,171,153

Control

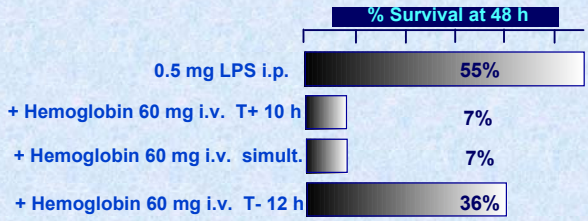
rBPI23 (5mg/kg) T0, +20, 40 & 60 min



0111:B4 and 07K1 lead to 1000 x higher blood bacterial count compared with J5

HEMOGLOBIN INCREASE MORTALITY FROM BACTERIAL ENDOTOXIN

Su et al. Infect. Immun. 1997,65,1258



ACYLOXYACYL HYDROLASE

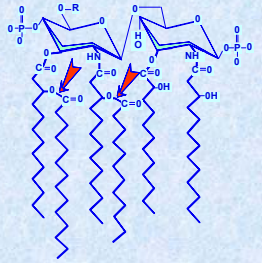
Munford et al.

52 - 60 kDa glycoprotein enzyme

2 subunits (50 & 14 kDa)

found in PMN & macrophages

found in plasma, and increased 16x upon i.v. injection



Deacylated LPS has a reduced capacity :

- Dermal Shwartzman reaction
- Tissue toxicity
- TNF production in whole blood
- Priming of PMN for superoxyde release

Deacylated LPS inhibits :

- PMN adherence to LPS-pretreated endothelial cells
- plasminogenactivator inhibitor-1, prostacyclin, and PGE2 production by LPS treated endothelial cells
- LPS induced mitogenesis

Serum Lipoproteins inhibit LPS binding to monocytes and LPS-induced cytokine production

Cavaillon et al. Infect.Immun. 1990,58,2375

	E.c. LPS (20 ng/ml)	LPS - LP	none
cell-associated IL-1α (pg/ml)	4930	220	190
released IL-1β (pg/ml)	3375	120	40
TNFα (u/ml)	710	< 5	< 5
IL-6 (u/ml)	1700	55	20

	bound cpm
[3H]-LPS N.m. (0.2µg)	5330
[3H]-LPS + LPS N.m. (2 µg)	218
[3H]-LPS - LP	92
[3H]-LPS - LP + LPS	97

In vivo Interactions Lipoprotein - LPS

Harris et al. J.Clin.Invest. 1990,86,696

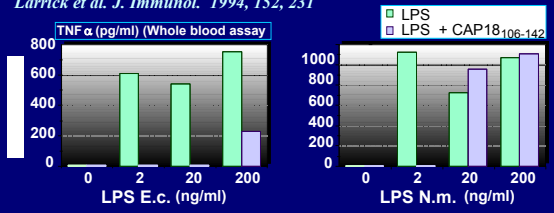
	200 ng / $GalNH_2$ treated mice	Survival
6 h incubation	LPS in saline	7 %
	LPS - VLDL (10 mg)	100 %
	LPS - LDL (0.5 mg total Cholest)	100 %
	LPS - HDL (0.05mg total cholest)	100 %
	LPS - Chylomicrons (13 mg)	100 %
No incubation	LPS - LDL (0.5 mg total Cholest)	50 %

Levine et al. P.N.A.S. 1993,90,12040

	Transgenic mice	% survival 48h LPS E.c. i.p. 30 mg/kg	TNF serum
HDL (mg/dl)	60 ± 7	12 %	130 u
	97 ± 3	19 %	
	120 ± 11	47 %	480 u

CATIONIC ANTIMICROBIAL PROTEIN -18 kDa CAP18$_{106-142}$

Larrick et al. J. Immunol. 1994, 152, 231

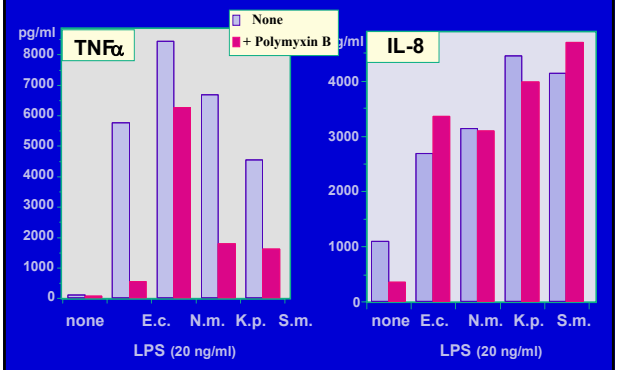


CAP18$_{106-142}$ inhibits :

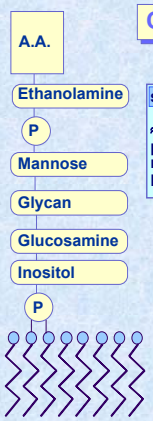
LAL coagulation
TNF, IL-1 & IL-6 production
NO induction

$GalNH_2$ C57Bl/6 mice	7 day survival
S.m. LPS(0.1µg)	1/12
LPS + CAP18$_{106-142}$ (1 µg)	9/10

EFFECT OF POLYMYXIN B ON LPS-INDUCED CYTOKINE PRODUCTION IN A WHOLE BLOOD ASSAY



CD14



sCD14

≈ 3 µg/ml plasma normal
Kd = 29x10^{-9}M

A phosphatidylinositol anchored molecule

gp 55 kDa (Proteic core = 53 kDa)

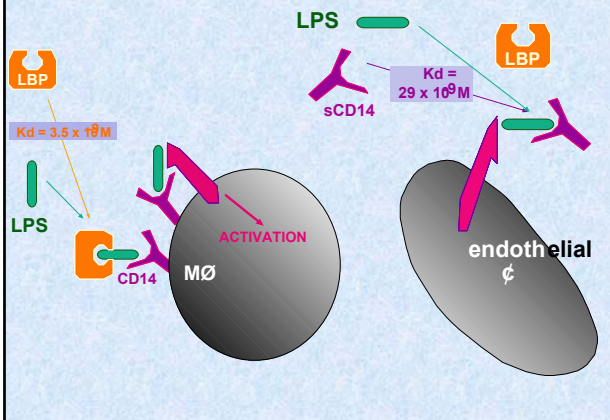
Present on cell surface of :
- monocytes / macrophages
- neutrophils
- Langerhans cells
- follicular dendritic cells

Absent or in low amounts in paroxysmal nocturnal hemoglobinuria patients

Binds : Lipoarabinomannan
uronic acid polymer
Gram + cell walls
LPS - LBP

Goyert et al. 1994

	MORTALITY	PLASMA TNF (U)
C57Bl/6 + 20 µg /gbw i.v.	100 %	2800
C57BL/6 CD14 K.O. + 20 µg /gbw i.v.	0 %	10
C57BL/6 CD14 K.O. + 200 µg /gbw i.v.	0 %	50



INVOLVEMENT OF CD14 IN STIMULATION OF CYTOKINE PRODUCTION BY POLYSACCHARIDE CONTAINING MOLECUL

Poly ß1-4-D-mannuronic acid derived from algae and Pseudomonas aeruginosa

Espevik et al. 1993
Eur. J. Immunol. 23, 255
Otterlei et al. 1993
Infect. Immun. 61, 1917

Poly-M binds to CD14

Anti-CD14 MAb blocks Poly-M-induced TNF

Poly-M inhibits LPS binding to monocytes

Staphylococcal peptidoglycans

Weideman et al. 1994
Infect. Immun. 62, 4709

Anti-CD14 MAb blocks PG-induced IL-1 & IL-6

Streptococcal Rhamnose-Glucose Polymer

Soell et al. 1995
J. Immunol. 154, 851

RGP binds to CD14

Anti-CD14 MAb blocks RGP-induced TNF

MUTATIONS IN TLR4 ABOLISH LPS RESPONSIVENESS

C3H/HeJ

Sultzer, Nature 1968, 219, 1253

LPS RESISTANT

C57Bl/10ScCr

Coutinho & Meo, Immunogenetics 1978, 7, 17

lps locus

mouse chromosome 4

Watson et al. J. Immunol. 1978, 120, 422

≡ *Tlr4*

Poltorak et al. Science 1998, 282, 2085
Quershi et al. J. Exp. Med. 1999, 189, 615

Increased sensitivity to Gram negative infections

O'Brien et al. J. Immunol. 1980, 124, 20

C3H/HeJ : codominant point mutation
C57Bl/10ScCr : gene deletion

Mutations in TLR4 with the extra-cellular domain (Asp299Gly & Thr399Ile) are associated with a reduced response to inhalated LPS inhalé in humans.

Arbour et al. Nature Genetics 2000, 25, 187

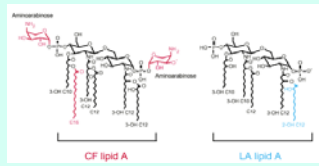
a

	NH$_2$ terminal region		Middle region	COOH terminal region
LRR :	A	B	C	TM
Human	260 aa		82 aa 248 aa	223 aa

Amino acid identity (%)

Pygmy chimp	100	98	100	100
Olive baboon	95	85	93	92
Horse	81	58	72	85
Cat	79	54	73	82
Hamster	72	45	65	82
Mouse	67	48	64	79
Rat	66	39	64	79

Hallar et al. Nature Immunol. 2002, 3, 354



Hallar et al. Nature Immunol. 2002, 3, 354

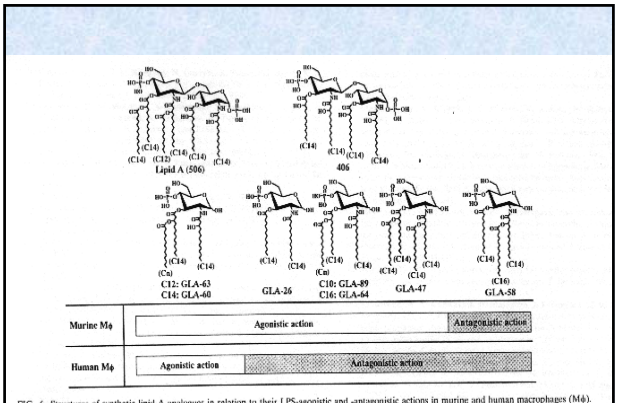
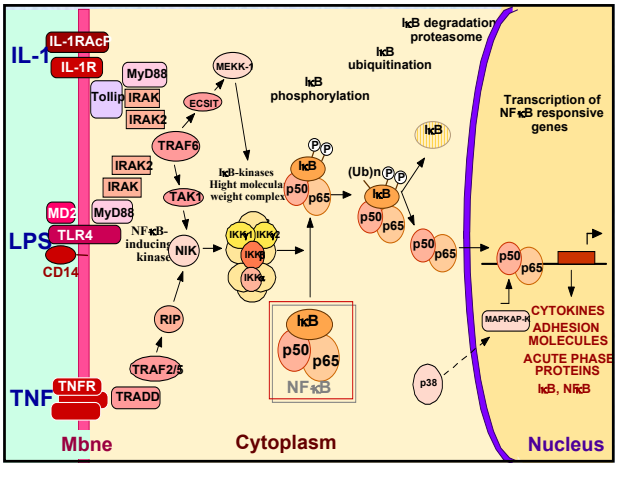
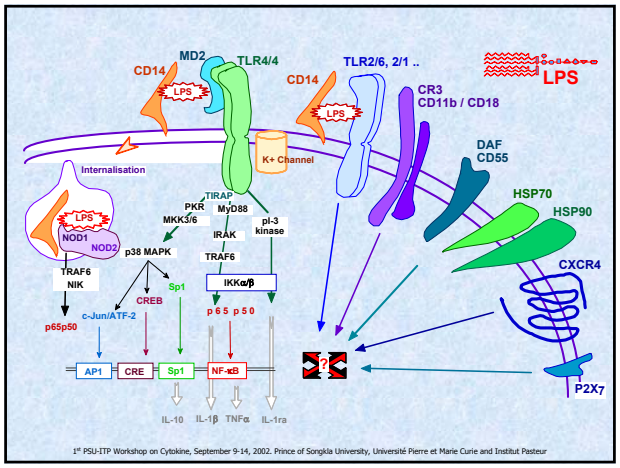


FIG. 6. Structures of synthetic lipid A analogues in relation to their LPS-agonistic and -antagonistic actions in murine and human macrophages (Mϕ).





Ogata et al. J. Exp. Med. 2000, 192, 23

anti-RP105
=> prolifération
=> protection against irradiation-induced apoptosis

LPS

RP105 MD1

RP105 -/- mice have a reduced B resp
TLR4 mice (C3H/HeJ) failed to respor

⇓

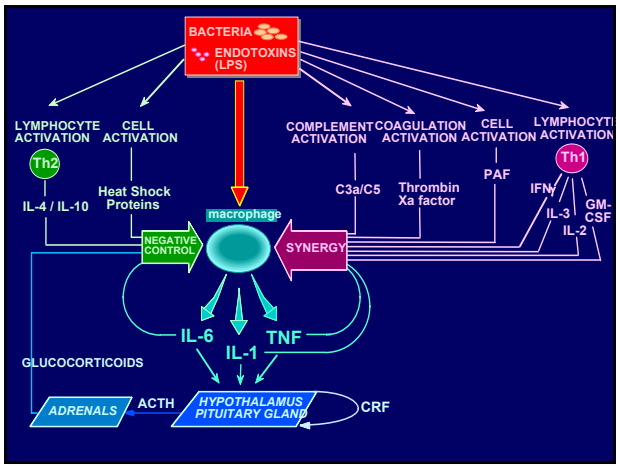
+ Cooperative role of TLR

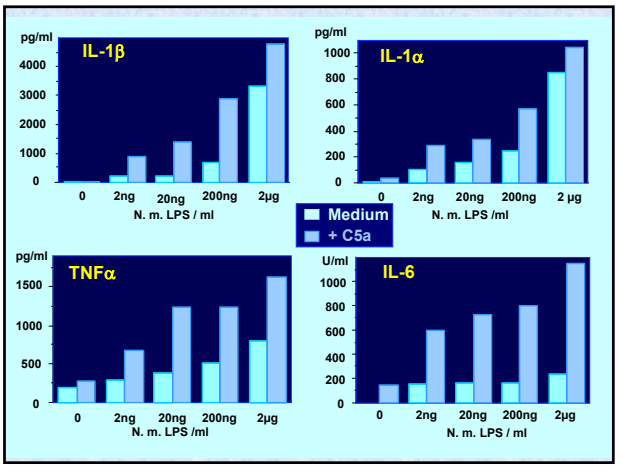
B cell

RP105 is largely restricted to mature peripheral B cells

Lyn
protein tyrosine kinase
PKCβI/II
ERK2
JNK1
p38
NFκB

Chan et al. J. Exp. Med. 1998, 18





BACTERIAL LIPOPROTEIN AND LPS ACT SYNERGISTICALLY TO INDUCE LETHAL SHOCK AND PRO-INFLAMMATORY CYTOKINES

Zhang et al. J. Immunol. 1997, 159, 4868

MORTALITY
GalNH model

	C3H/HeN
LP 9 µg/kg	25 %
LPS 0.6 µg/kg	17 %
LP + LPS	83 %

	C3H/HeJ
LP 48 µg/kg	20 %
LPS 200 µg/kg	0 %
LP + LPS	91 %
heat killed E. coli	90 %
E. coli LP - / -	50 %

TNF *in vitro* (pg/ml)

per. MØ	C3H/HeN	C3H/HeJ
LP 250 ng/ml	350	65
LPS 250 ng/ml	200	0
LP + LPS	1000	155

GalNH model
C3H/HeN
1 h

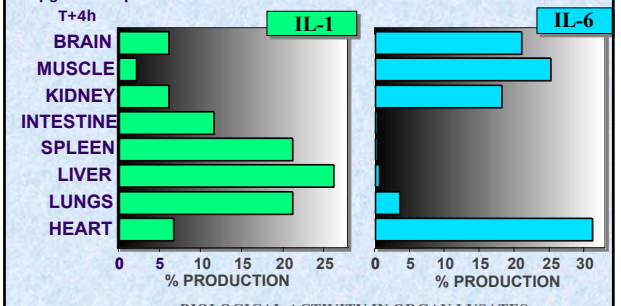
TNF *in vivo* (pg/ml)

LP 80 µg/kg	100
LPS 80 µg/kg	200
LP + LPS	470

COMPLEMENTARY ORGAN EXPRESSION OF IL-1 & IL-6 IN LPS-INJECTED MICE

10 µg LPS i.p.
T+4h

Hacham et al. Cytokine 1996,8,21



BIOLOGICAL ACTIVITY IN ORGAN LYSATES

ENDOTOXIN TOLERANCE

CLINICAL RELEVANCE

SEPSIS

SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)

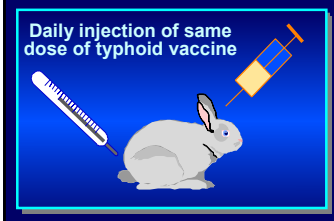
- deactivation
- adaptation
- desensitization
- anergy
- refractoriness
- reprogrammation

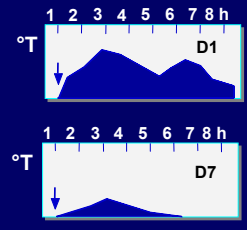
- Immunodepression
- Immunosuppression
- Immunoparalysis

ENDOTOXIN TOLERANCE

ENDOTOXIN TOLERANCE WAS FIRST DEFINED BY A REDUCED FEVER RESPONSIVENESS

Paul B. Beeson, 1946
Proc. Soc. Exp. Biol. Med. 61, 248





ENDOTOXIN TOLERANCE CAN PROTECT AGAINST A LETHAL CHALLENGE OF ENDOTOXIN

FEVER

CLINICAL RELEVANCE

ENDOTOXIN TOLERANCE IN INFECTED PATIEN

IN PATIENTS WITH PYELONEPHRITIS
McCabe 1963 J. Clin. Invest. 42, 618

IN PATIENTS CONVALESCENT FROM TYPHOID AND PARATYPHOID FEVER
Neva et al. 1950 J. Lab. Clin. Med. 35, 911

IN PATIENTS RECOVERING FROM MALARIA
Heyman et al. 1949 J.Lab.Clin.Med. 34, 1400

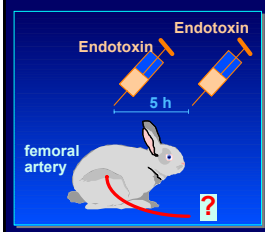
Reduced fever as compared to controls in response to endotoxin or killed bacteria

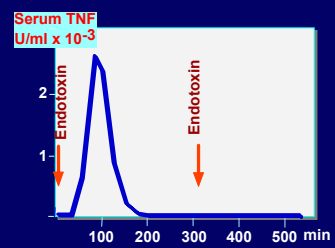
ENDOTOXIN TOLERANCE

CYTOKINES

Adaptation to bacterial LPS controls LPS-induced TNF production in rabbit macrophages

Mathison et al. 1990
J. Clin. Invest. 85, 1108

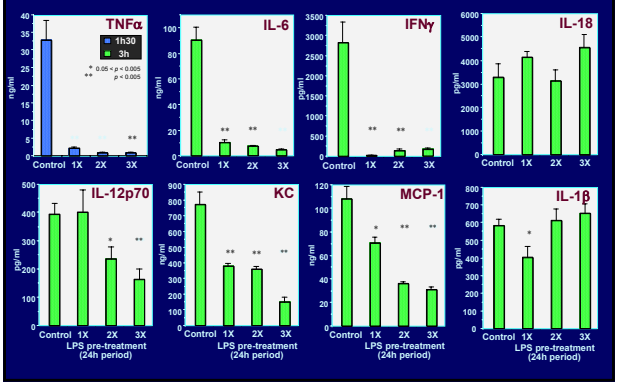




ENDOTOXIN TOLERANCE

EFFECT OF ENDOTOXIN TOLERANCE OF LPS-INDUCED CIRCULATING CYTOKINES

Rayhane et al. J. Endotoxin Res. 1999, 5, 319



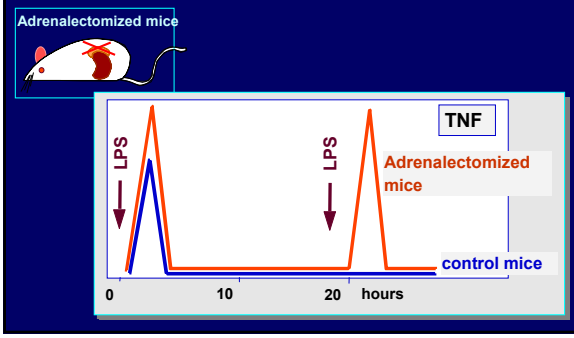
Consequences of endotoxin tolerance on various LPS-induced activities

REDUCED RESPONSIVENESS	Fever and lethality Hypotension and hypoglycaemia Procoagulant activity Induction of iNO synthase Synthesis of TXB2, TXA2, 6-keto-PGF1α Production of TNF, IL-8, IFNγ, IL-12
NO EFFECT OR ENHANCEMENT	G-CSF, GM-CSF, IL-1ra, PGE2 production Central sensitivity to LPS : Neuroendocrine activatio Corticosterone induction; Food intake Phagocytic activity Liver cell injury
CONTROVERSIALS DATA	IL-1, IL-6, IL-10 & NO productions

ENDOTOXIN TOLERANCE

Glucocorticoid-dependent and -independent mechanism involved in LPS tolerance

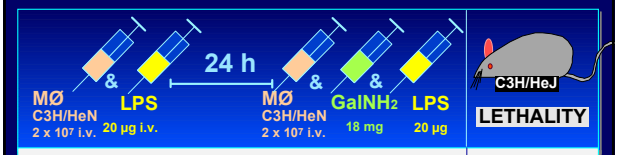
Evans & Zuckerman Eur. J. Immunol. 1991, 21, 1973



ENDOTOXIN TOLERANCE

Role of macrophages in LPS tolerance

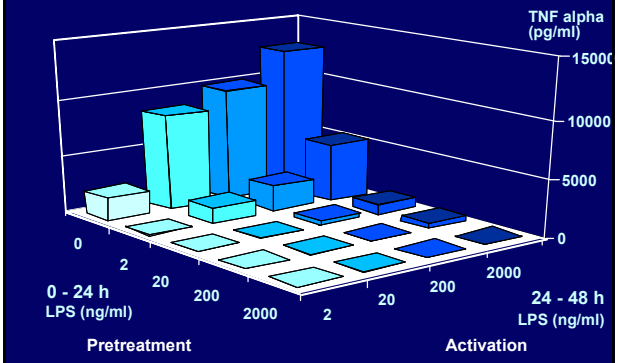
Freudenberg & Galanos Infect. Immun. 1988, 56, 1352



					LETHALITY
0	0	0	+	+	0 %
0	0	+	+	+	100 %
0	+	+	+	+	100 %
+	+	+	+	+	0 %

ENDOTOXIN TOLERANCE

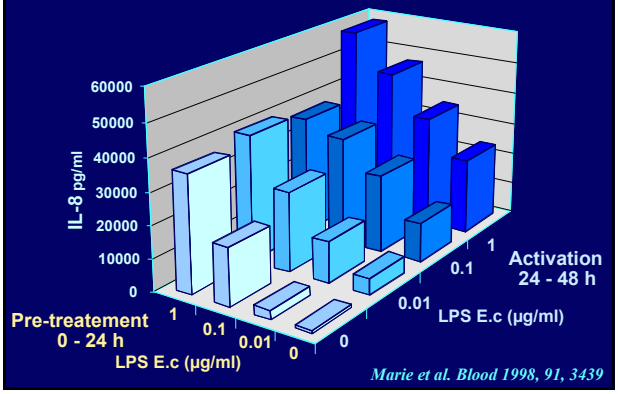
IN VITRO TOLERIZATION OF MACROPHAGES



Cavaillon et al. J. End. Res. 1994, 1, 21

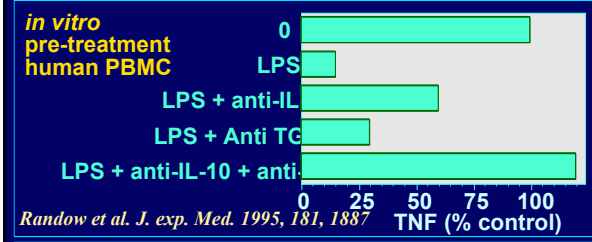
ENDOTOXIN TOLERANCE

Human neutrophils cannot be "tolerized" *in vitro*



Marie et al. Blood 1998, 91, 3439

ENDOTOXIN TOLERANCE



Randow et al. J. exp. Med. 1995, 181, 1887

in vivo
IL-10 KO mice

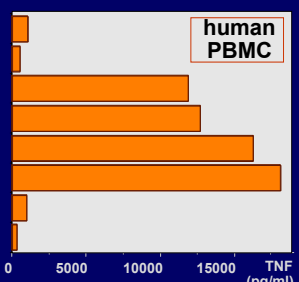
	LPS T0	LPS T24h	SURVIVAL
Wild Type	0	200 µg	0 %
	25 µg	200 µg	72 %
IL-10 - / -	0	10 µg	0 %
	1 µg	10 µg	87 %

Berg et al. J. Clin. Invest 1995, 96, 2339

ENDOTOXIN TOLERANCE

REVERSAL OF ENDOTOXIN TOLERANCE BY IFNγ & GM-CSF

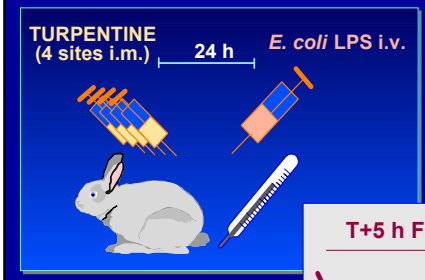
0 - 24h	24-48h	48-72h
0	0	LPS
LPS	0	LPS
0	IFNγ	LPS
LPS	IFNγ	LPS
0	GM-CSF	LPS
LPS	GM-CSF	LPS
0	IL-12	LPS
LPS	IL-12	LPS



Randow et al. 1997 J. Immunol. 158, 2911

ENDOTOXIN TOLERANCE IS NOT A LPS-SPECIFIC PHENOMENON

Greisman et al. J. Clin. Invest. 1969, 48, 613



T+5 h FEVER INDEX :

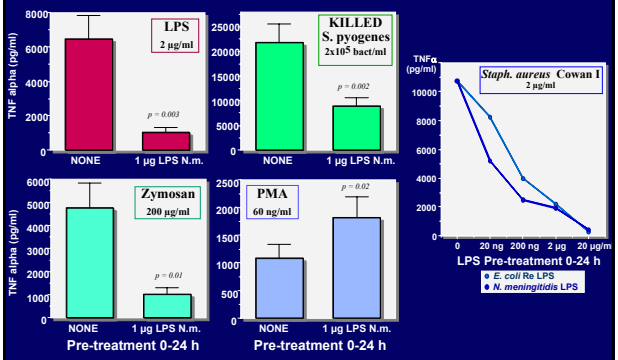
71% *versus* CONTROL

ENDOTOXIN TOLERANCE IS NOT A LPS-SPE PHENOMENON AND CAN BE INDUCED BY

- TNF
- IL-1
- Alcohol
- Complet Freund adjuvant
- Sodium arsenite, PMA
- Heat stress
- Exercice ...

ENDOTOXIN TOLERANCE

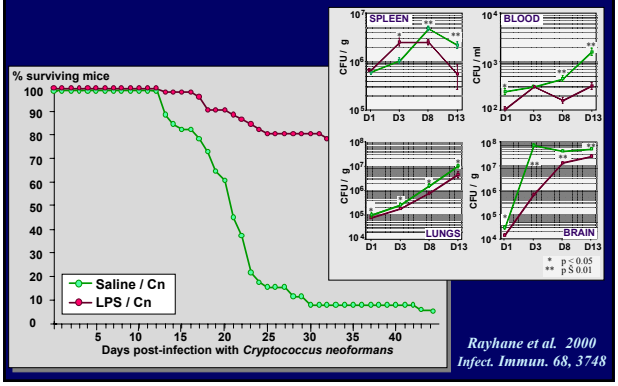
ENDOTOXIN-INDUCED TOLERANCE ON HUMAN MONOCYTES IS NOT A LPS SPECIFIC PHENOMENON



Cavaillon et al. J. End. Res. 1994, 1, 21

ENDOTOXIN TOLERANCE

Endotoxin tolerance protects against fungal infection



Rayhane et al. 2000 Infect. Immun. 68, 3748

SIGNALING

A. Cross *et al.* J. End. Res. 2002, 8, 83

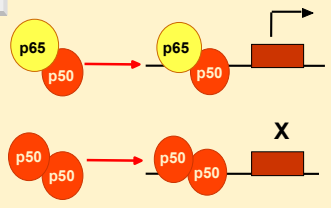
M. West *et al.* Crit. Care Med. 2002, 30(S1), S64

ENDOTOXIN TOLERANCE

Heterodimer : activator

NF- κ B

Homodimer : inhibitor



Endotoxin tolerance :

Modification of NF-κB expression

- Global decrease of NF-κB (both hetero- & homodimers) *Blackwell et col.*
- Decrease of the ratio p65p50 / p50p50 *Ziegler-Heitbrock et col.*