

KKU International Teaching Platform

UNIVERSITÉ PIERRE & MARIE CURIE
sciences & santé

Khon Kaen University

Cytokine network and techniques for detection of cytokines

Jean-Marc Cavaillon
Unit Cytokines & Inflammation

INSTITUT PASTEUR

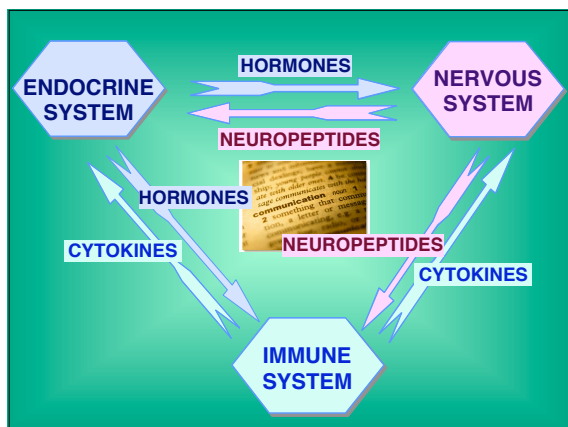
KKU International Teaching Platform

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Khon Kaen University

- 1/ DEFINITIONS
- 2/ RECEPTORS
- 3/ FUNCTIONS
- 4/ LIFE WITHOUT CYTOKINES
- 5/ PRODUCTION (homeostasis vs activation)
- 6/ THE CYTOKINE NETWORK
- 7/ INDIVIDUAL HETEROGENEITY
- 8/ PARAMETERS THAT AFFECT FUNCTIONS & PRODUCTION

INSTITUT PASTEUR



NEOLOGISM

1957 : INTERFERON

1969 : LYMPHOKINES

1974 : CYTOKINES

1979 : INTERLEUKINS

1992 : CHEMOKINES

ELSEVIER

Cytokine 28 (2004) 242–247

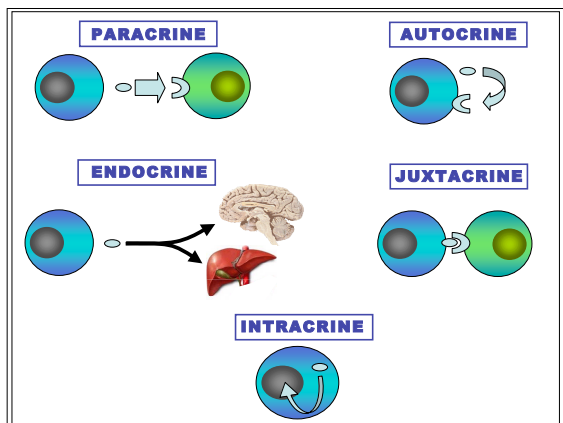
www.elsevier.com/locate/mbs/1043466

Cytokine: more than a new word, a new concept
proposed by Stanley Cohen thirty years ago

**MACROPHAGE
MIGRATION
INHIBITORY
FACTOR (MIF)**

1966 : Découverte du MIF (HSR; T-cell)

	SOURCES	TARGET	ACTIVITIES	ACTION
HORMONES	Secreted by a specialized cell	Specificity rather limited to one single type of target cell (Except insulin)	Single action	endocrine
CYTOKINES	Produced by many cell types	Numerous target cells	wide spectrum of activity Redundancy	juxtacrine paracrine autocrine endocrine



CYTOKINES	
INTERFERONS	IFN α , IFN β , IFN γ , IFN κ , IFN τ , IFN λ
INTERLEUKINS	IL-1 α , IL-1 β , IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17A-E, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33
COLONY STIMULATING FACTORS	M-CSF, G-CSF, GM-CSF
CHEMOKINES	CCL1, CCL2, CCL3.... CCL28 XCL1, XCL2 CXCL1, CXCL2, CXCL3... CXCL16 CX3CL1
TNF family	TNF, Lta, Lt β , NGF, CD27L, CD30L, CD40L, CD137L FasL, LIGHT, TRAIL, RANKL, TWEAK, APRIL, BLYS
TRANSFORMING GROWTH FACTORS	TGF α , TGF β 1,2,3
Leukemia Inhibitory Factor (LIF), Cardiotrophin, Oncostatin M, CNTF Migration inhibitory factor (MIF), Stem cell factor (c kit ligand)	

THE STORY OF DISCOVERIES	
1948 - early 70 's	BIOLOGICAL ACTIVITIES
Late 70 's - early 80 's	BIOCHEMICAL CHARACTERIZATION
Mid 80 's - mid 90 's	MOLECULAR CLONING
Late 90 's - 2000 's	RESEARCH IN DATA BANKS OF GENE HOMOLOGY AND CLONING
1984 : 10 000 L of activated Jurkat cell supernatant -> 30 mg IL-2	
1985 : 10 L of recombinant <i>E. coli</i> supernatant -> 1 g	
1985 : first clinical application of IL-2	

Biological activities

ENDOGENOUS PYROGEN 1948

OSTEOCLAST ACTIVATING FACTOR

HEMOPOIETIN-1

CATABOLIN 1972

LYMPHOCYTE ACTIVATING FACTOR

1977 - Biochemical characterization
=> 17.5 kDa ; 2 l.p. = 5.0 & 7.0

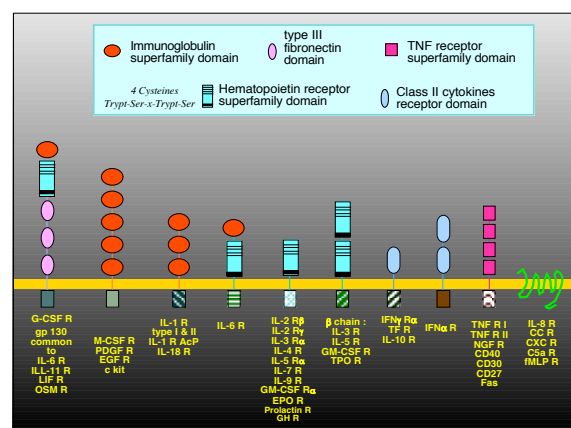
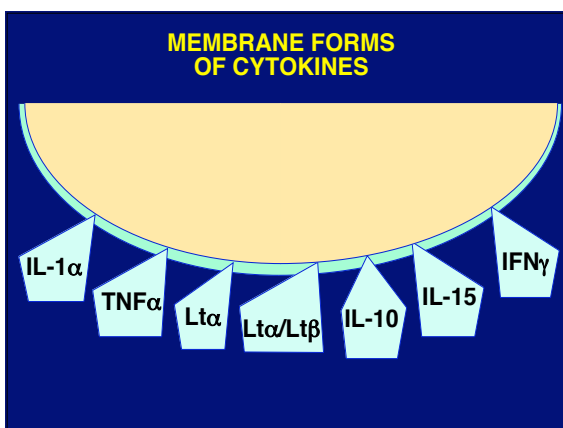
1979 - INTERLEUKIN-1

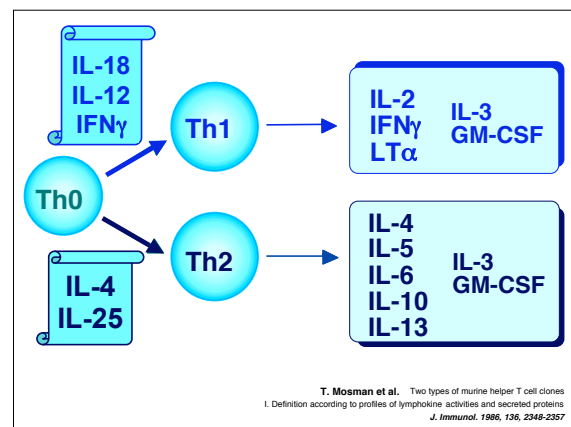
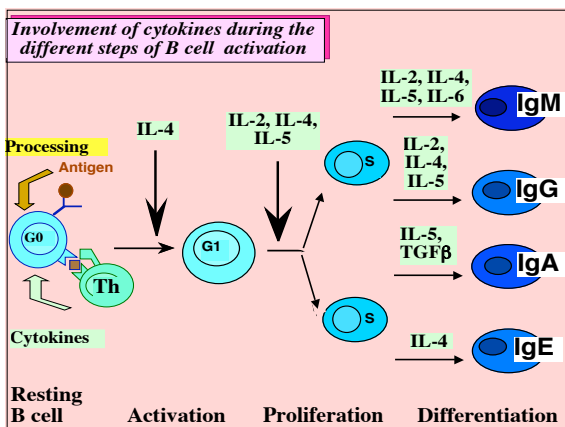
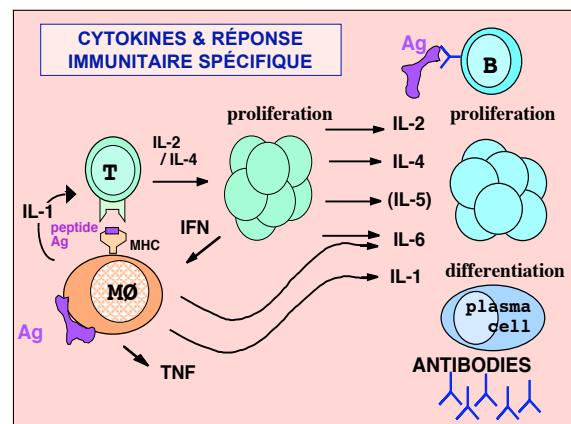
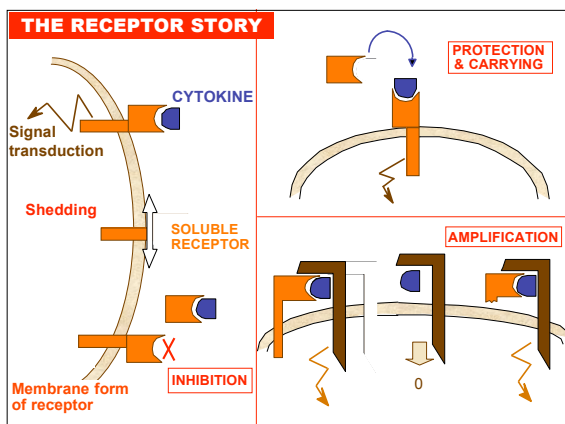
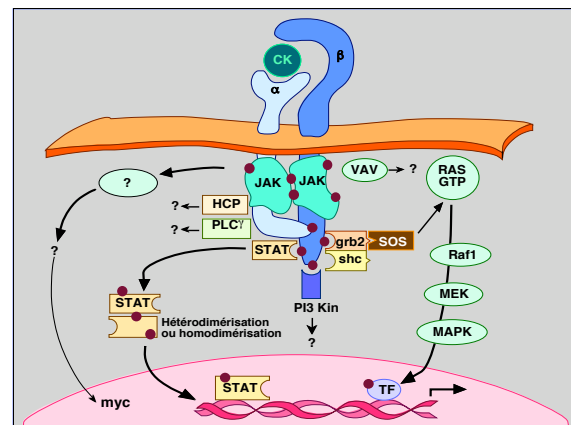
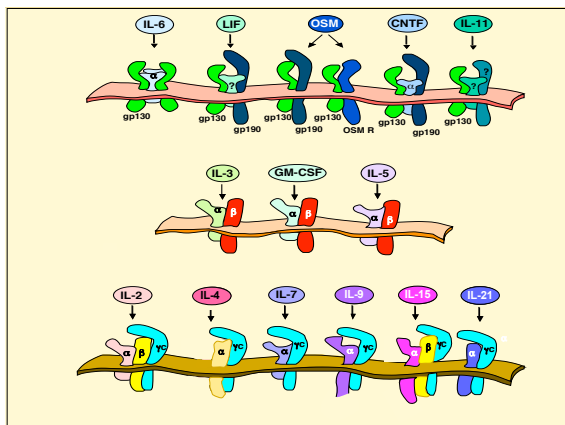
1984 / 85 - CLONING OF GENES
(6 coding exons)

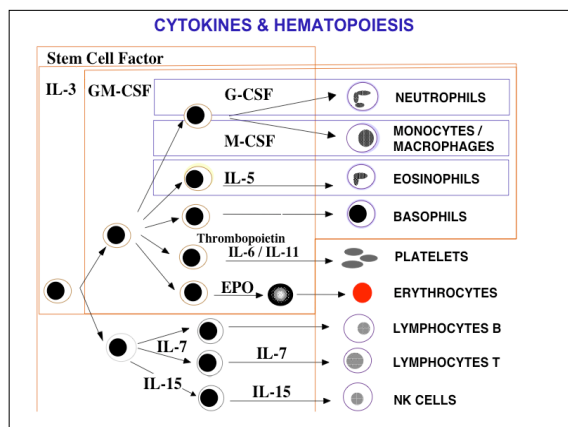
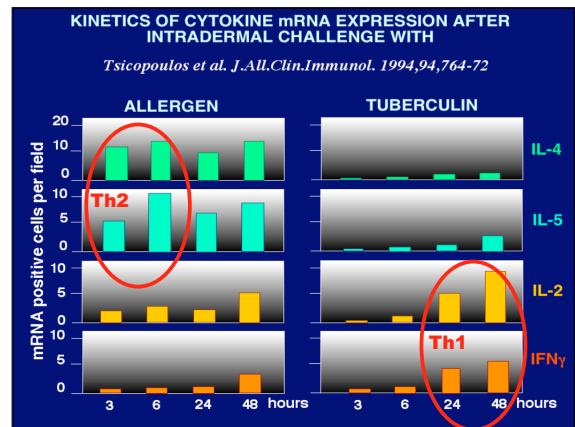
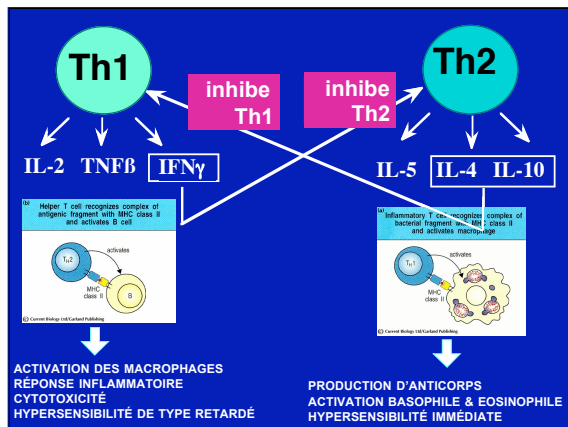
Chromosome 2 [human & mouse]

PRECURSOR FORM = 30 kDa

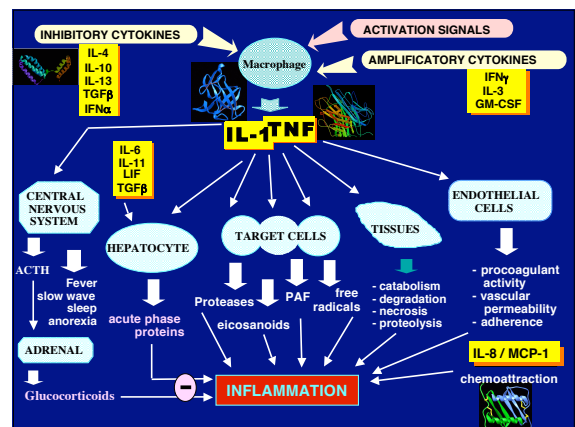
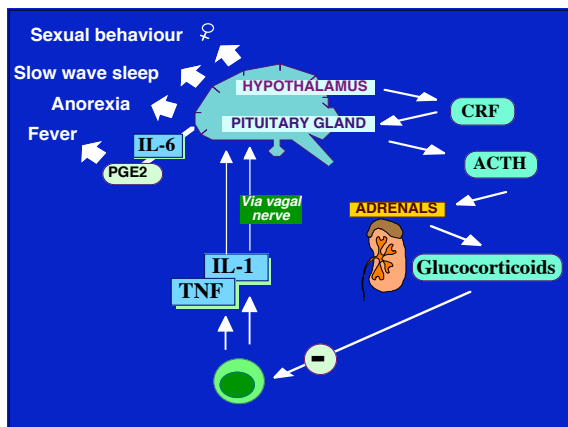
2 GENES IL-1 α & IL-1 β (26% homology)







CYTOKINES & REPRODUCTION			
SPERMATOGENESIS			OVOGENESIS
	Leydig c	Sertoli c	Germinal c
IL-1	Source & Target	Source & Target	Source & Target
IL-6	Source & Target	Source & Target	Target
TNF	Target	Target	Target
IFN	Source & Target	Source & Target	?
TGF β	Target	Source & Target	Target
GESTATION			EMBRYOGENESIS
Endometrium	IL-1, -3, -4, -6, -8, -10		CARDIOTROPHINE : heart development LYMPHOTOXINE : secondary lymphoid organs
Placenta	M-CSF, GM-CSF, LIF, TGF β , IFN α , β , τ , TNF, Lta		
implantation / parturition			SDF1 (CXCR4) : ventricular septum, neuronal c migration in cerebellum gut vasculature, B lymphopoiesis



LYMPHOTOXIN- α DEFICIENT MICE

De Togni et al. Science 1994, 264, 703

- * Absence of lymph nodes and Peyer's patches
- * Increased number of IgM+ cells in spleen and in blood stream
- * Abnormal segregation of B and T lymphocytes within the white pulp of spleen

normal % normal of CD4+ & CD8
normal cytotoxic T cell activity

	Splenocytes + Con A	
	LT α +/+	LT α -/-
TNF units	128 u	32 u

IL-6 DEFICIENT MICE

Kopf et al. Nature 1994, 368, 339

IMMUNE RESPONSE

Stomatitis Vesicular Virus : Low levels of IgG antibodies

Vaccinia Virus : Low cytotoxic T cell activity
-> Increased number of virus in the lungs

Listeria monocytogenes : High frequency of bacteria in liver and lungs

INFLAMMATORY RESPONSE

	Haptoglobin mg/ml		α -1 Acid Glycoprotein mg/ml		Serum amyloid A mRNA	
	IL-6+/+	IL-6-/-	IL-6+/+	IL-6-/-	IL-6+/+	IL-6-/-
Control	<0,1	<0,1	0,4	0,3	<0,2	<0,2
Turpentine	4,0	0,9	1,2	0,3	100	1
LPS	1,7	0,7	1,2	0,7	55	25
IL-6	0,5	0,9	0,6	0,6	12	14

GM-CSF - lacking mice

Dranoff et al. 1994
Science 264, 713

HEMATOPOIESIS :

- normal numbers of :
 - peripheral blood cells
 - bone marrow progenitors
 - tissue hematopoietic populations

NOT ESSENTIAL
AS A GROWTH
FACTOR FOR
BASAL
HEMATOPOIESIS

LUNG

- accumulation of surfactant lipids and proteins in the alveolar space
- lymphoid hyperplasia

CRITICAL ROLE IN
PULMONARY
HOMEOSTASIS

TGF β 1 KO mice

MULTIFOCAL INFLAMMATORY DISEASE

Shull et al. Nature 1992, 359, 693

Age at death

24 days

Site of inflammation
cell infiltration / necrosis

stomach / Liver / pancreas /
myocardium & endocardium /
striated muscle / serosa

blood leukocytes

increased number of monocytes
and immature neutrophils

PCR analysis of cytokines (spleen, liver, lung)

	TGF β 1	IFN γ	TNF α	MIP-1 α	IL-1 β
Normal	+	-	-	-	+
TGF β 1 KO	-	+	+	+	+

(++ in liver)

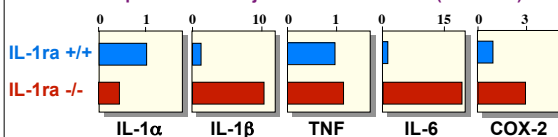
DEVELOPMENT OF CHRONIC INFLAMMATORY ARTHROPATHY IN IL-1 α DEFICIENT MICE

Horai et al. J. Exp. Med. 2000, 191, 313

INCIDENCE OF ARTHRITIS

Weeks	BALB/c		C57Bl/6
	+/+	-/-	-/-
8	0	80%	0
16	0	100%	0

mRNA expression in the joints of BALB/c mice (16 weeks)



SPONTANEOUS EXPRESSION OF CYTOKINES WITHIN CELLS

MAST CELLS

IL-1; IL-4; IL-6;
IL-13; TNF...

KERATINOCYTES

IL-1 α ; IL-1 α ; IL-8; GM-CSF

LEUKOCYTES

MIF

SPONTANEOUS EXPRESSION OF CHEMOKINES IN TISSUES

THYMUS

CCL17 (TARC) ; CCL25 (TECK)

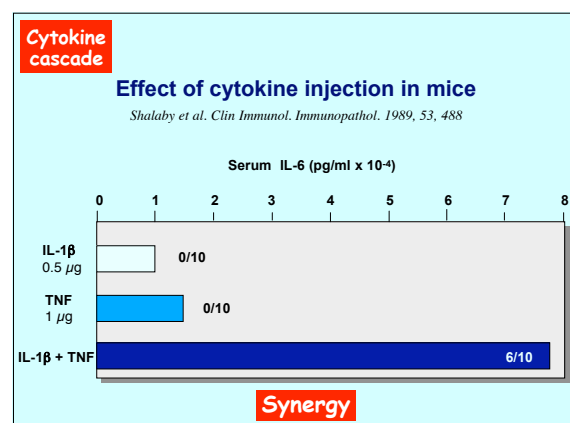
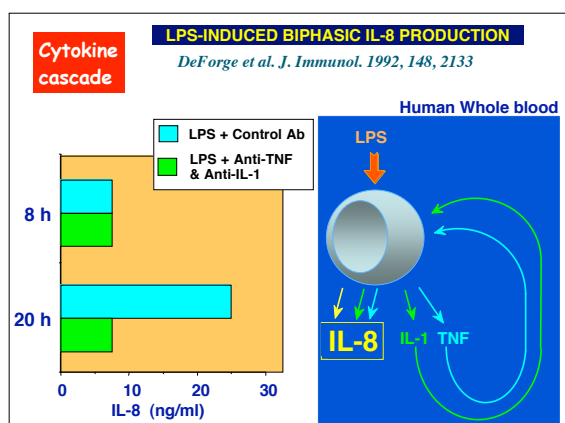
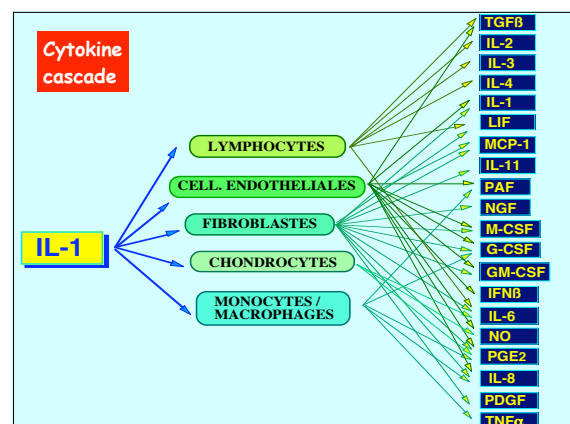
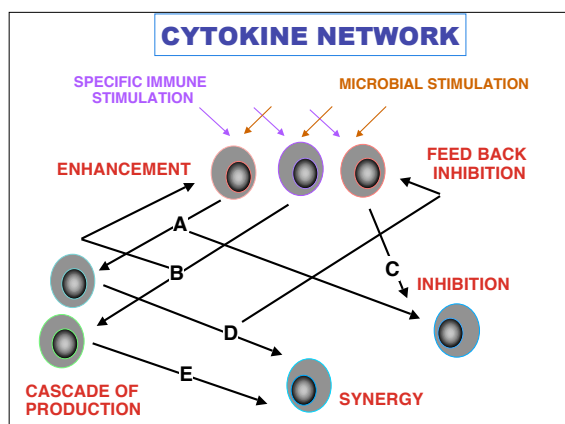
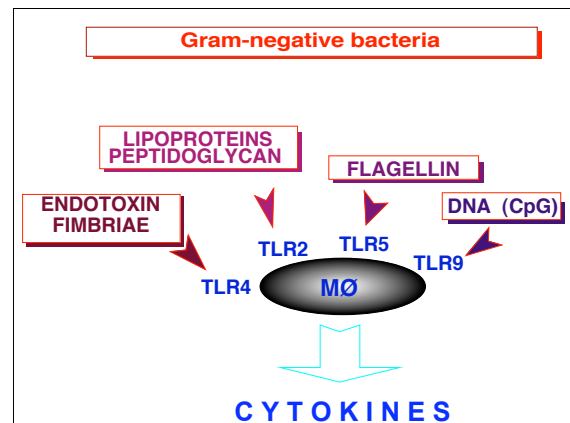
LYMPH NODES &
PEYER'S PATCHES

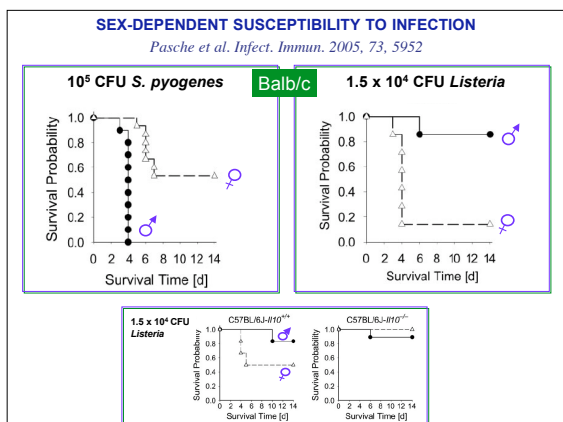
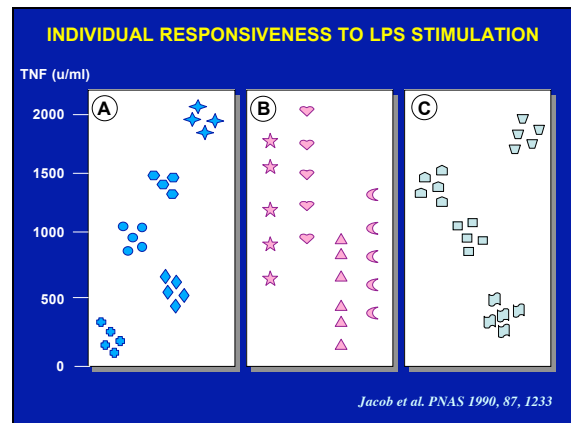
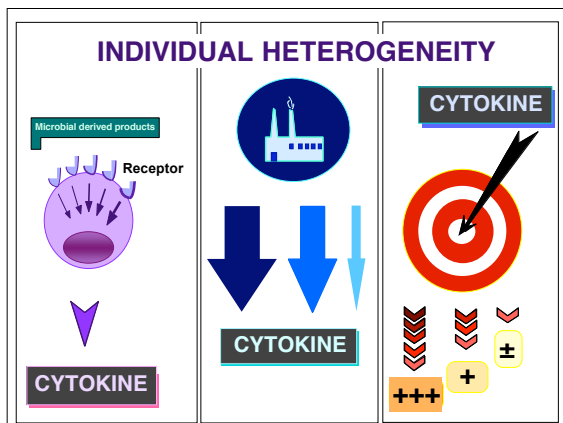
CCL21 (exodus-2)
CXCL13 (BLC)

Numerous tissues

CXCL12 (SDF-1)

SPONTANEOUS EXPRESSION OF CYTOKINES IN BIOLOGICAL FLUIDS	
SWEAT	IL-1 α ; IL-8
TEARS	IL-1 α ; IL-1 β ; IL-6 ; IL-8 ; GM-CSF ; TGF β
SALIVA	IL-2 ; IL-8 ; TNF α ; TGF β ; sTNFR
SEMINAL FLUID	IL-2 ; IL-6 ; IL-8 ; IL-10 ; IL-12 ; TNF ; sIL-2R ; sIL-6R
COLOSTRUM / MILK	IL-1 β ; IL-1 α ; IL-2 ; IL-4 ; IL-5 ; IL-6 ; IL-8 ; IL-10 ; IL-12 ; IL-13 ; IL-18 ; TNF α ; IFN γ ; TGF β ; GRO α ; MCP-1 ; RANTES ; PDGF ; sFasL ; sTNFR I & II ; sCD30
PLASMA	IL-1RA ; TGF β ; RANTES ; sTNFR



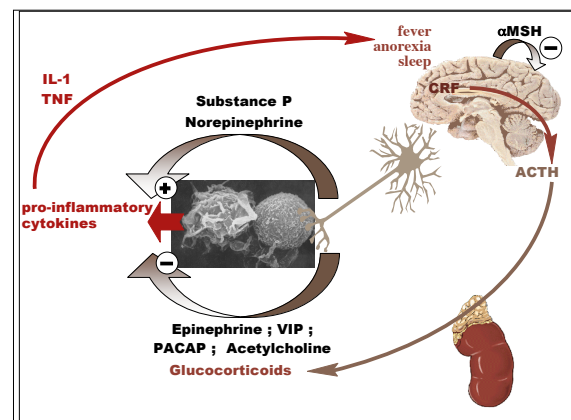
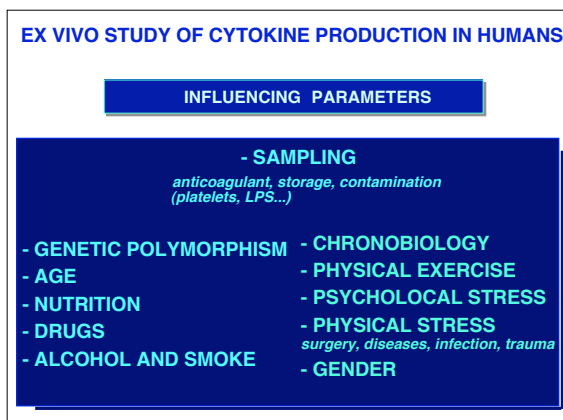


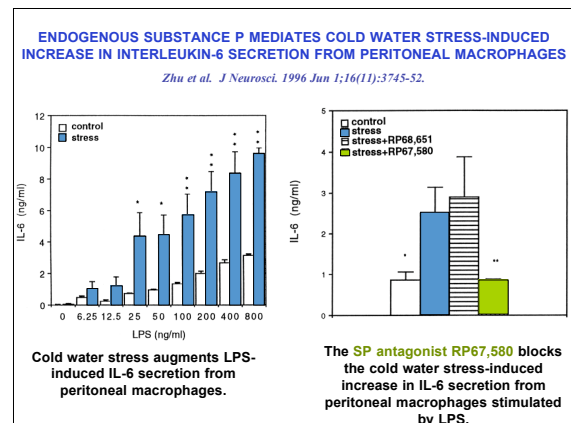
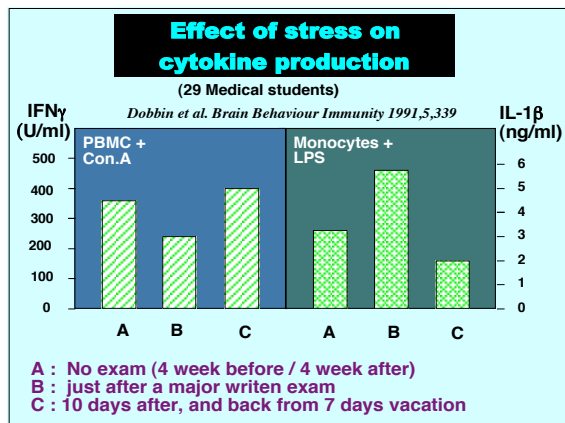
A GENOMIC POLYMORPHISM WITHIN THE TNF LOCUS INFLUENCES PLASMA TNF α CONCENTRATIONS AND OUTCOME OF PATIENTS WITH SEVERE SEPSIS

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

polymorphic site of the restriction enzyme <i>Nco</i> I	TNFB1 homozygotes	TNFB1/B2 heterozygotes	TNFB2 homozygotes
frequency	10 %	48 %	42 %
APACHE II	21	22	22
mean TNF α (pg/ml) ¹	150	250	600
Survivors	3 / 4	12 / 19	2 / 17

¹ every 6h during the first 48h, every 12h thereafter until 96h





CYTOKINE ANALYSIS IN HUMANS
WHERE ?

NATURAL BIOLOGICAL FLUIDS
- Plasma, synovial fluid, crevicular fluid, urine, cerebrospinal fluid, pleural effusion, sputum, etc...
INDUCED BIOLOGICAL FLUIDS
- broncho-alveolar or peritoneal lavages
BLOOD LEUKOCYTES
- <i>Ex vivo</i>
- After <i>in vitro</i> culture
- whole blood, isolated cells
- spontaneous or induced production
TISSUES BIOPSIES

CYTOKINE ANALYSIS IN HUMANS
HOW ?

BIOLOGICAL ASSAYS	NORTHERN
IMMUNOHISTOCHEMISTRY	mRNADOT ANALYSIS
IN SITU HYBRIDIZATION	RT PCR
ELISpot	IMMUNOBLOT
CYTOMETRY	ELISA

