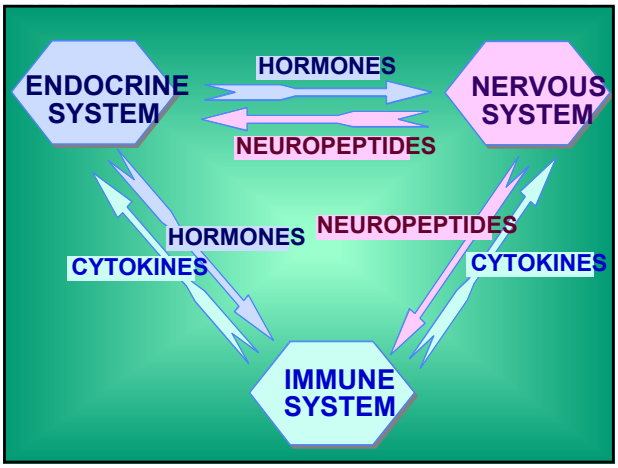
CYTOKINES

1/ DEFINITIONS
2/ RECEPTORS
3/ FUNCTIONS
- a- immune response
- b- hematopoiesis
- c- reproduction
- d- link with the central nervous system
- e- inflammation

4/ LIFE WITHOUT CYTOKINES
5/ PRODUCTION (homeostasis vs activation)
6/ THE CYTOKINE NETWORK
7/ INDIVIDUAL HETEROGENEITY
8/ PARAMETERS WHICH AFFECT FUNCTIONS AND PRODUCTION



	SOURCES	TARGET	ACTIVITIES	ACTION
HORMONES	Secreted b a specialyze 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	juxtracrine paracrine autocrine endocrine

NEOLOGISM

1957 : INTERFERON
1969 : LYMPHOKINES
1974 : CYTOKINES
1979 : INTERLEUKINS
1992 : CHEMOKINES

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 *E. coli* supernatant -> 1 g

1985 : first clinical application of IL-2

CYTOKINES

INTERFERONS IFNα, IFNβ, IFNγ, IFNω, IFNκ, IFNτ

INTERLEUKINES IL-1α, IL-1β, 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-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23

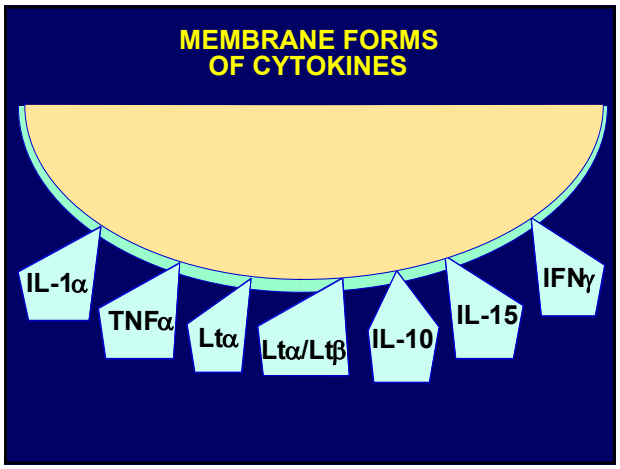
COLONY STIMULATING FACTORS M-CSF, G-CSF, GM-CSF

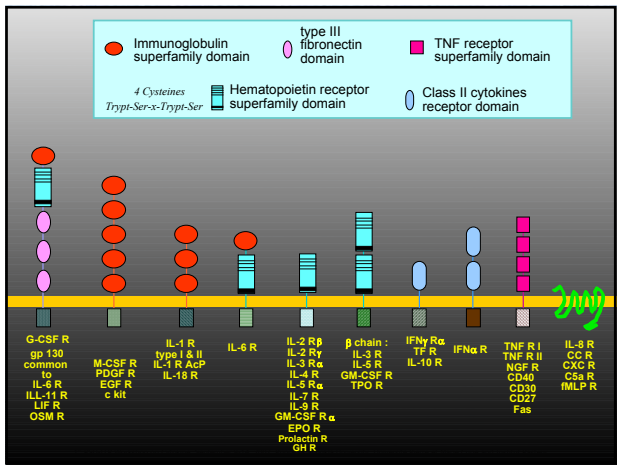
TNF family TNFα, TNFβ (LTα), LTβ, NGF, FasL, CD40L, CD30L, CD27L

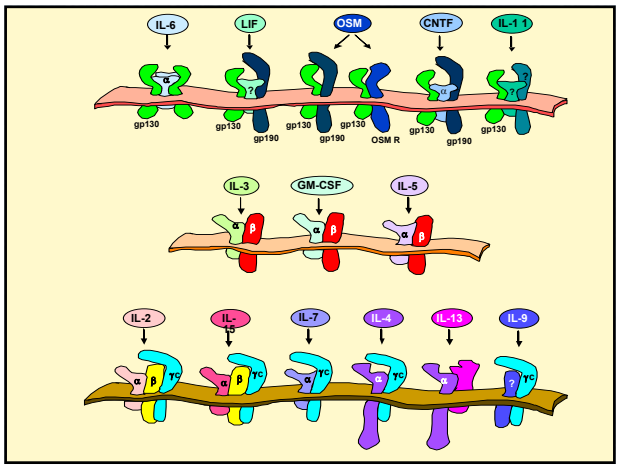
CHEMOKINES CCL1, CCL2, CCL3.... CCL27 XCL1, XCL2
CXCL1, CXCL2, CXCL3... CXCL15 CX3CL1

TRANSFORMING GROWTH FACTORS TGFα, TGFβ 1, 2, 3

Migration inhibitory factor (MIF), Stem cell factor (c kit ligand)
Leukemia Inhibitory Factor (LIF), Oncostatin M, CNTF







IL-2 R, IL-4 R, IL-7 R, IL-9 R, IL-15 R gamma chain -/-

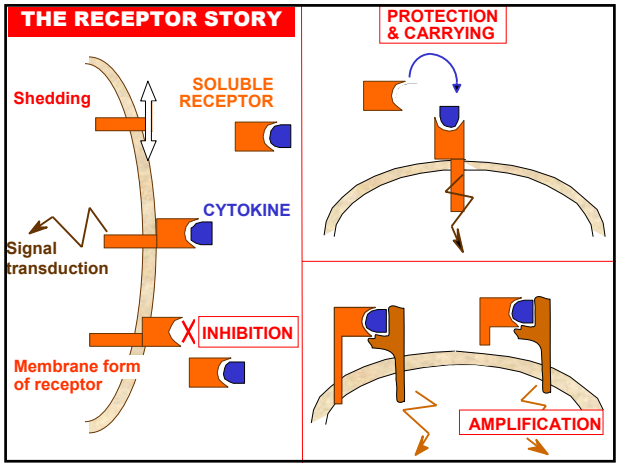
Human X-linked severe combined immunodeficiency (XSCID)

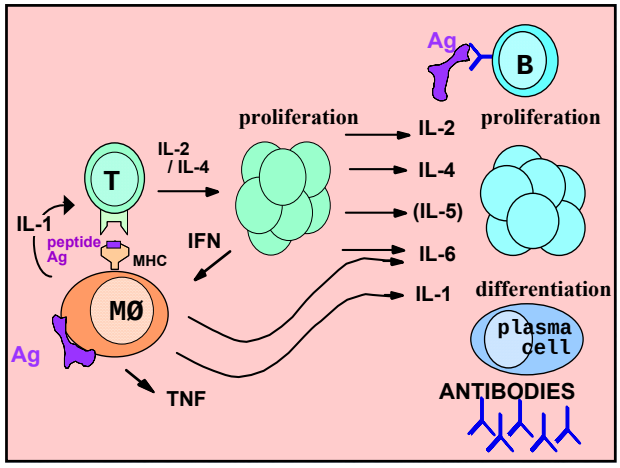
- dimished cell-mediated and humoral immunity
- death occuring in the first year of life in the absence of bone marrow transplant

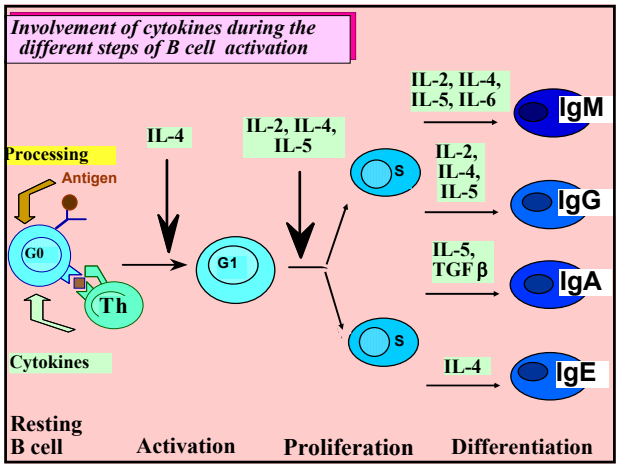
MICE LACKING THE COMMON CYTOKINE RECEPTOR GAMMA CHAIN

Cao et al. 1995
Immunity 2, 223

- Hypoplastic thymus
- reduced splenic T-cells at 3 weeks of age (increase at 4-9 weeks)
- overalldecrease in B cells
- absence of : NK cells
  - γ/δ intestinal intraepithelial lymphocytes
  - Thy1+ γ/δ dendritic epidermal T cells
  - peripheral lymph node
  - gut associated lymphoid tissue
- inflammation of cecum and colitis in γc-/Y males
- Mitogenic responses of thymocytes :
  (PMA + ionomycin) & (anti-CD3 + anti-CD28) ↓ (PMA + IL-4)↓
- Mitogenic responses of splenocytes : LPS / IL-4 + anti-μ / PMA + ionomyc
  anti-CD3 + anti-CD28↓



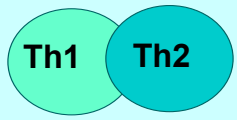


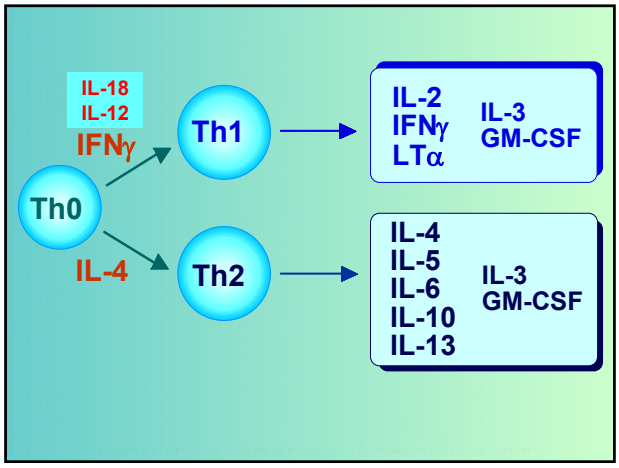


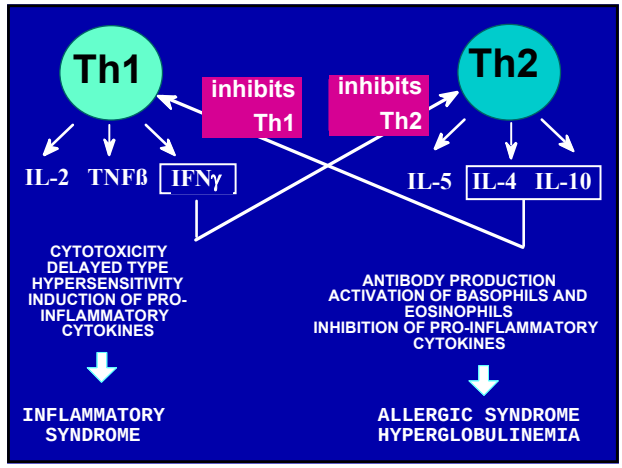
T. Mosman et al.

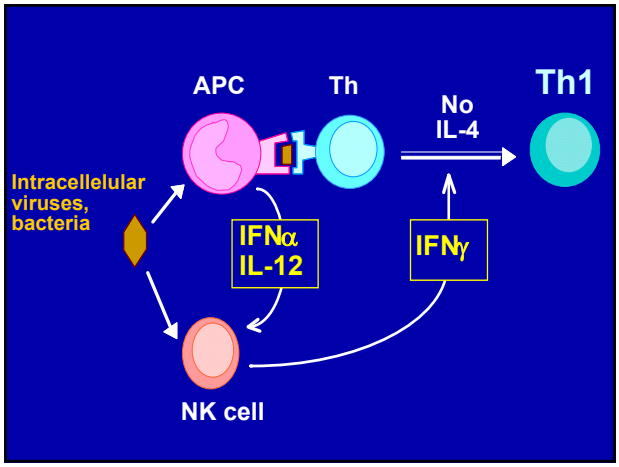
Two types of murine helper T cell clones
I. Definition according to profiles of lymphokine activities and secreted proteins

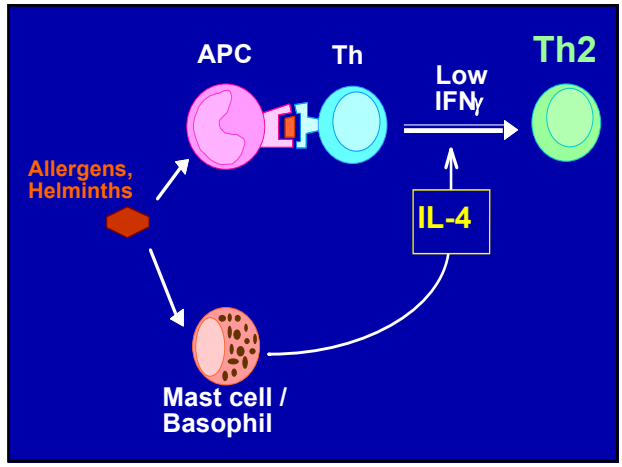
J. Immunol. 1986, 136, 2348-2357





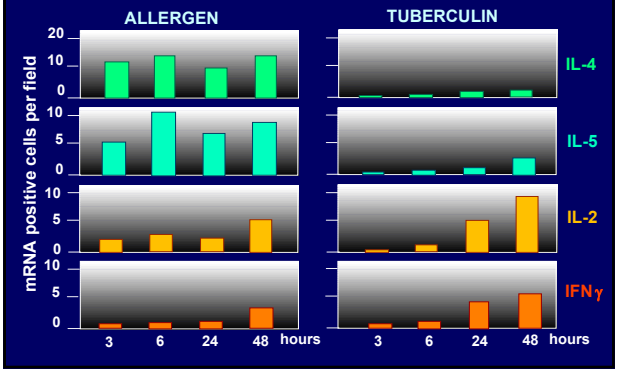






KINETICS OF CYTOKINE mRNA EXPRESSION AFTER INTRADERMAL CHALLENGE WITH

Tsicopoulos et al. J.All.Clin.Immunol. 1994,94,764-72



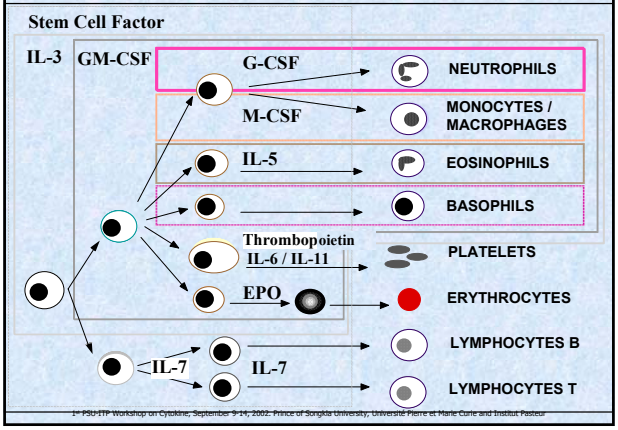
Chemokines influence adaptative immune response

CHEMOKINE RECEPTORS ON HUMAN T CELLS

Sallusto et al. J. Exp. Med. 1998, 187, 875

Naive T cells	memory/ activated T cells	Th0	Th1	Th2
CXCR4	majority : CXCR3 minority : CCR3 & CCR5	CXCR3 +++ IP-10 & MIG responsiveness CCR5 +++	CXCR3 +++	CXCR3 ± CCR3 & CCR4 +++ Eotaxin & TARC responsiveness CCR5 ++ or -

CYTOKINES & HEMATOPOIESIS



CYTOKINES & REPRODUCTION

SPERMATOGENESIS

	Leydig ¢	Sertoli ¢	Germinal ¢
IL-1	Source & Target	Source & Target	Source & Target
IL-6	Source & Target	Source & Target	Target
TNF	Target	Target	Source & Target
IFN	Source & Target	Source & Target	?
TGFß	Target	Source & Target	Target

OVOGENESIS

Follicule ovulatoire { IL-1, -1ra, -2, -6, IFNγ, TNFα

Foliculogenesis
ovulation
hormonal interactions

GESTATION

Endometrium / Placenta { IL-1, -3, -4, -6, -8, -10 M-CSF, GM-CSF, LIF, TGFβ, IFNα,β,τ, TNF, Lα

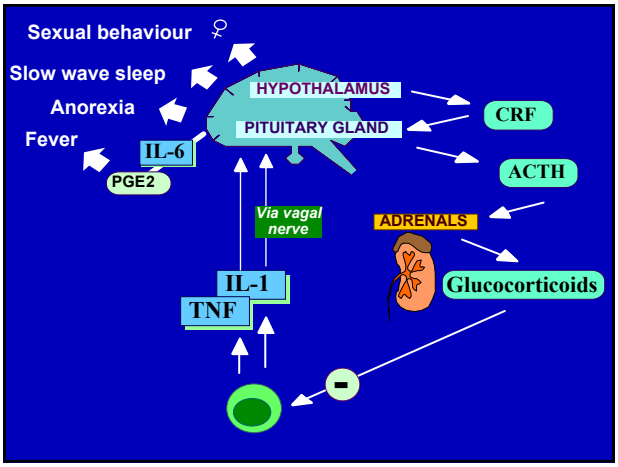
implantation / parturition

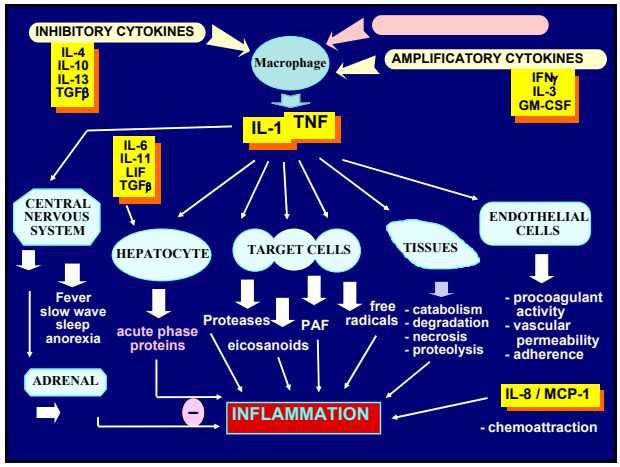
EMBRYOGENESIS

CARDIOTROPHINE : heart developme

LYMPHOTOXINE : secundary lympho organs

SDF1 (CXCR4) : ventricular septum, neuronal ¢ migration in cerebellum gu vasculature, B lymphopoiesis ....





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

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	
	LTα +/+	LTα -/-
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

CONSTITUTIVE IN VIVO CYTOKINE EXPRESSION IN THE BONE MARROW OF HEALTHY INDIVIDUALS

Cluitmans et al Blood 1995, 85, 2038

+++	+++ / ++	++ / +	+ / 0	0
M-CSF IL-1β IL-4 IL-7 IL-1ra MIP1α IGF	Steel Factor IL-6 ICE TNFβ TGFβ	TNFα	IL-10	IL-1α IL-2 IL-3 IL-5 IL-8 IL-9 IL-12 IL-13 IFNγ GM-CSF G-CSF LIF

+++, ++; + : gene expression detected after 32, 40, or 60 PCR amplification, respectively.

CYTOKINE mRNA EXPRESSION IN MOUSE IN PHYSIOLOGICAL CONDITIONS

RT-PCR detection

Organs	IL1β	IL-2	IL-3	IL-4	IL-5	IL-6	TNFα	IFNα	IFNγ
Spleen	+	+	-	++	-	+	++	++	+
Lungs	++	-	-	-	+	+	++	+	++
Liver	+	-	-	-	-	-	-	-	-
Kidney	+	-	-	-	-	-	-	+	-
Brain	+	-	-	-	-	-	-	-	-
Spinal cord	+	-	-	-	-	-	-	-	-

Kita et al. C.R.Soc.Biol.1993,187,414

SPONTANEOUS EXPRESSION OF IL-6

ELISpot	BONE MARROW	0.5 %	50% MØ
	SPLEEN	0.1 %	38 % B
	MESENTERIC LYMPH NODES	0.01 %	

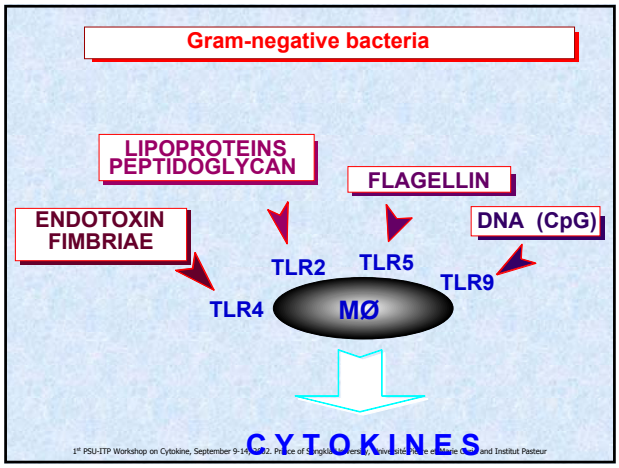
Shirai et al. J. Immunol 1993, 150, 793

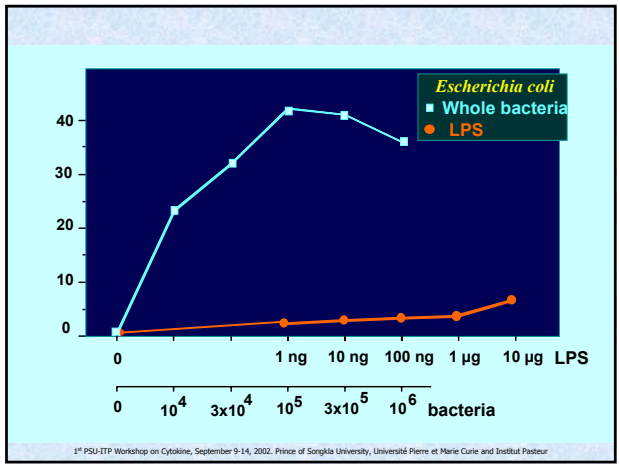
In situ hybridization
PCR
Immuncytochemistry

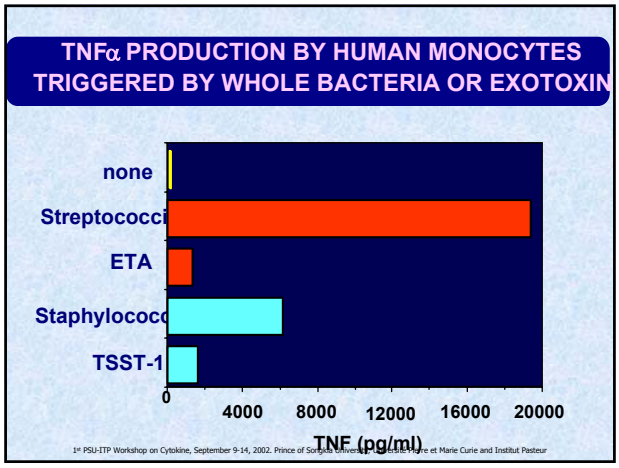
EOSINOPHILS (8% mRNA)
NEUTROPHILS

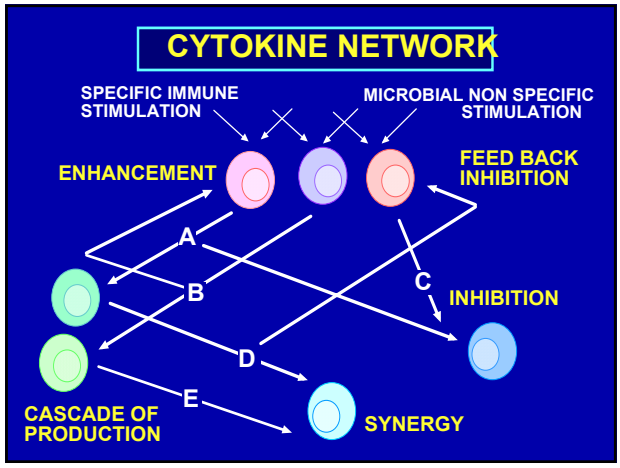
Hamid et al Blood 1992, 80, 1496
Melani et al Blood 1993, 81, 274

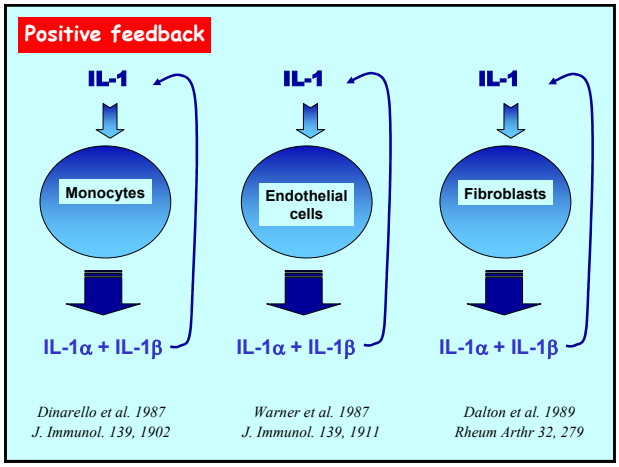
EPIDERMAL CELLS / TROPHOBLAST / HUMAN MILK /
ANTERIOR PITUITARY CELLS / SMOOTH MUSCLE CELLS /
BONE MARROW STROMAL FIBROBLASTS ...

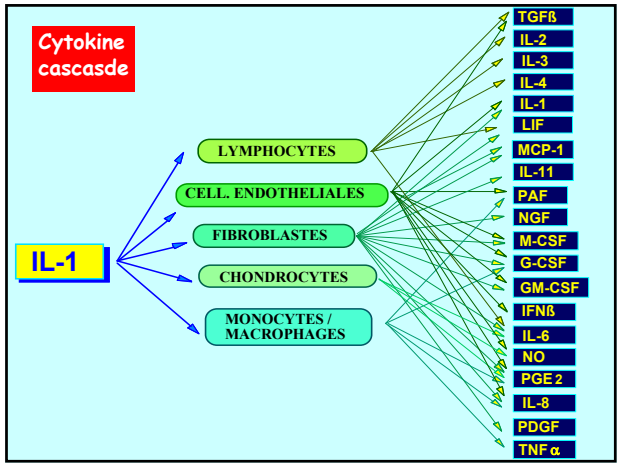

Gram-negative bacteria
LIPOPROTEINS
PEPTIDOGLYCAN
FLAGELLIN
ENDOTOXIN
FIMBRIAE
DNA (CpG)
TLR2
TLR5
TLR9
TLR4
MØ
CYTOKINES

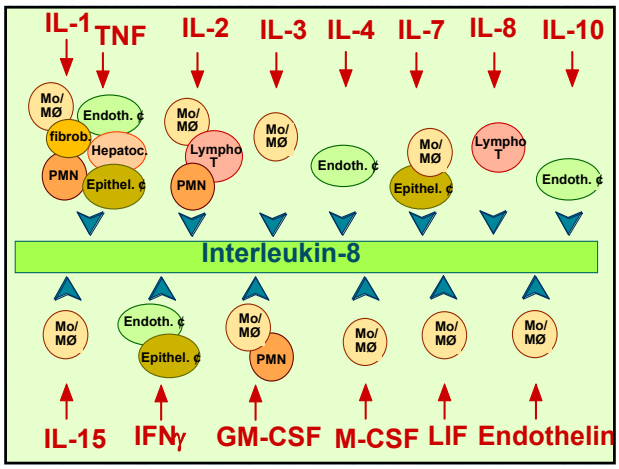

Escherichia coli
Whole bacteria
LPS
40
30
20
10
0
0
1 ng
10 ng
100 ng
1 µg
10 µg
LPS
0
10^4
3x10^4
10^5
3x10^5
10^6
bacteria

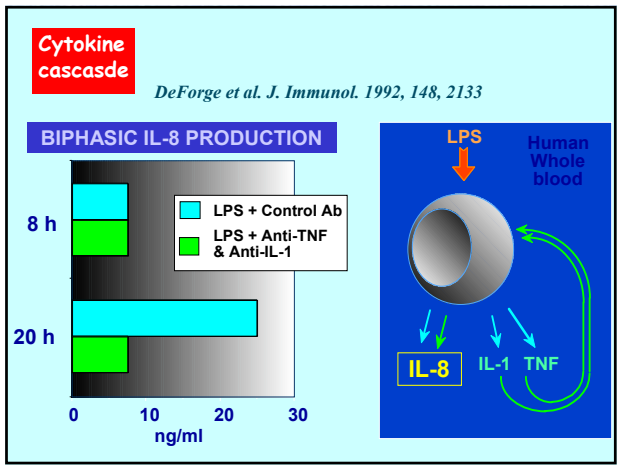

TNFα PRODUCTION BY HUMAN MONOCYTES
TRIGGERED BY WHOLE BACTERIA OR EXOTOXIN
none
Streptococci
ETA
Staphylococ
TSST-1
0
4000
8000
12000
16000
20000
TNF (pg/ml)

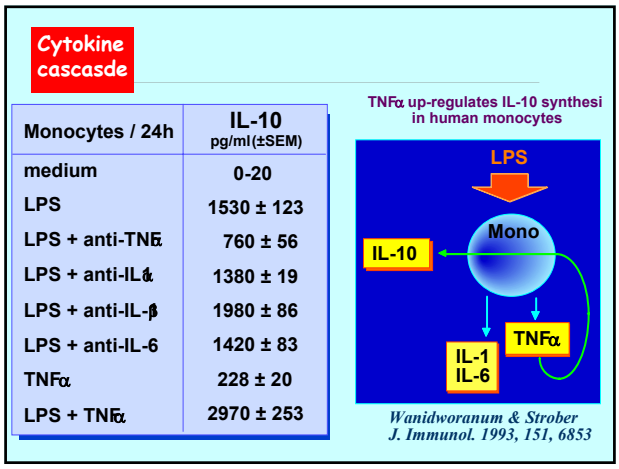

CYTOKINE NETWORK
SPECIFIC IMMUNE
STIMULATION
MICROBIAL NON SPECIFIC
STIMULATION
ENHANCEMENT
FEED BACK
INHIBITION
A
B
C
D
E
INHIBITION
CASCADE OF
PRODUCTION
SYNERGY

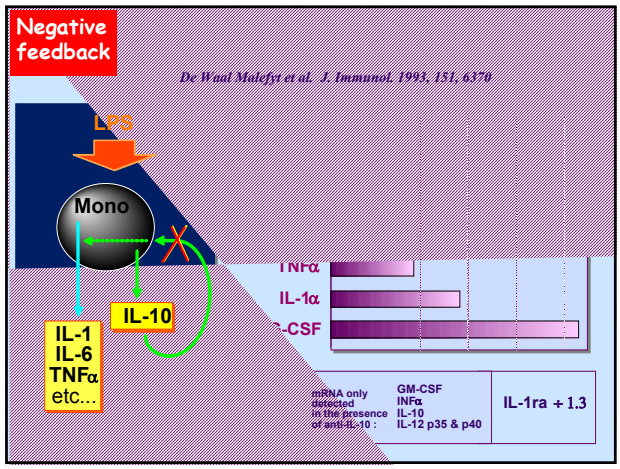

Positive feedback
IL-1
IL-1
IL-1
Monocytes
Endothelial
cells
Fibroblasts
IL-1α + IL-1β
IL-1α + IL-1β
IL-1α + IL-1β
Dinarello et al. 1987
J. Immunol. 139, 1902
Warner et al. 1987
J. Immunol. 139, 1911
Dalton et al. 1989
Rheum Arthr 32, 279

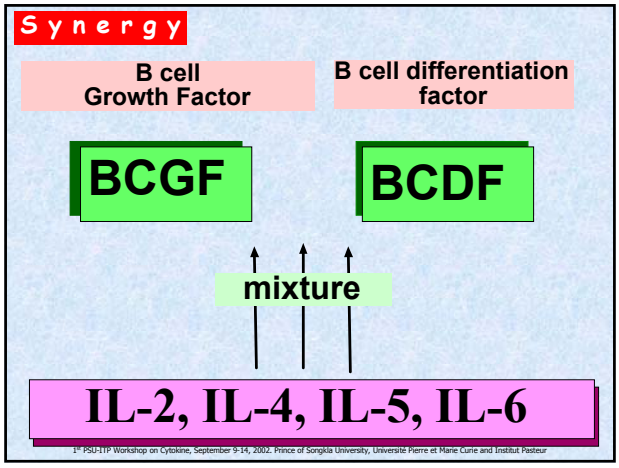

Cytokine
cascasde
IL-1
LYMPHOCYTES
CELL. ENDOTHELIALES
FIBROBLASTES
CHONDROCYTES
MONOCYTES /
MACROPHAGES
TGFß
IL-2
IL-3
IL-4
IL-1
LIF
MCP-1
IL-11
PAF
NGF
M-CSF
G-CSF
GM-CSF
IFNß
IL-6
NO
PGE2
IL-8
PDGF
TNFα

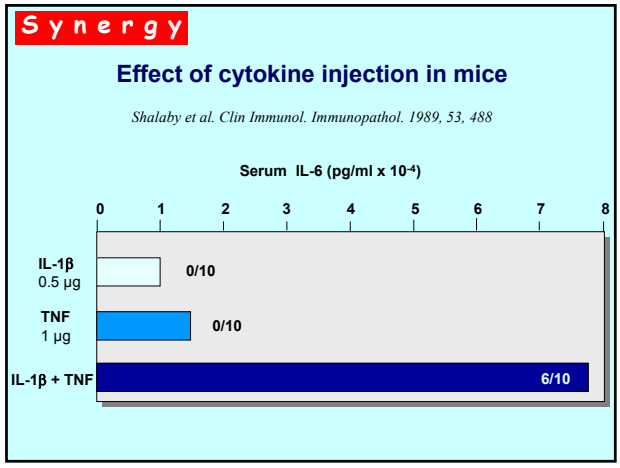

IL-1
TNF
IL-2
IL-3
IL-4
IL-7
IL-8
IL-10
Mo/ MØ
Endoth. ¢
fibrob.
Hepatoc.
PMN
Epithel. ¢
Lympho T
Interleukin-8
IL-15
IFNγ
GM-CSF
M-CSF
LIF
Endothelin


Cytokine cascasde
DeForge et al. J. Immunol. 1992, 148, 2133
BIPHASIC IL-8 PRODUCTION
8 h
20 h
LPS + Control Ab
LPS + Anti-TNF & Anti-IL-1
0
10
20
30
ng/ml
LPS
Human Whole blood
IL-8
IL-1
TNF


Cytokine cascasde
Monocytes / 24h
IL-10 pg/ml(±SEM)
medium 0-20
LPS 1530 ± 123
LPS + anti-TNFα 760 ± 56
LPS + anti-IL1α 1380 ± 19
LPS + anti-IL-β 1980 ± 86
LPS + anti-IL-6 1420 ± 83
TNFα 228 ± 20
LPS + TNFα 2970 ± 253
TNFα up-regulates IL-10 synthesi in human monocytes
LPS
Mono
IL-10
IL-1
IL-6
TNFα
Wanidworanum & Strober J. Immunol. 1993, 151, 6853


Negative feedback
De Waal Malefyt et al. J. Immunol. 1993, 151, 6370
LPS
Mono
IL-10
IL-1
IL-6
TNFα
etc...
TNFα
IL-1α
-CSF
mRNA only detected in the presence of anti-IL-10 :
GM-CSF
INFα
IL-10
IL-12 p35 & p40
IL-1ra + 1.3


Synergy
B cell Growth Factor
B cell differentiation factor
BCGF
BCDF
mixture
IL-2, IL-4, IL-5, IL-6
1st PSU-ITP Workshop on Cytokine, September 9-14, 2002. Prince of Songkla University, Université Pierre et Marie Curie and Institut Pasteur


Synergy
Effect of cytokine injection in mice
Shalaby et al. Clin Immunol. Immunopathol. 1989, 53, 488
Serum IL-6 (pg/ml x 10-4)
0
1
2
3
4
5
6
7
8
IL-1β 0.5 µg
0/10
TNF 1 µg
0/10
IL-1β + TNF
6/10

Synergy

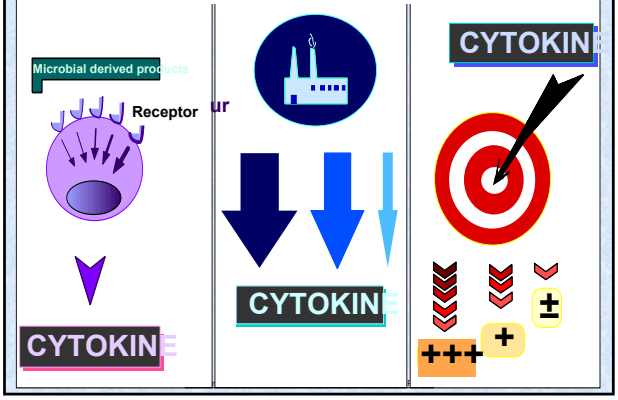
EVIDENCE FOR IFN γ AS A MEDIATOR OF THE LETHALITY OF ENDOTOXIN & TNF α

Doherty et al. J.Immunol. 1992,149,1666

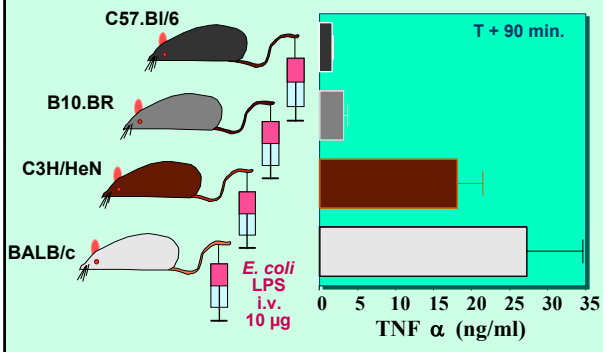
TNF α 300 µg/kg	*0 % lethality*
IFN γ 50,000 U	*0 % lethality*
TNF α + IFN γ	*100 % lethality*

1 to 4 LD	TNF α 100 (1400 µg/kg)
ANTI-IFN γ	*Protection*

INDIVIDUAL HETEROGENEITY

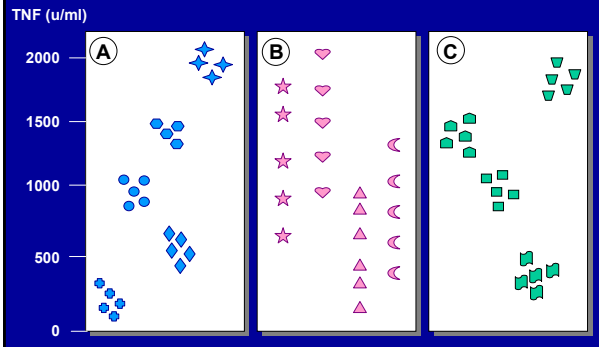


GENETIC CONTROL OF IN VIVO TNF PRODUCTION IN MICE



Yamakawa et al. 1996
Clin. Immunol. Immunopath. 79, 256

INDIVIDUAL RESPONSIVENESS TO LPS STIMULATION



Jacob et al. PNAS 1990, 87, 1233

CORRELATION BETWEEN TNF & MHC GENES AND SECRETION OF TNF α

Pociot et al. Eur.J.Immunol. 1993,23,224

TNFB genotype (NcoI restriction fragment length polymorphism)		LPS-stimulated TNF α secretion
TNFB1/TNFB1		2.4 ± 0.3
TNFB1/TNFB2		3.1 ± 0.2
TNFB2/TNFB2		3.3 ± 0.2
TNFa microsatellite (13 alleles)		
Highest	a2 (n=46)	3.4 ± 0.2
lowest	a6 (n=21)	2.4 ± 0.3
DR subtypes (DR1-w8)		
Highest	DR3	3.8 ± 0.4
lowest	DR5	1.2 ± 0.4

INTER-SUBJECT VARIATION AND RELATIONSHIP TO AN IL1-Ra GENE POLYMORPHISM

Danis et al. 1995
Clin. Exp. Immunol. 99, 303

86 bp variable repeat polymorphism in intron 2 of IL-1ra gene	Frequency
Allele A1 (410 bp)	0.73
Allele A2 (240 bp)	0.25
Allele A4 (325 bp)	

GM-CSF stimulated production by monocy

Genotype	IL-1ra secreted	IL-1ra cell-associated	IL-1α secreted	IL-1α cell-associated
A1 A1 or A1 A4	++	++	±	++++
A2 A2 or A2 A1	++++	++	±	++

POLYMORPHISM IN THE INTERLEUKIN-10 GENE PROMOTER

Turner et al. Eur. J. Immunogen. 1997, 24, 1

Identification of three single base pair substitutions (-1082, -81

	IL-10 1A + (A at position -1082)	IL-10 1A - (G at position -108
FREQUENCY (n = 238)	49%	51%
IL-10 PRODUCTION (ConA) (n = 37)	1297 ± 101 pg/ml	1720 ± 184 pg/ml
	p = 0.035	

	Allele frequencies (%) TNF2 homozygotes	TNF2 heterozygotes
mild malaria (n=332)	1.8	26.8
severe malaria anaemia (n=111)	1.8	31.5
cerebral malaria (1-10 yrs)		
all cases (n=376)	4.5	26.6
deaths & sequelae (n=99)	8.1	25.3

TNF POLYMORPHISM IN TRAUMA PATIENT

Flach et al. Cytokine 1999, 11, 173

Allotype	Non septic (n = 30)	Septic Complications (n = 10)
TNFB1	10%	10%
TNFB2	30%	90%
TNFB1/TNFB2	60%	0%

IN VITRO IL-6 & TNF PRODUCTION IS HIGHER IN THE COMPLICATION GF

CYTOKINE GENE POLYMORPHISMS AND DISEASES

RHEUMATOID ARTHRITIS	→ Non coding region of IFNγ gene
CROHN'S DISEASE	→ IL-10
SUDDEN INFANT DEATH SYNDROME	→ IL-10
MULTIPLE SCLEROSIS	→ microsatellite allele of TNF gene
ALZHEIMER	→ IL-1α
SCHIZOPHRENIA	→ Interleukin-1 gene complex
SINGLE VESSEL CORONARY DISEASE	→ IL-1ra
INFECTIOUS NEPHROPATHY	→ low CXCR1 expression
HIV RESISTANCE	→ ² CCR5

GENETICALLY DISTINCT ENDOTHELIAL CELL LINES DERIVED FROM HUMAN UMBILICAL VEIN (n = 35)

Bender et al. P.N.A.S. 1994,91,3994

		HIGH RESPONDER	INTERMEDIATE RESPONDER	LOW RESPONDER
IL-1 ACTIVATION	ELAM-1	+++	++	±
	ICAM-1	+++	++	±
	VCAM-1	+++	++	±
	NFκB	+++	+++	+++
		46%	40%	14%

EX VIVO STUDY OF CYTOKINE PRODUCTION IN HUMANS

INFLUENCING PARAMETERS

- SAMPLING

anticoagulant, storage, contamination (platelets, LPS...)

- GENETIC POLYMORPHISM
- AGE
- NUTRITION
- DRUGS
- ALCOHOL AND SMOKE
- CHRONOBIOLOGY
- PHYSICAL EXERCISE
- PSYCHOLOCAL STRESS
- PHYSICAL STRESS *surgery, diseases, infection, trauma*
- GENDER

CYTOKINE ANALYSIS IN HUMAN
WHERE ?

NATURAL BIOLOGICAL FLUIDS

- Plasma, synovial fluid, crevicular fluid, urine, cerebrospinla fluid, pleural effusion, sputum, etc...)

INDUCED BIOLOGICAL FLUIDS

- broncho-alveolar or peritonéal lavages

BLOOD LEUKOCYTES

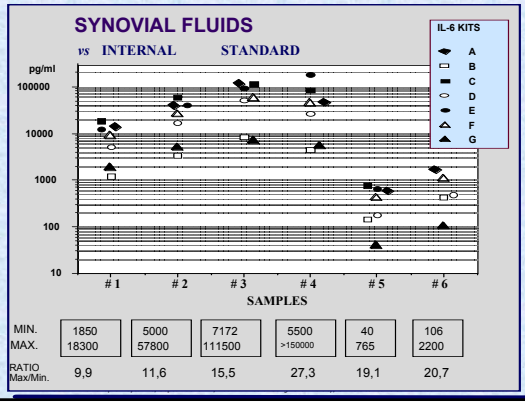
- *Ex vivo*
- After *in vitro* culture
  - whole blood, isolated cells
  - spontaneous or induced production

TISSUES BIOPSIES

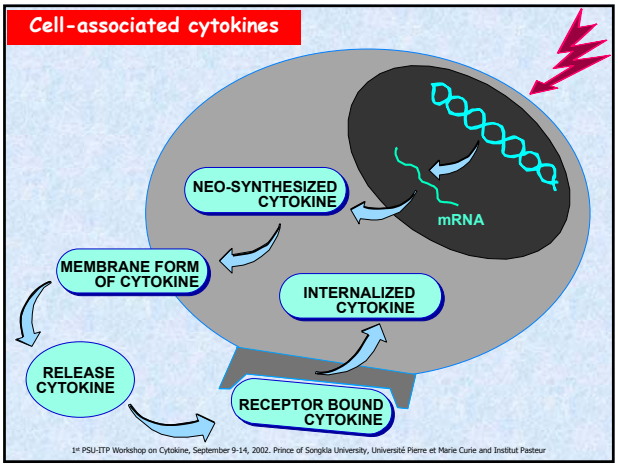
CYTOKINE ANALYSIS IN HUMAI
HOW?

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

CYTOKINE MEASUREMENT BY ELISA



	# 1	# 2	# 3	# 4	# 5	# 6
MIN.	1850	5000	7172	5500	40	106
MAX.	18300	57800	111500	>150000	765	2200
RATIO Max/Min.	9,9	11,6	15,5	27,3	19,1	20,7



Circulating cytokines : the tip of the iceberg ?

Cavaillon et al. Circ. Shock 1992, 38, 145