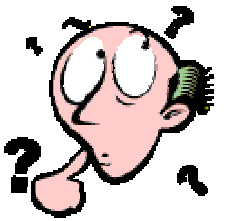
MEDIZINISCHE UNIVERSITÄT WIEN

Warum ist ein effektives Immunmonitoring notwendig?

? Existiert ein effektives Immunmonitoring ?



Andreas Spittler
Chirurgische Forschung, Wien

Ernährung 2004
Jahrestagung der DGEM, AKE, GESKES

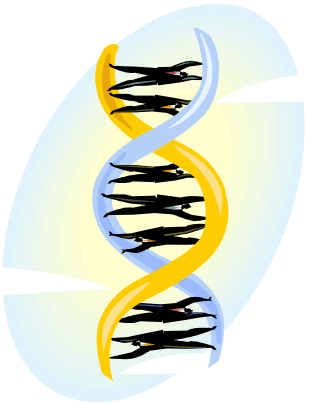
München, 6.-8. Mai 2004
Klinikum Großhadern

Ziel des immunologischen Monitorings

- ✓ Identifikation von Patienten mit hohem Risiko
- ✓ Bestimmung des Schweregrades und des Verlaufs der Sepsis mit raschen und genauen Methoden
- ✓ Durchführung einer adäquaten Therapie
- ✓ Reduktion der intensivmedizinischen Kosten

Mortalitätsrate von ICU Patienten

SIRS	24%
Sepsis	40%
Septic Shock	64%



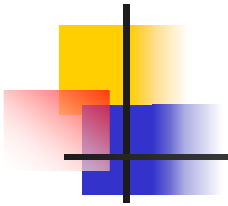
Gender differences in sepsis: genetically determined?

Schroder J. et al. *Shock* 2000; 14:307

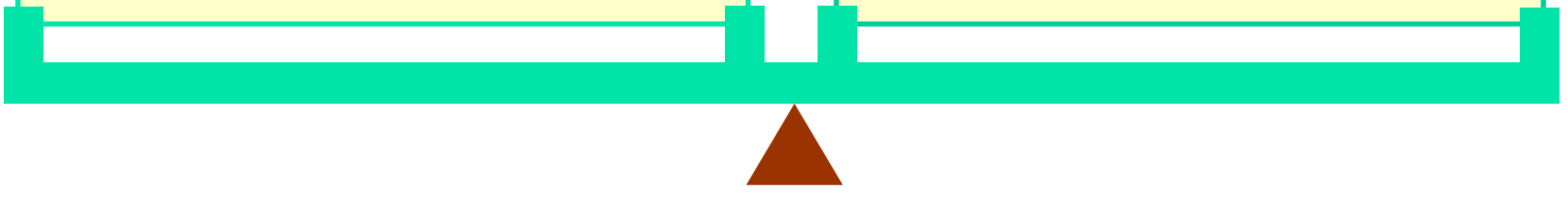
Genotyp	♂ n=133	% Mortalität	♀ n=68
TNFB1/TNFB1	42		33
TNFB1/TNFB2	47		33
TNFB2/TNFB2	72*		53

*signifikant unterschiedlich von anderen Genotypen des selben Geschlecht

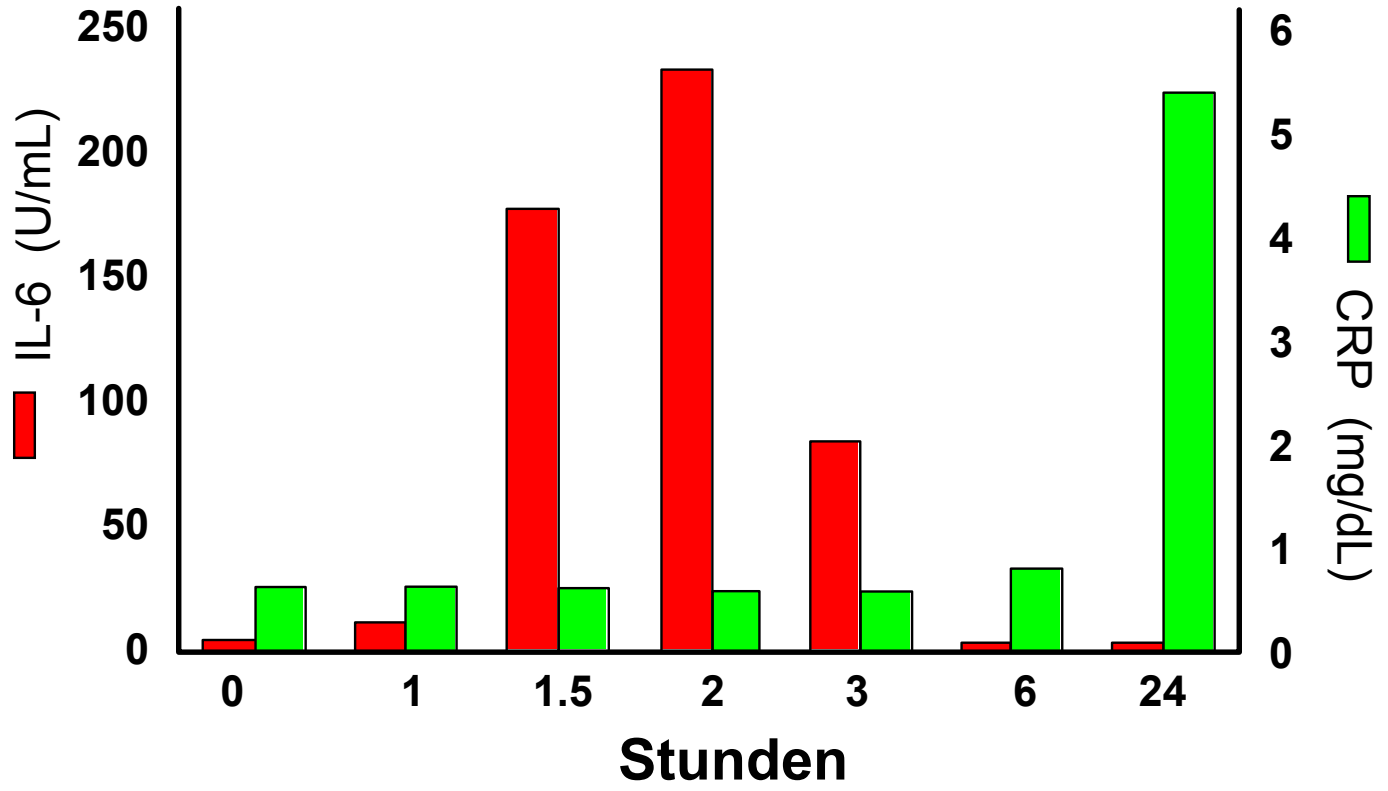
Balance zwischen pro- und anti-inflammatorischen Zytokinen



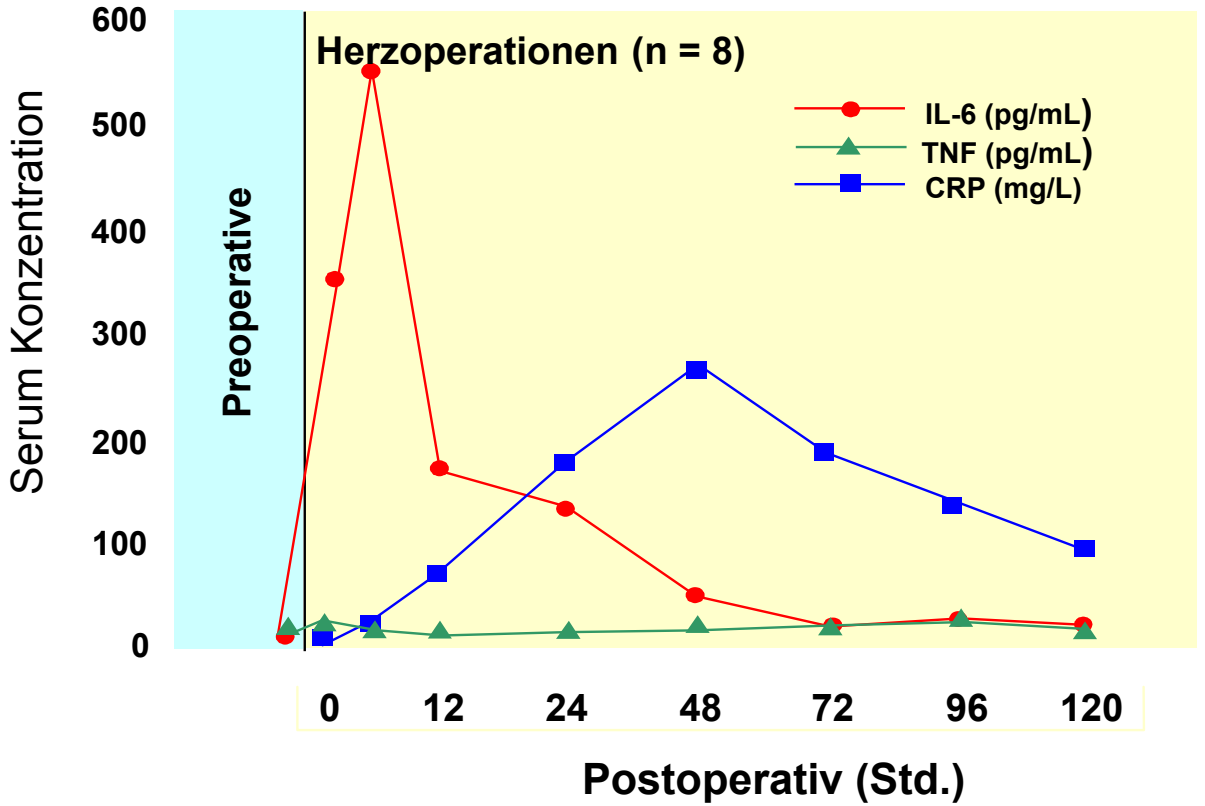
Pro-inflammatorische Substanzen	Anti-inflammatorische Substanzen
TNF-α (Tumor nekrosis factor-α), IL (Interleukins), IFN-γ (Interferon-γ), LTα (Lymphotoxin α), C‘ (Complement), LB_4 (Leukotrien B_4), PAF (Platelet activating factor), Kinins (Bradykinine), NO (Nitric oxid), GM-CSF (Granulocyte-Macrophage colony stimulationg factor), MIF (Macrophage inhibiting factor)	IL-1ra (Interleukin-1 receptor antagonist), IL (Interleukins), sTNFR (soluble Tumor necrosis factor receptor), sIL-1R (soluble Interleukin-1 receptor), TGF-β (transforming growth factor-β), PGE_2 (Prostaglandin E_2), G-CSF (Granulocyte colony stimulating factor), IFN- α/β (Interferon α/β



Freisetzung von IL-6 und CRP nach LPS Injektion (*E. coli*, 4 ng/kg)

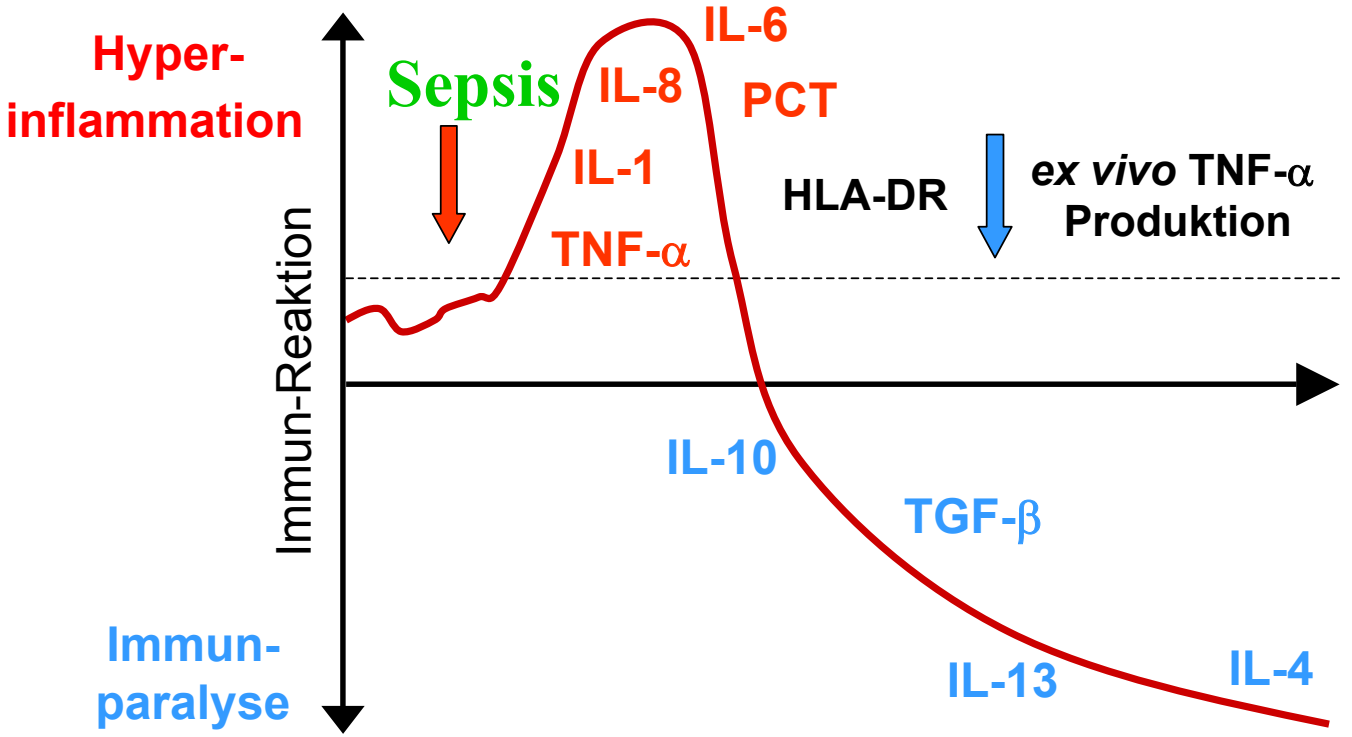


IL-6, CRP und TNF-α Serumkonzentrationen in Patienten nach Herzoperationen

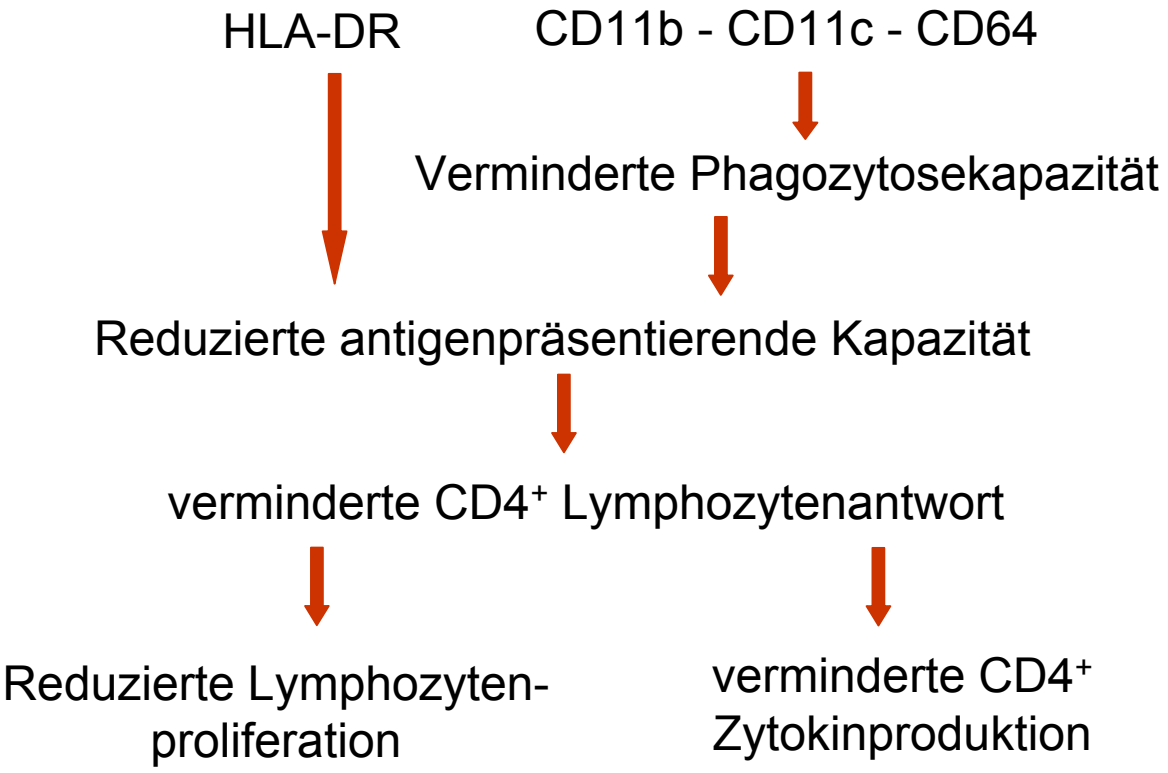


(Kragsbjerg, P. et al., Eur. J. Surg. 1995)

Hyperinflammation - Immunparalyse

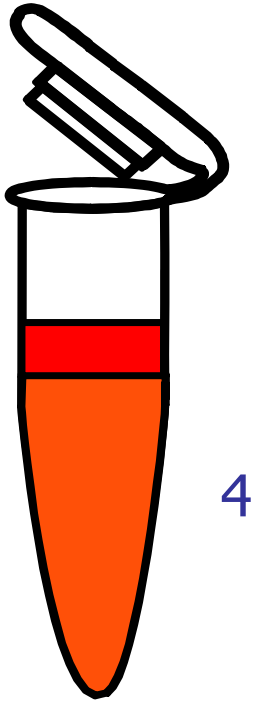


Reduzierte Monozytenfunktion



Spittler A, et al. *Blood* 1995, 86: 1564

Ex vivo Vollblut Stimulation Test



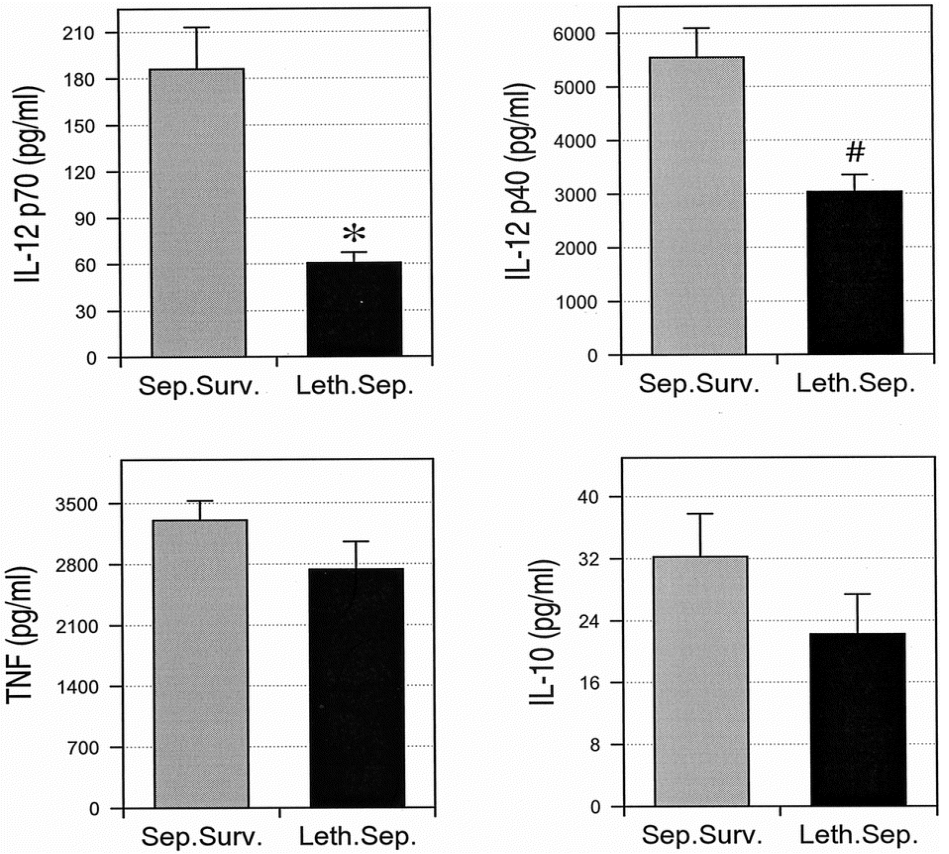
50 µL Vollblut

450 µL RPMI + LPS

- ✓ mischen
- ✓ 4h bei 37° C inkubieren
- ✓ zentrifugen
- ✓ TNF-α im Überstand messen

Impaired Monocyte IL-12 Production Before Surgery as a Predictive Factor for the Lethal Outcome of Postoperative Sepsis

Holzmann B. et al. *Ann Surg* 2002, 235: 560–567



Downregulation of Proinflammatory Cytokine Release in Whole Blood from Septic Patients

Ertel W, et al. *Blood* 1995; 5: 1341-1347

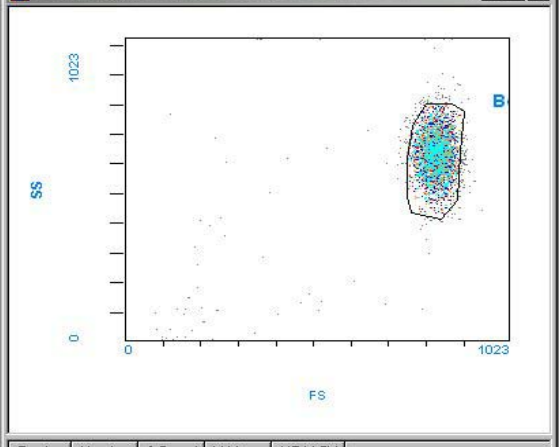
	TNF-α (U/ml)		IL-1β (ng/ml)		IL-6 (x10^3 U/ml)	
Time (h)	Control	Sepsis	Control	Sepsis	Control	Sepsis
0	0 ± 0	1.3 ± 0.7	0 ± 0	0.1 ± 0.1	0 ± 0	0.2 ± 0.1
1	98.1 ± 21.2	25.6 ± 11.3	1.2 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.8 ± 0.4
2	246.5 ± 39.6	80.5 ± 24.5	3.4 ± 0.5	0.6 ± 0.2	5.7 ± 1.7	1.5 ± 0.4
4	538.4 ± 69.0	101.0 ± 34.5	31.9 ± 1.7	3.9 ± 0.9	31.4 ± 5.4	4.7 ± 1.2
8	535.9 ± 75.0	71.2 ± 23.1	45.0 ± 2.2	3.9 ± 0.8	90.2 ± 23.1	6.2 ± 1.7
24	170.9 ± 31.6	1.0 ± 0.6	44.9 ± 1.3	3.8 ± 0.9	120.0 ± 28.4	9.7 ± 3.9

Vollblut wurde mit 1µg/ml LPS für die angegebenen Zeitpunkte stimuliert.
Kontrolle (n = 15), Septische Patienten (n = 20)

Expo32 ADC : s:\ImmunMonitoring\LMD\00006505 030.LMD

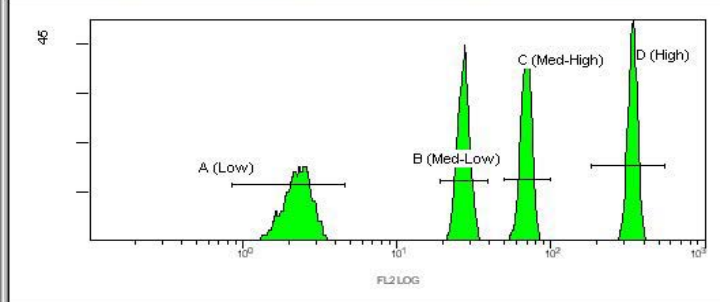
File Edit View Insert Tools Plots Analysis FlowPAGE Cytometer Window Help

(F1)[Ungated] 00006505 030.LMD : FS Lin/SS Lin



Region	Number	%Gated	X-Mean	HP X-CV
Beads	3989	93.99	828.3	2.1

(F1)[Beads] 00006505 030.LMD : FL2 Log



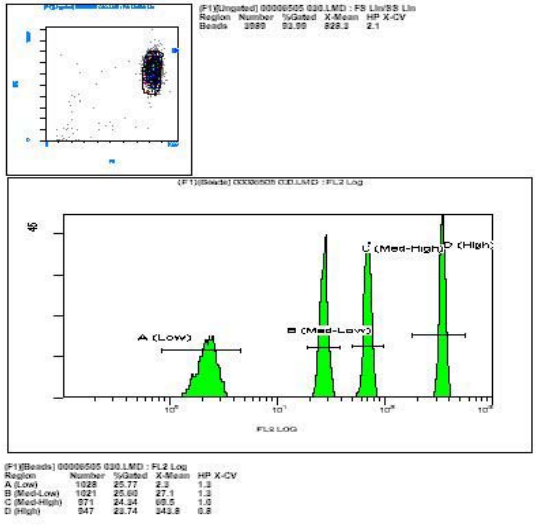
Region	Number	%Gated	X-Mean	HP X-CV
A (Low)	1028	25.77	2.3	1.3
B (Med-Low)	1021	25.60	27.1	1.3
C (Med-High)	971	24.34	69.5	1.0
D (High)	947	23.74	343.8	0.8

PAGE 1

Monozyten HLA-DR Quantifizierung (Beads)

Patient: _____

Datum: _____



Auswertung:

Ready

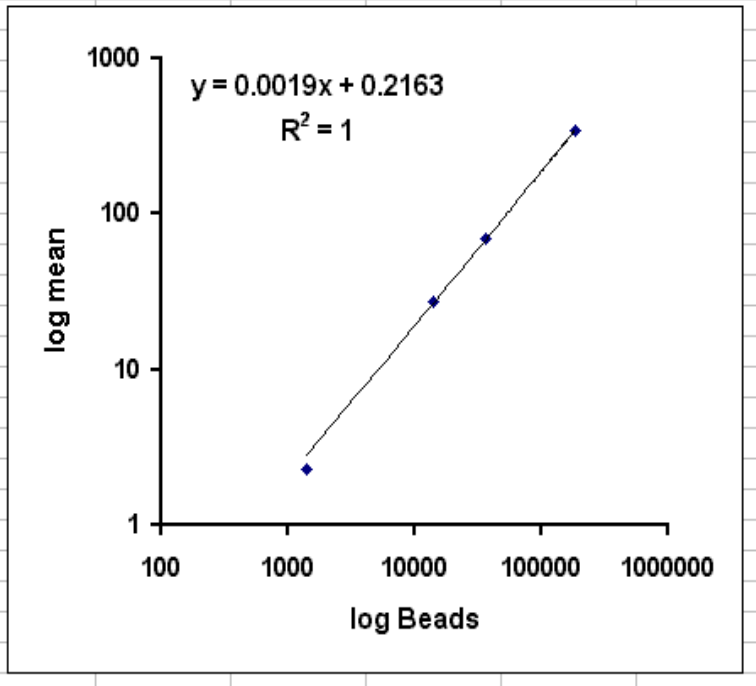
NUM

Start | Expo32 ADC : s:\ImmunM...

2:48 PM

1400	2.3	
14000	27.1	
36600	69.5	
182000	343.8	

Datum: 19.04.2001

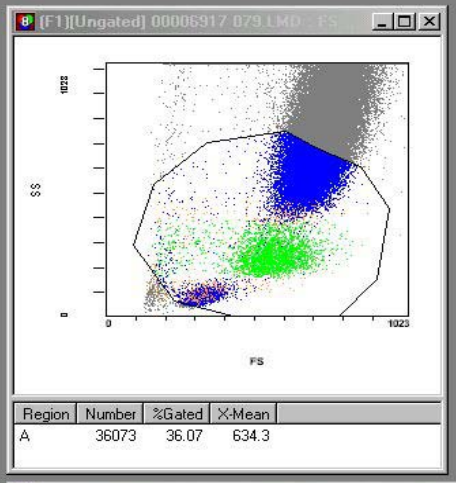


Eichgerade | Statistik | Tabelle3

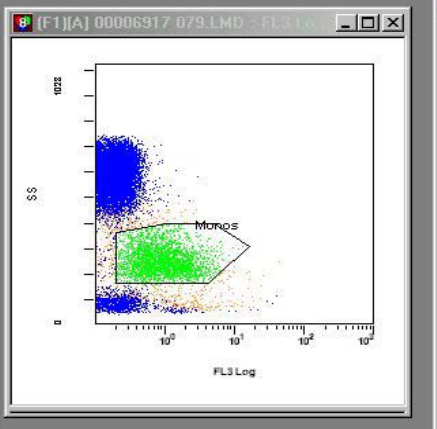
Bereit

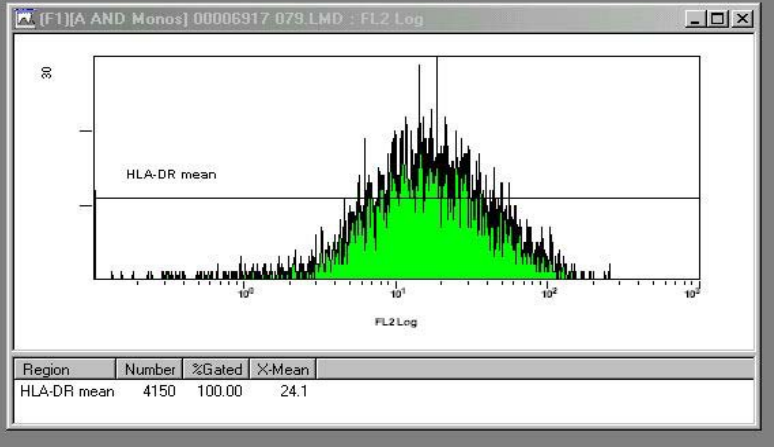
Expo32 ADC : s:\ImmunMonitoring\LMD\00006917 079.LMD

File Edit View Insert Tools Plots Analysis FlowPAGE Cytometer Window Help



Region	Number	%Gated	X-Mean
A	36073	36.07	634.3





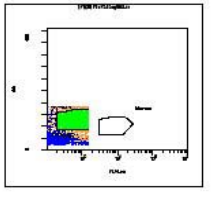
Region	Number	%Gated	X-Mean
HLA-DR mean	4150	100.00	24.1

PAGE 1

Monozyten HLA-DR Quantifizierung

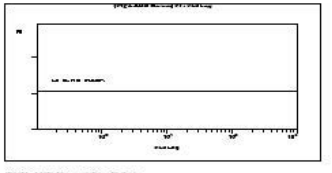
Patient: _____

Datum: _____



(F1)[A] F1 : FL4 Log/SS Lin

Region	Number	%Gated	X-Mean
Monos	0	0.00	0.0



(F1)[A AND Monos] F1 : FL2 Log

Region	Number	%Gated	X-Mean
HLA-DR mean	0	0.00	0.0

Auswertung: (F1)[Ungated] F1 : Legend

Formel der Regressionsgeraden:

HLA-DR Antigene pro Monozyt:

(F1)[Ungate

Ready

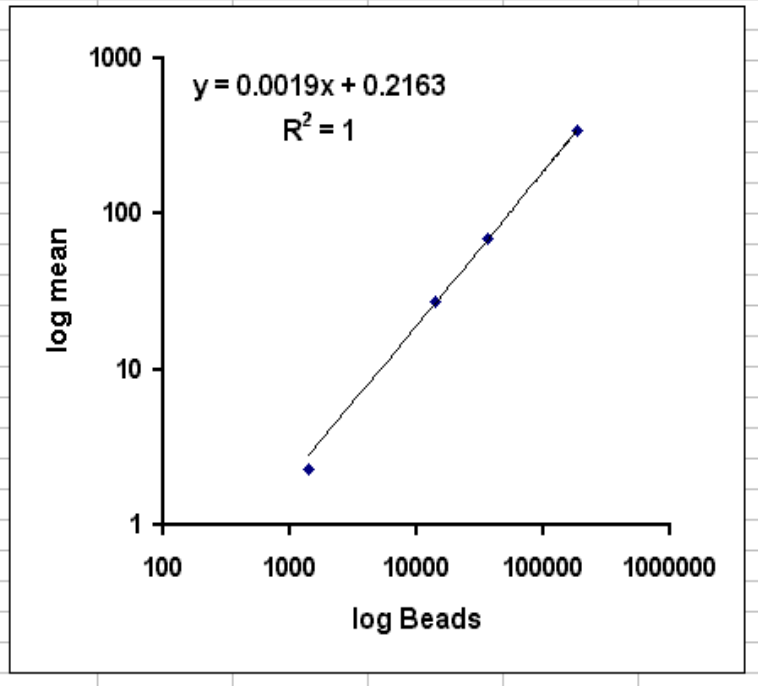
NUM

Start | Expo32 ADC : s:\ImmunM...

2:55 PM

1400	2.3	
14000	27.1	
36600	69.5	
182000	343.8	

Datum: 19.04.2001



$y = 0{,}0019x + 0{,}2163$

$x = (y - 0{,}2163) / 0{,}0019$

$y = 24{,}1$

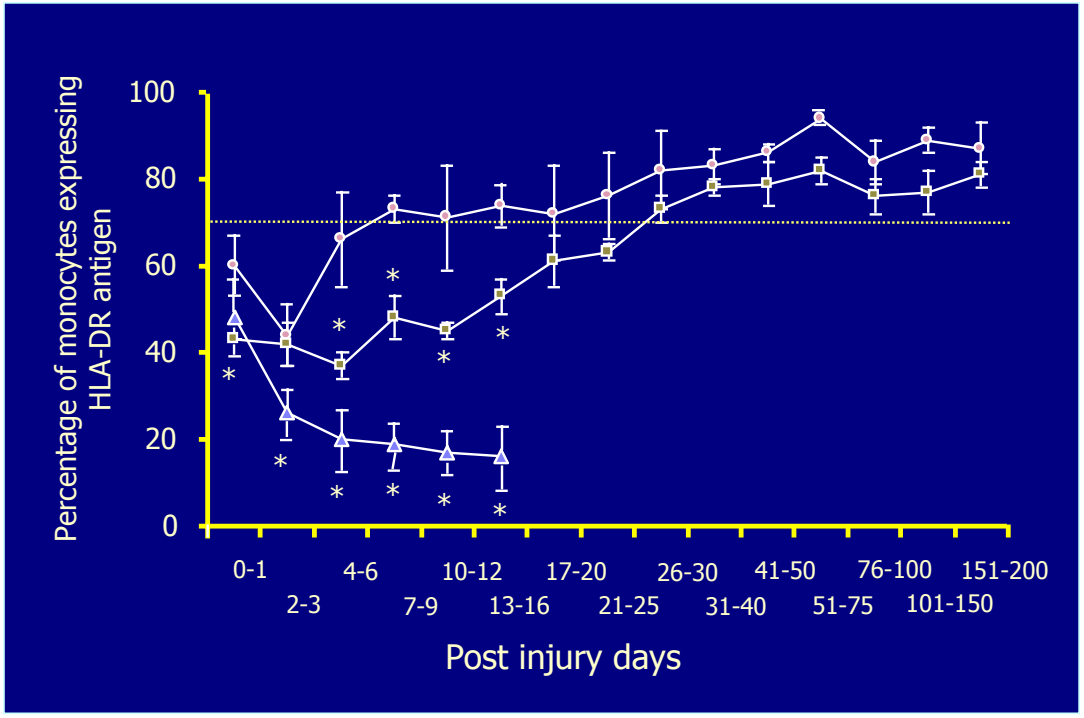
12.570 $= (24{,}1 - 0{,}2163) / 0{,}0019$

Eichgerade | Statistik | Tabelle3

Bereit

Monocyte HLA-DR Antigen Expression Characterizes Clinical Outcome in the Trauma Patient

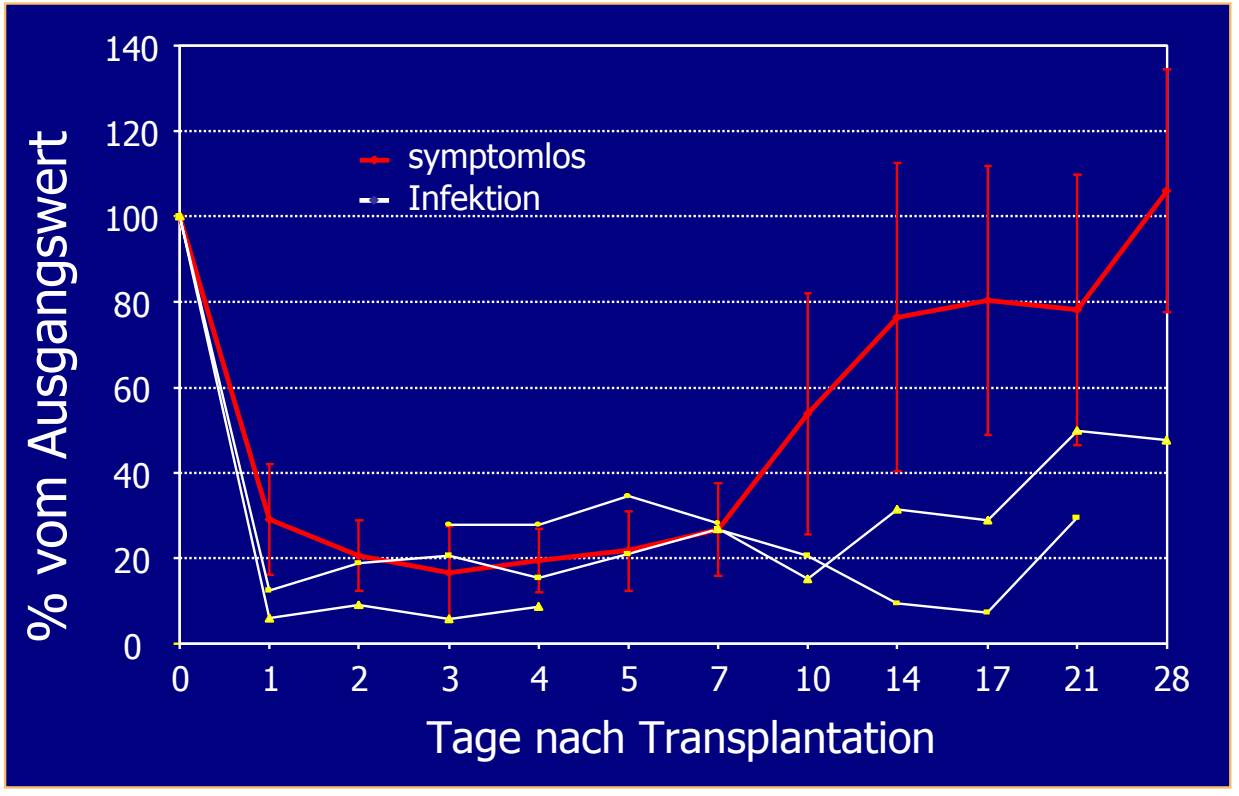
Hershman MJ, et al. *Br J Surg* 1990, 77:204



Percentages of monocytes that expressed HLA-DR antigen after severe injury: **upper line**, uneventful recovery group; **middle line**, major sepsis; **lower line**, group of patients who died

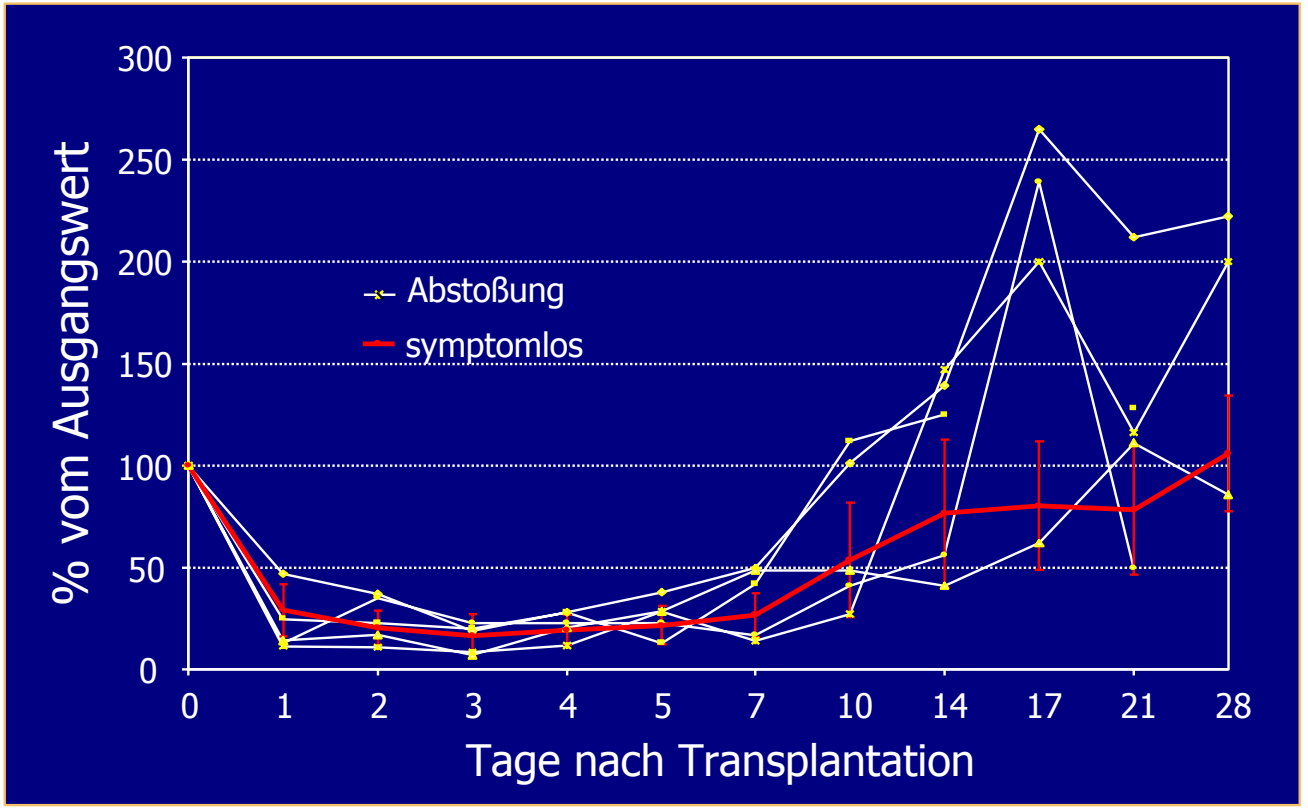
Immune Monitoring in Cardiac Transplant Recipients

Kocher A, Spittler A, et al.. *Transplantation Proceedings* 1997; 29: 2895-2898



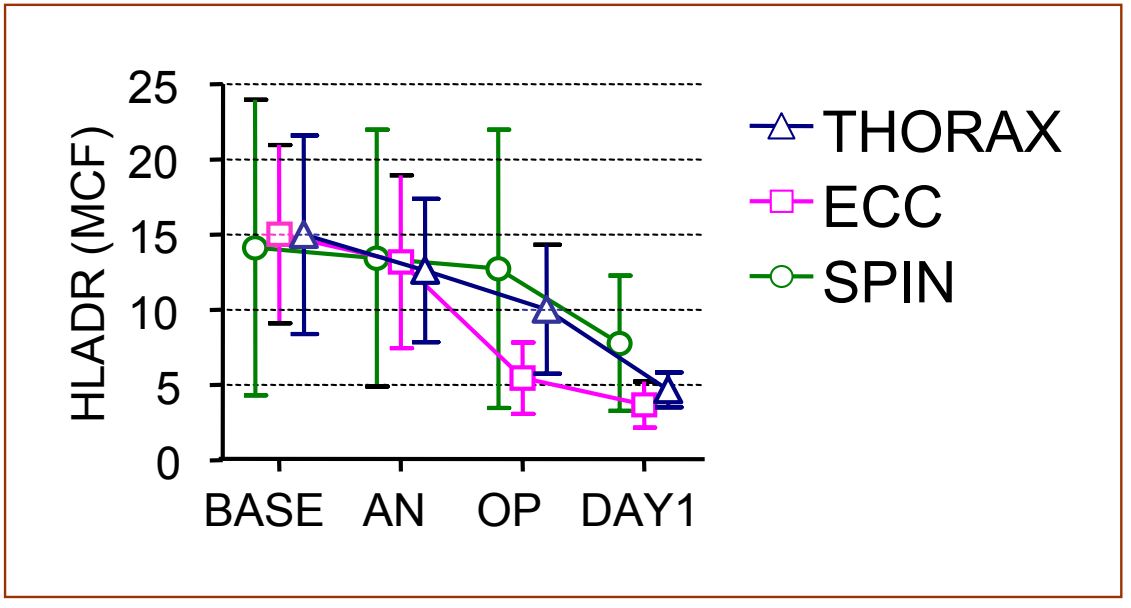
Immune Monitoring in Cardiac Transplant Recipients

Kocher A, Spittler A, et al.. *Transplantation Proceedings* 1997; 29: 2895-2898



Alterations in the number of circulating leukocytes, phenotype of monocyte and cytokine production in patients undergoing cardiothoracic surgery

Hiesmayr MJ, et al. *Clin Exp Immunol* 1999, 115: 315



THORAX = thoracic surgery, ECC = extracorporale circulation, SPIN = spinale anesthesia

Intensive Care Med (2003) 29:1245–1252
DOI 10.1007/s00134-003-1686-2

ORIGINAL

Sara E. Perry
Sobhy M. Mostafa
Richard Wenstone
Alan Shenkin
Paul J. McLaughlin

Is low monocyte HLA-DR expression helpful to predict outcome in severe sepsis?

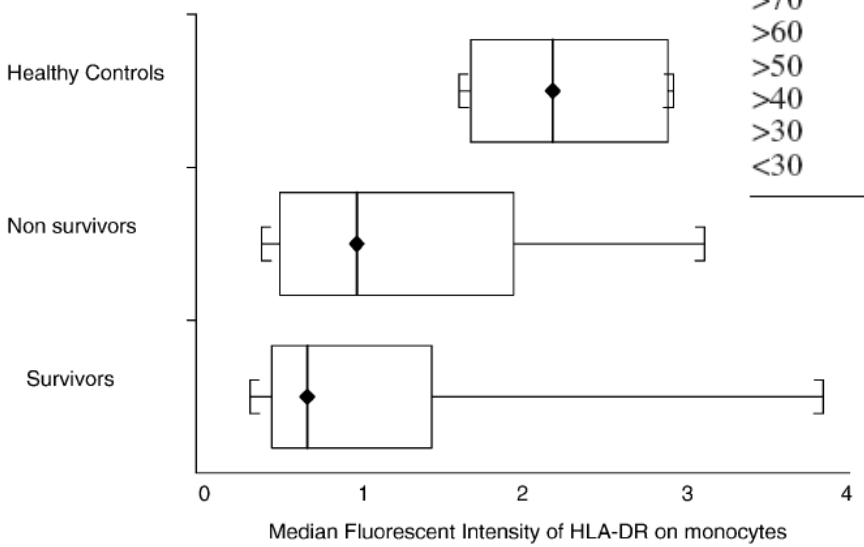


Table 6 The mortality of patients categorised by the percentage of monocytes expressing HLA-DR

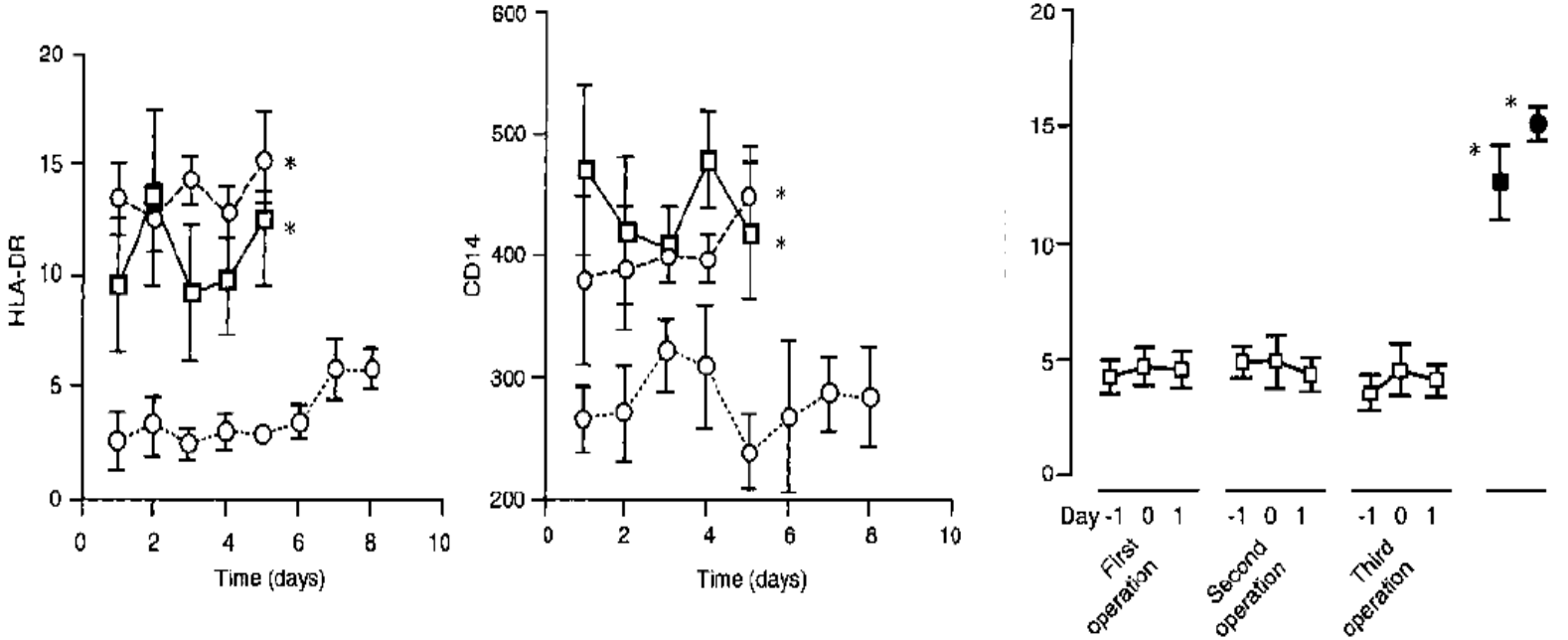
HLA-DR expression group (%)	Percentage mortality (number of patients died)
>70	38 (8/21)
>60	33.3 (10/30)
>50	31.6 (12/38)
>40	34.1 (14/41)
>30	30.4 (14/46)
<30	37.5 (9/24)

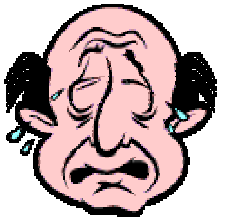
Severe Acute Pancreatitis Causes Alterations in HLA-DR and CD14 Expression on Peripheral Blood Monocytes Independently of Surgical Treatment

Peter Götzinger, Thomas Sautner, Andreas Spittler, Murat Barlan, Peter Wamser, Erich Roth, Raimund Jakesz and Reinhold Függer

From the Department of Surgery, University of Vienna, Vienna, Austria

Eur J Surg 2000; 166: 628–632





Intensive Care Med (2003) 29:1211–1213
DOI 10.1007/s00134-003-1820-1

EDITORIAL

Andreas Spittler
Erich Roth

Is monocyte HLA-DR expression predictive for clinical outcome in sepsis?

ESICM Working Group Immunomonitoring

E.M.S.I.C.

The European Multicenter Study of Immunomonitoring in Critical Care

Study Protocol (Rev. 8/2003)

Aim of the study is to evaluate a concept of immunomonitoring in established human sepsis and to characterize its predictive potential concerning outcome and nosocomial infections. The study will focus especially on the peripheral HLA-DR expression on monocytes at study entry and over time course.

Mandatory parameters:

- Admission dx., discharge dx.

Assessment of severity:

- APACHE II, SAPS II (initially, ICU admission)
- SOFA score daily (study entry till discharge/death)

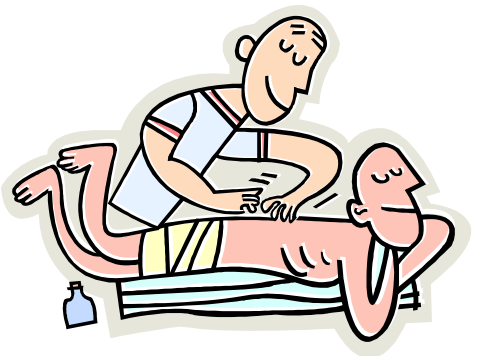
Assessment of blood parameters

- QuantiBRITE™ Anti–HLA-DR PE /Anti-Monocyte PerCP-Cy5.5 Antibodies (Catalog No. 340827); performed as recommended by BD
- Differential white blood count at the time of sampling for HLA-DR
- Plasma IL 10 (Heparin Plasma)
- Plasma IL 6 (Heparin Plasma)
- Plasma PCT (Serum)
- Plasma GM CSF (storage of blood samples for central measurement)

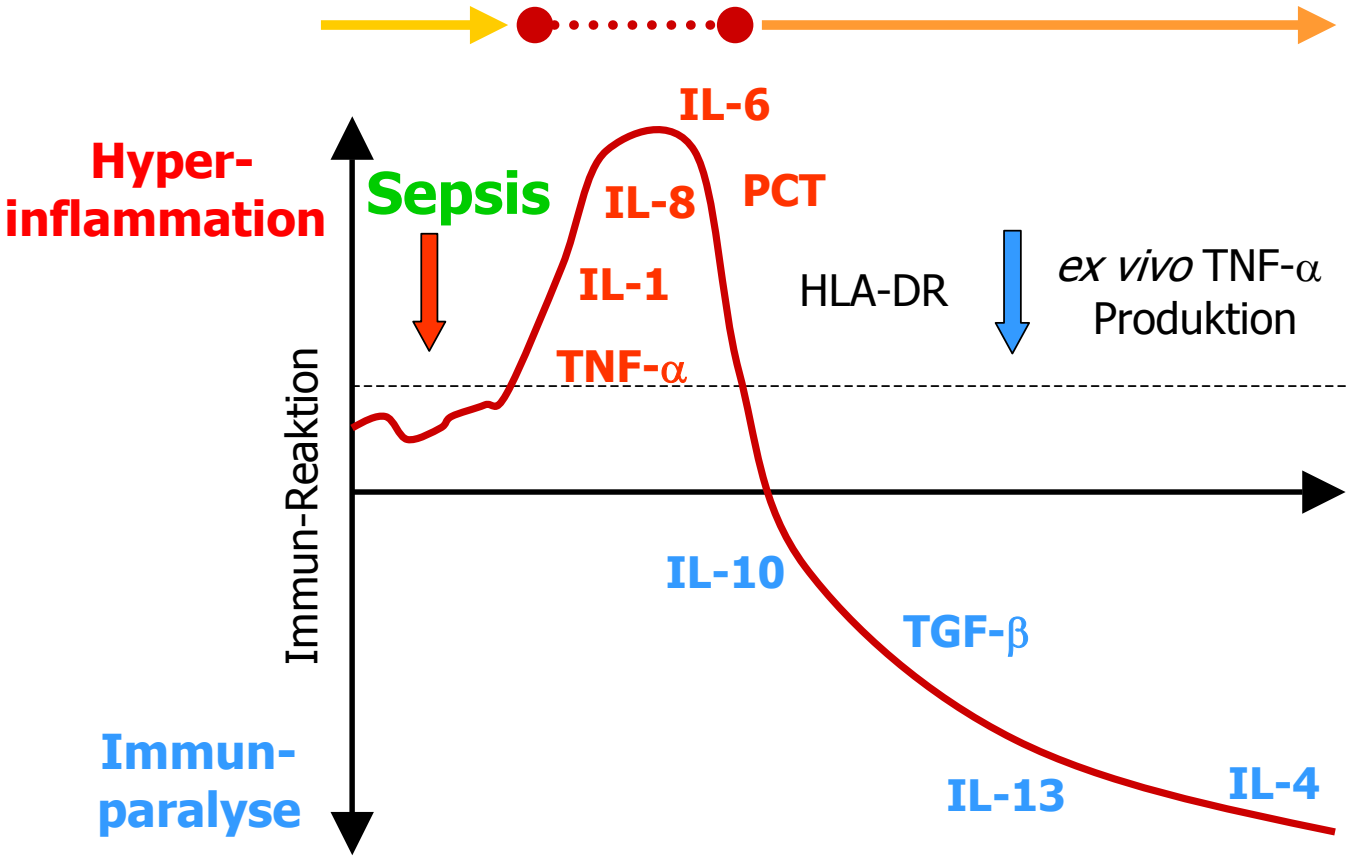
Outcome variables

- Length of stay/mortality (Hospital)
- Length of stay/mortality (ICU)
- Mortality (28 day)
- Incidence of infection (CDC criteria)
- Delta SOFA, total max. SOFA

Therapeutische Möglichkeiten ???

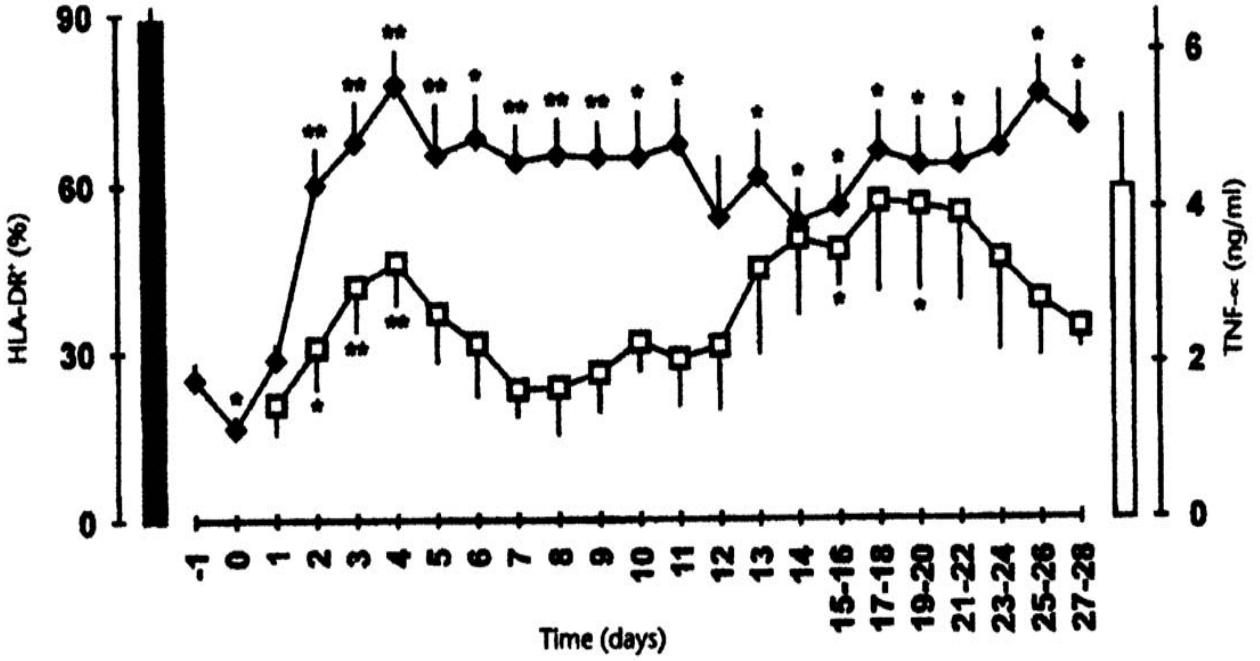


Immunologische Phasen im Verlauf der Sepsis



Monocyte Deactivation in Septic Patients: Restoration by IFN-γ Treatment

Döcke W-D, et al. *Nature Medicine* 1997, 3: 678-681

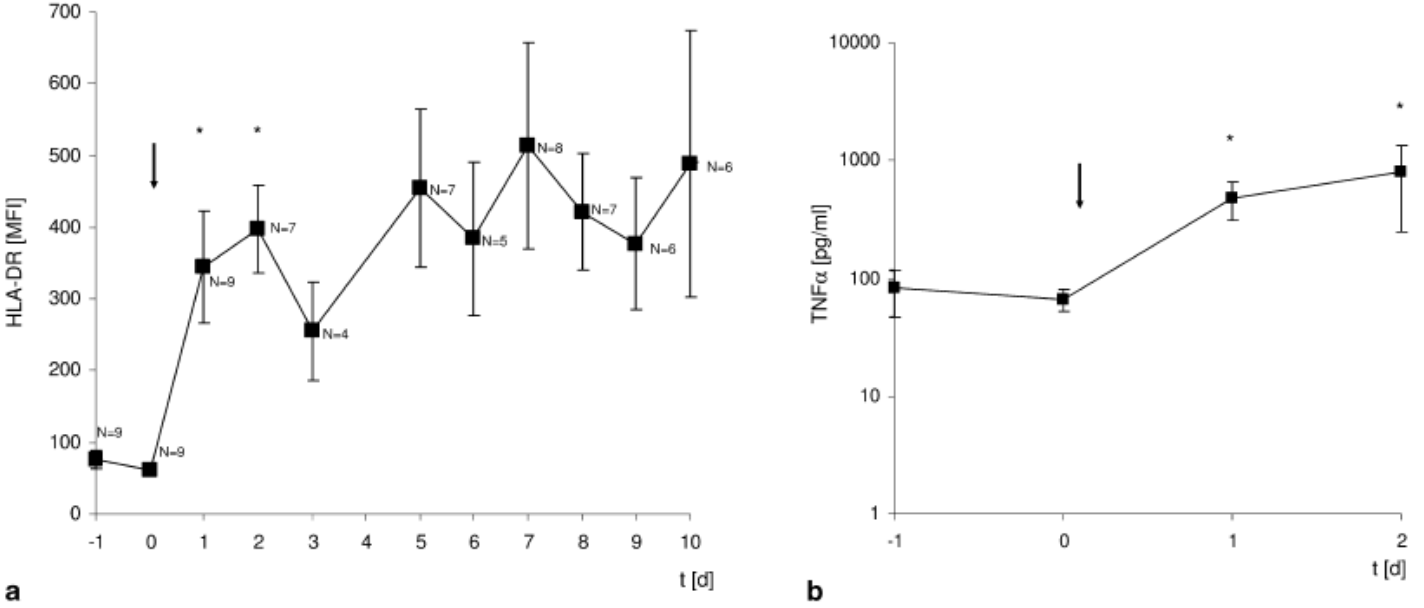


Intensive Care Med (2003) 29:646–651
DOI 10.1007/s00134-003-1666-6

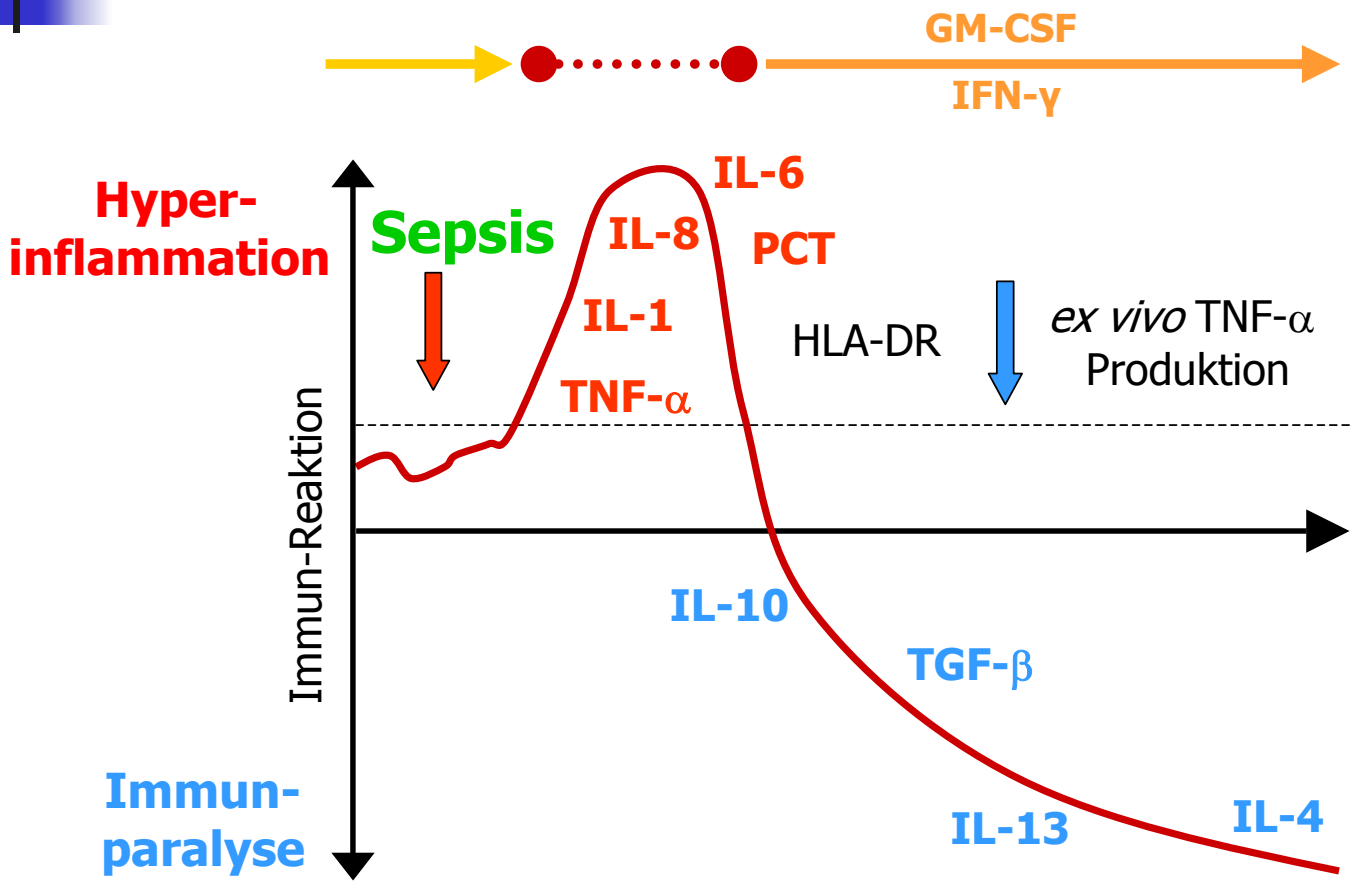
BRIEF REPORT

Axel Nierhaus
Barbara Montag
Nicole Timmler
Daniel P. Frings
Kai Gutensohn
Roman Jung
Claus G. Schneider
Werner Pothmann
Anne K. Brassel
Jochen Schulte am Esch

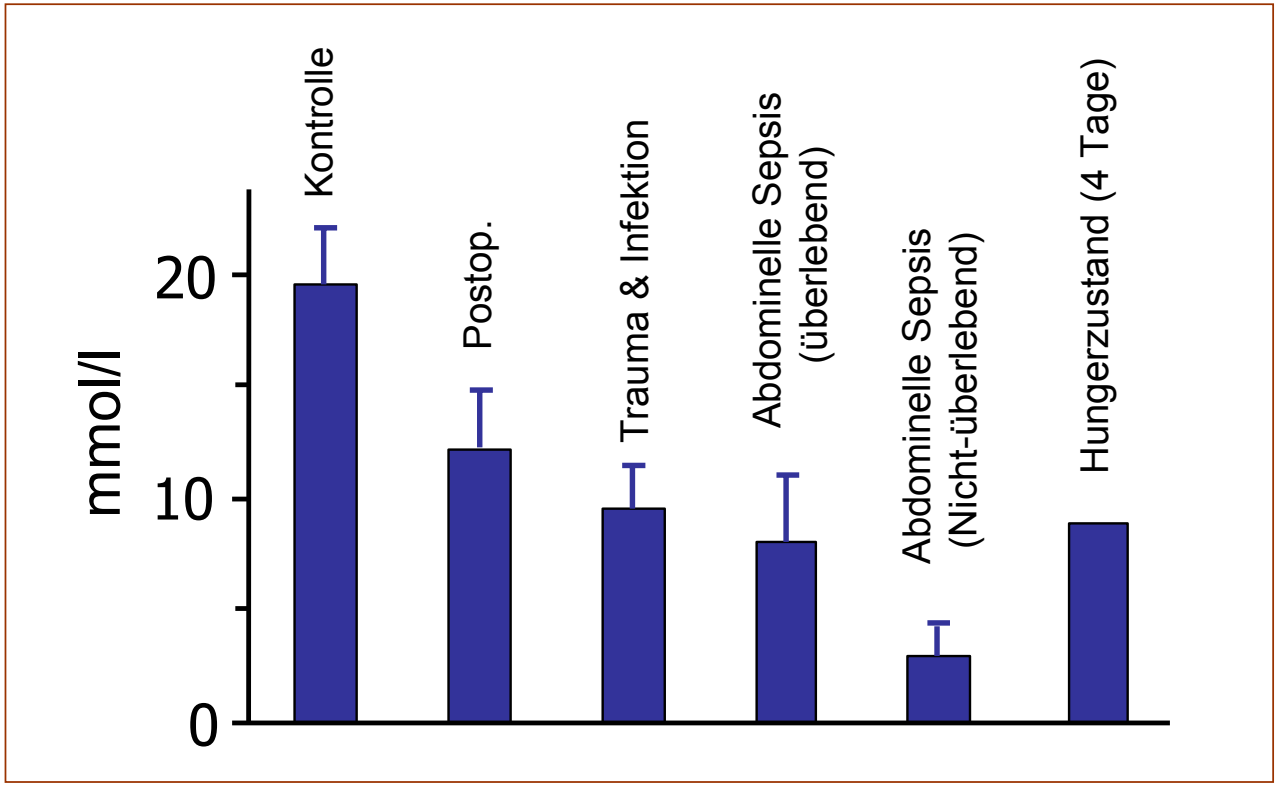
Reversal of immunoparalysis by recombinant human granulocyte-macrophage colony-stimulating factor in patients with severe sepsis



Unterschiedliche Sepsisphasen brauchen unterschiedliche therapeutische Interventionen

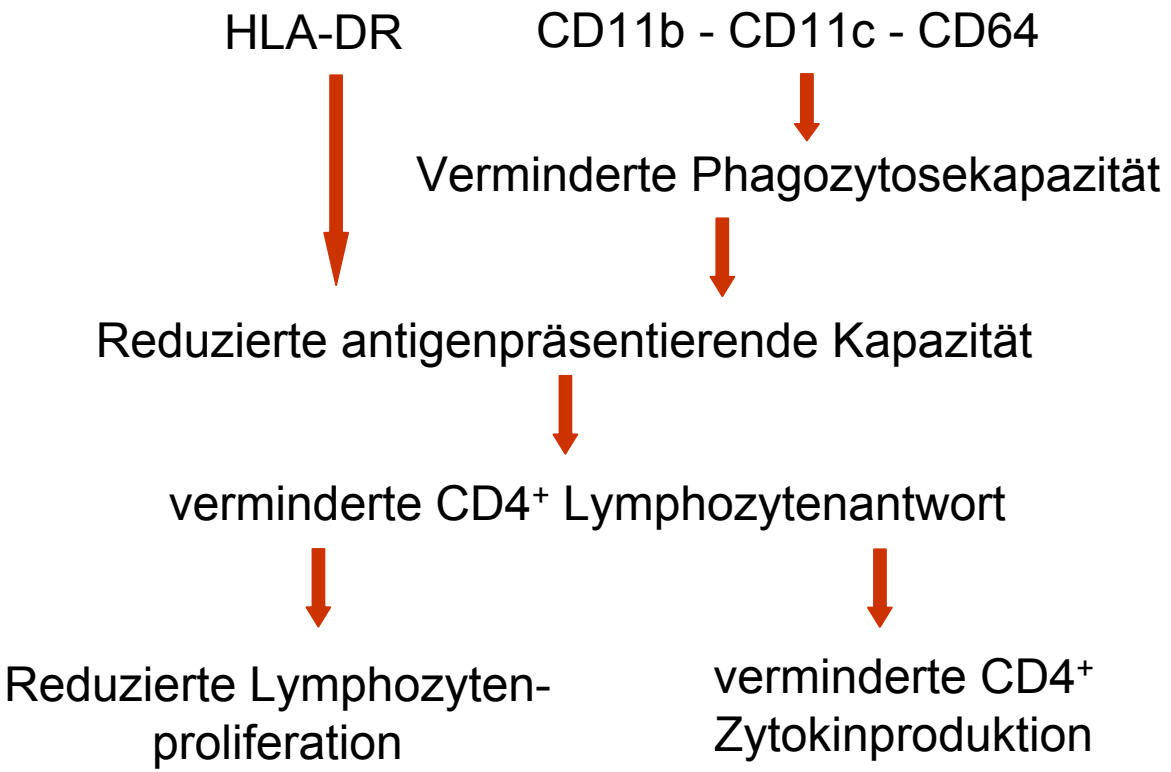


Glutamin im Muskel



- Roth E, et al.: Clin Nutr, 25-41, 1982
- Vinnars E, et al.: Ann Surg 182:665, 1975
- Askanazi J, et al.: Ann Surg 192:78, 1980
- Elwyn DH, et al.: in Walser M & Williamson JR (eds.) Metabolism and Clinical Implications of Branched Chain Amino Acids and Ketoacids. Elsevier NY 1981, pp 547-552

Glutaminmangel = reduzierte Monozytenfunktion

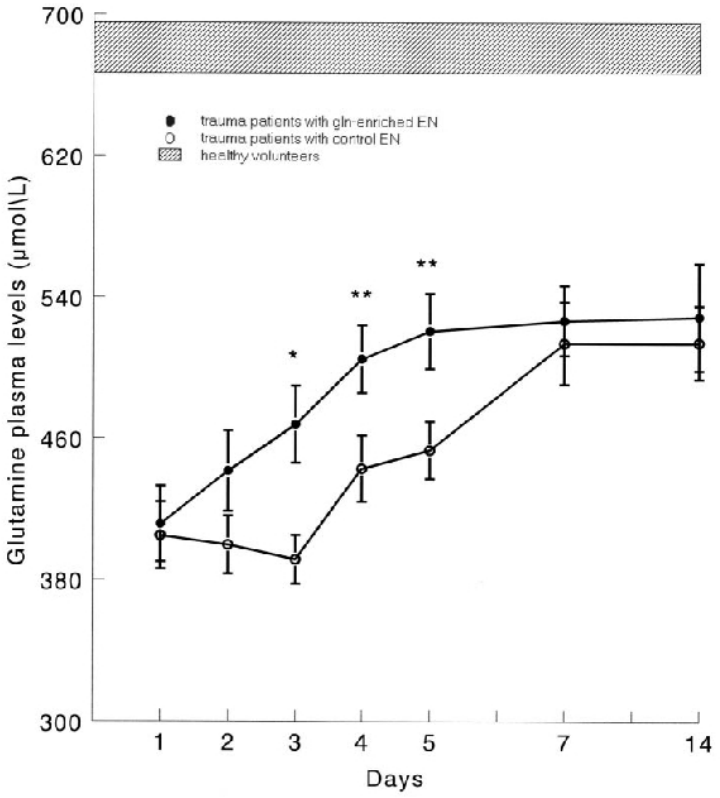


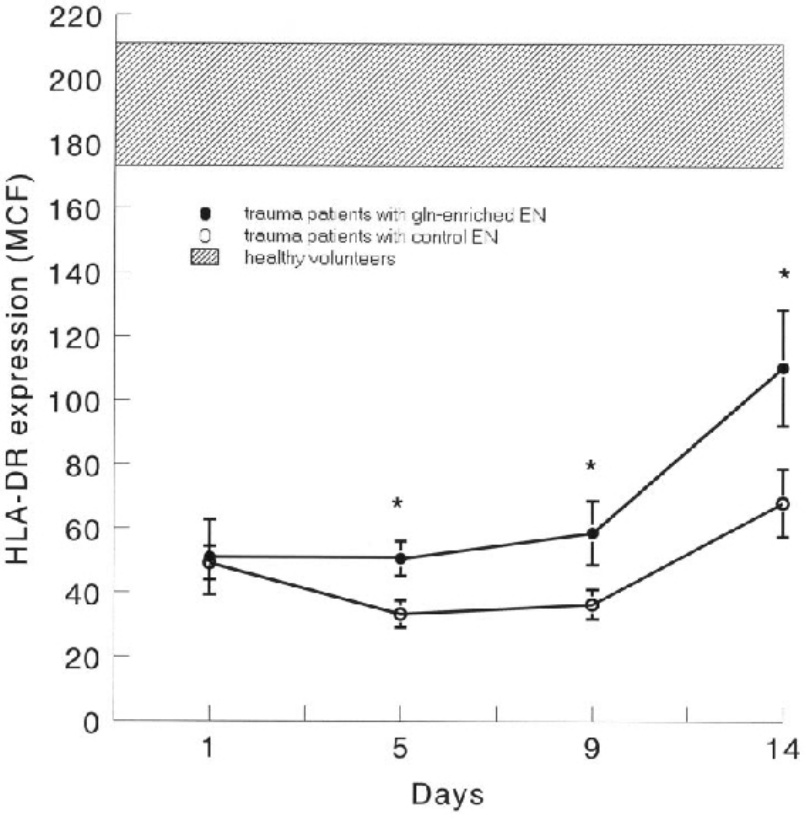
Infektion Sepsis

Spittler A, et al. *Blood* 1995, 86: 1564

Glutamine-Enriched EN Increases HLA-DR Expression on Monocytes of Trauma Patients

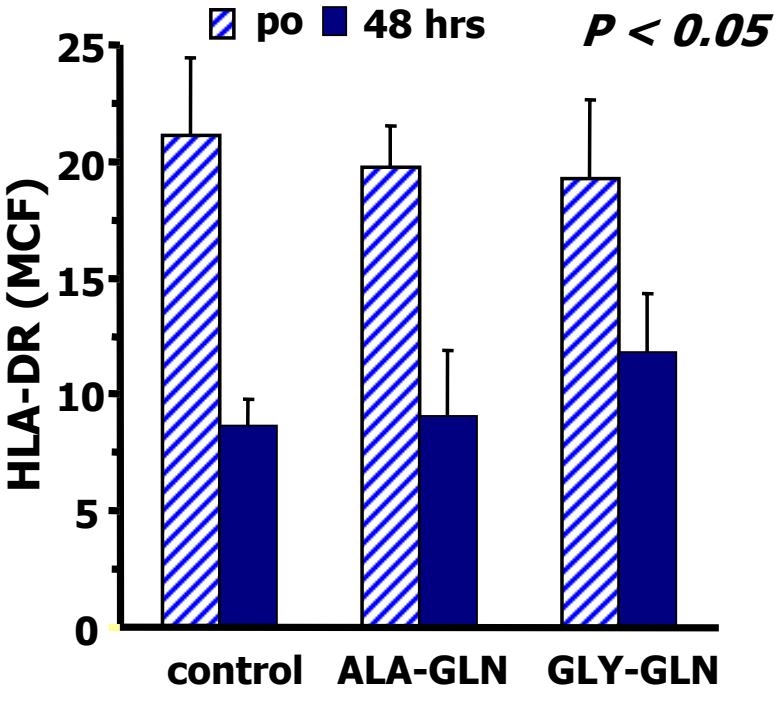
Boelens PG, et al. *Journal of Nutrition* 2002; 132: 2580-2586





Postoperative GLY-GLN infusion reduces immunosuppression: partial prevention of the surgery induced decrease in HLA-DR expression on monocytes

Spittler A, et al. *Clinical Nutrition* 2001, 20: 37



Patienten: 30 Patienten mit mittelschweren abdominalchirurgischen Eingriffen

Kontrollgruppe: 1500ml Vamin

ALA-GLN: Vamin und 500ml Alanyl-Glutamin (35g GLN)

GLY-GLN: Vamin und 500 ml Glycyl Glutamin (35g GLN)

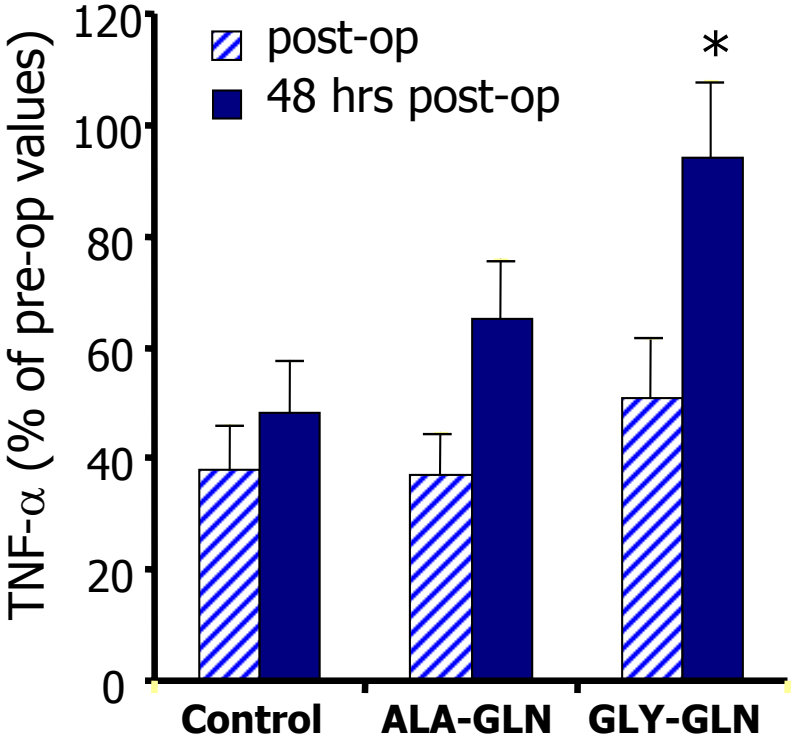
Infusion: kontinuierlich über 48 Stunden postoperativ

Blutproben:
1. Direkt nach der Operation,
2. Nach 48 Stunden

Messungen: HLA-DR Expression auf Monozyten mittels Durchflusszytometrie.

Perioperative GLY-GLN infusion diminishes the surgery induced immuno-suppression: accelerated restoration of the LPS stimulated TNF-α response

Exner R, et al. *Annals of Surgery* 2003, 237: 110



Patienten: 45 Patienten mit mittelschweren abdominalchirurgischen Eingriffen

Control: 1500ml Vamin

ALA-GLN: Vamin und 500ml Alanyl-Glutamin (35g GLN/24 Stunden)

GLY-GLN: Vamin und 500ml Glycyl-Glutamine (35g GLN/24 Stunden)

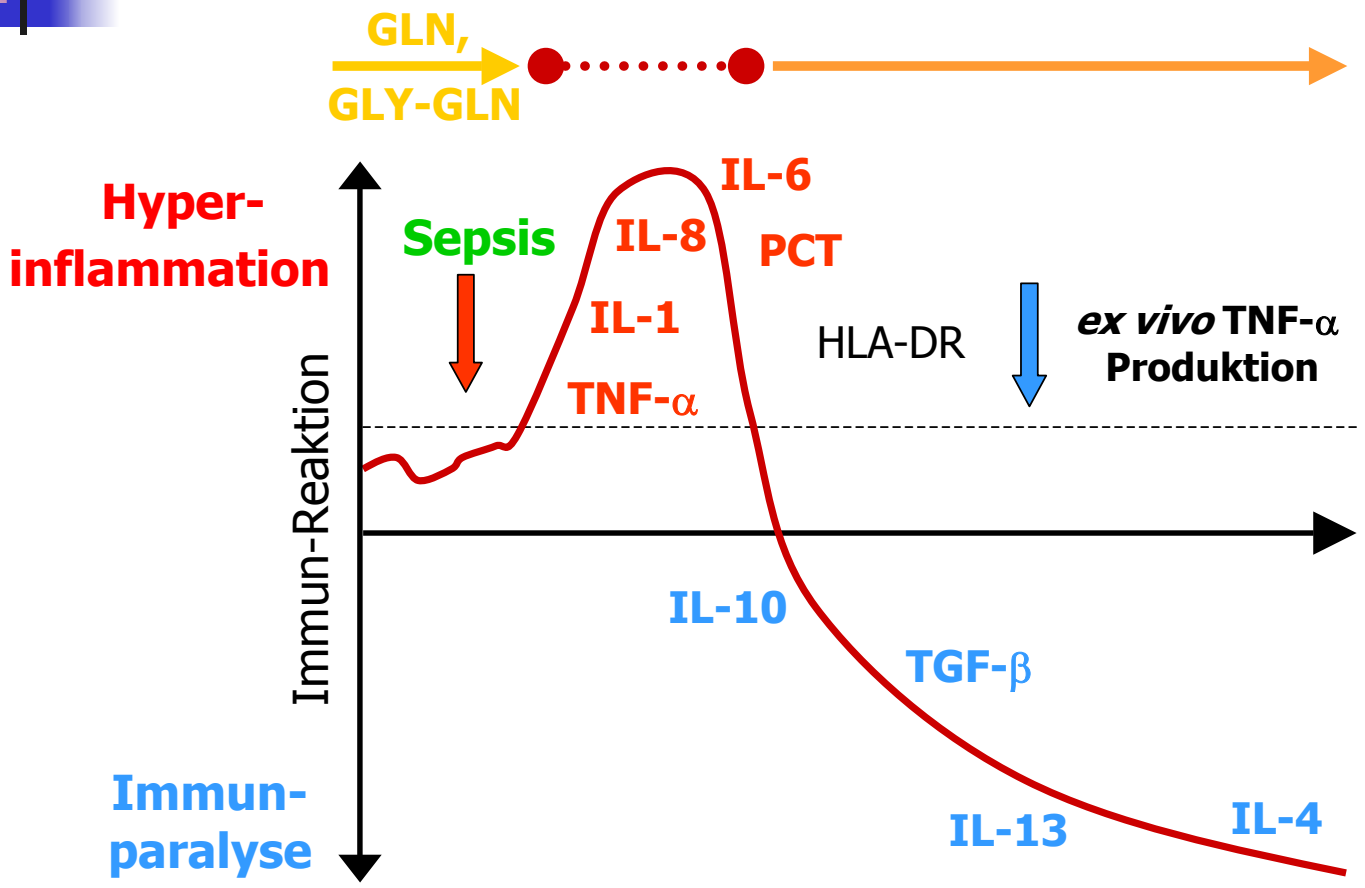
Infusion: kontinuierlich über 72 Stunden, beginnend 24 Stunden vor der Operation

Blutproben:

1. 24 Stunden vor der Operation
2. Direkt nach der Operation
3. Nach 48 Stunden

Messungen: LPS induzierte TNF-a Sekretion mittels ELISA.

Unterschiedliche Sepsisphasen brauchen unterschiedliche therapeutische Interventionen



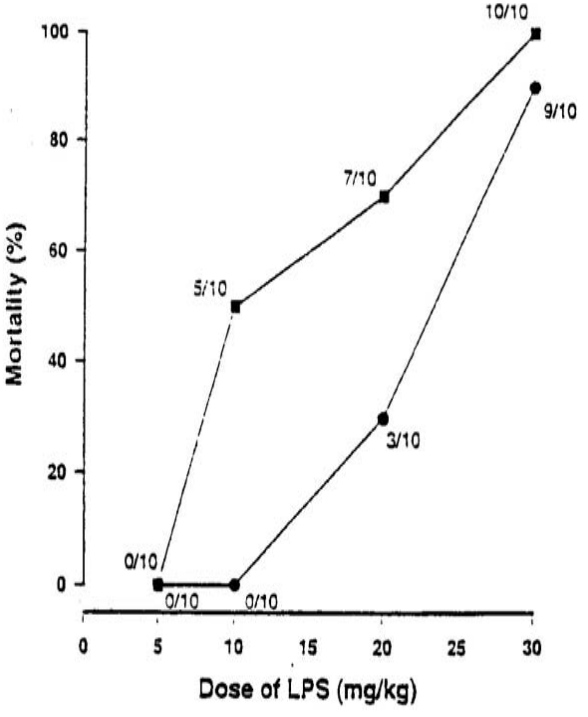
Effekte von Glycine

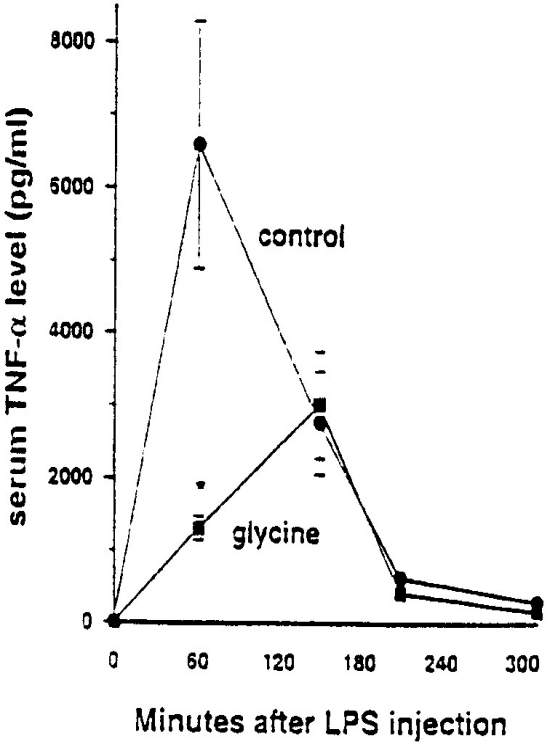
- Schützt vor Ischämie-Reperfusionsschäden, besonders in Leber und Niere
- Schützt vor Hypoxieschäden
- Hemmt die Produktion von freien Radikalen
- Schützt vor MODS und verbessert das Überleben in Tiermodellen mit induziertem Schock

A diet containing glycine improves survival in endotoxin shock in the rat

Ikejima K, Iimuro Y, Forman DT, Thurman RG

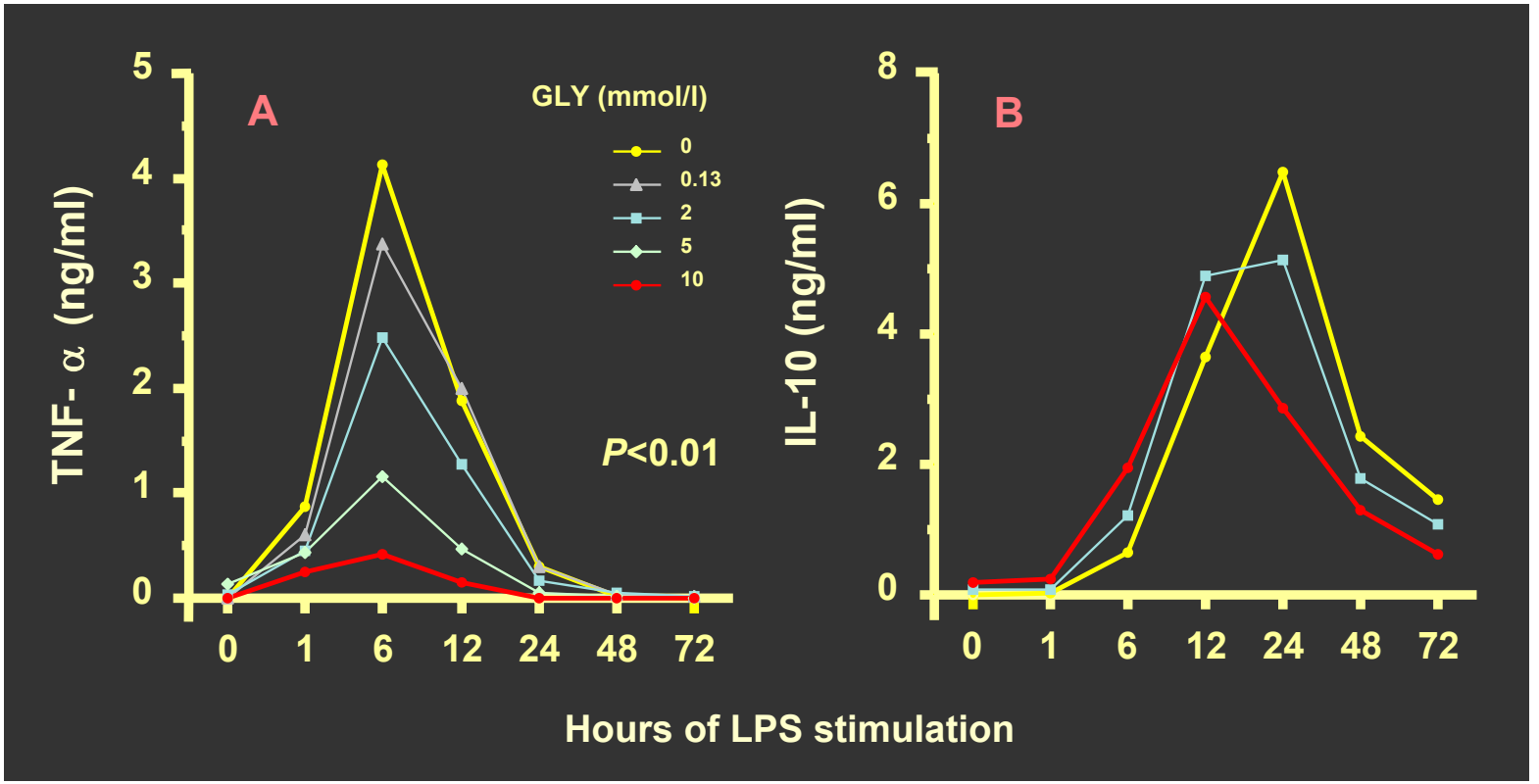
Am J Physiol Gastrointest Liver Physiol 271, G97-G103, 1996





Immunomodulatory effects of glycine on LPS-treated monocytes: reduced TNF-α production and accelerated IL-10 expression

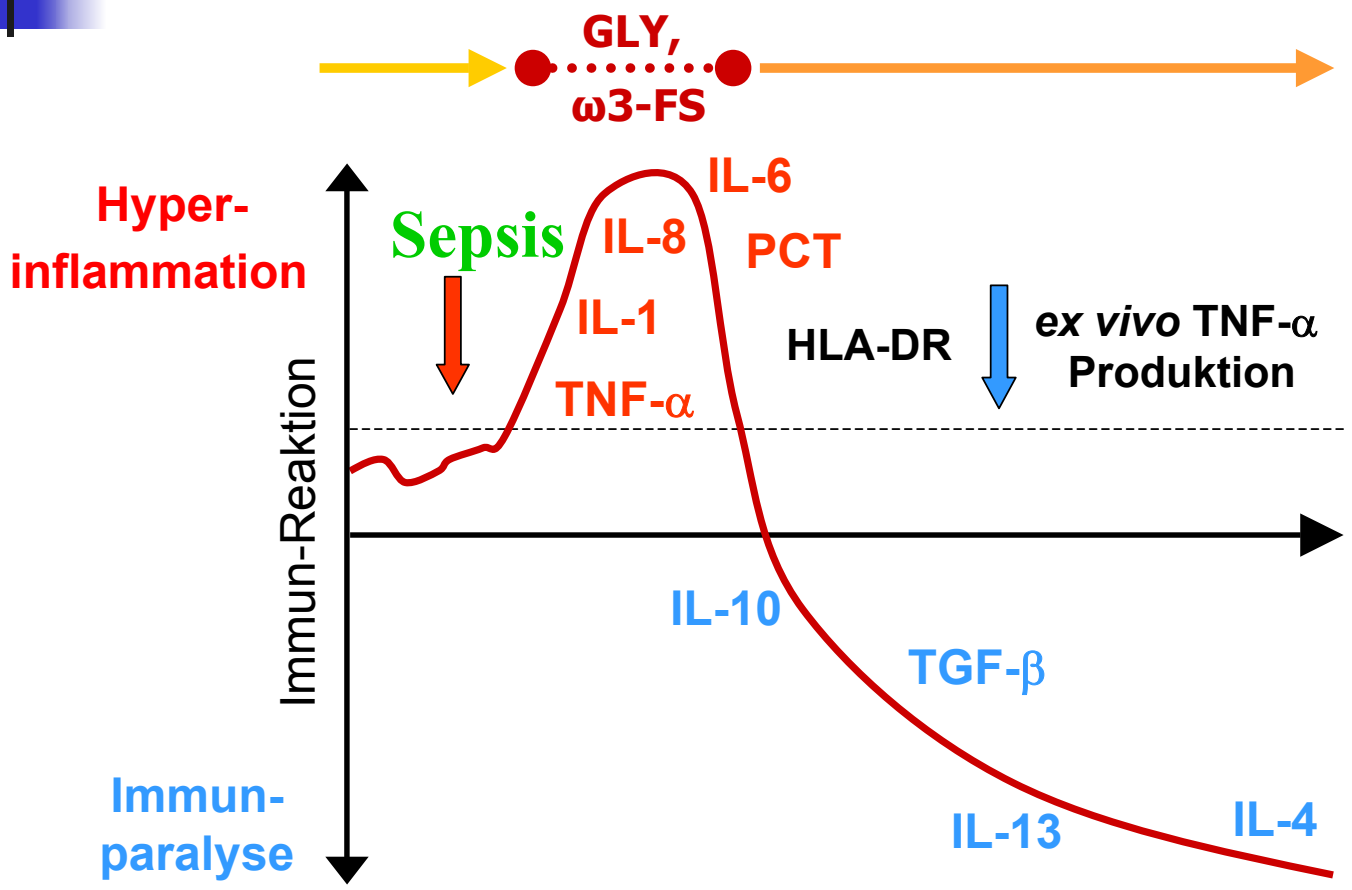
Spittler A., et al. *FASEB Journal* 1999;13:563



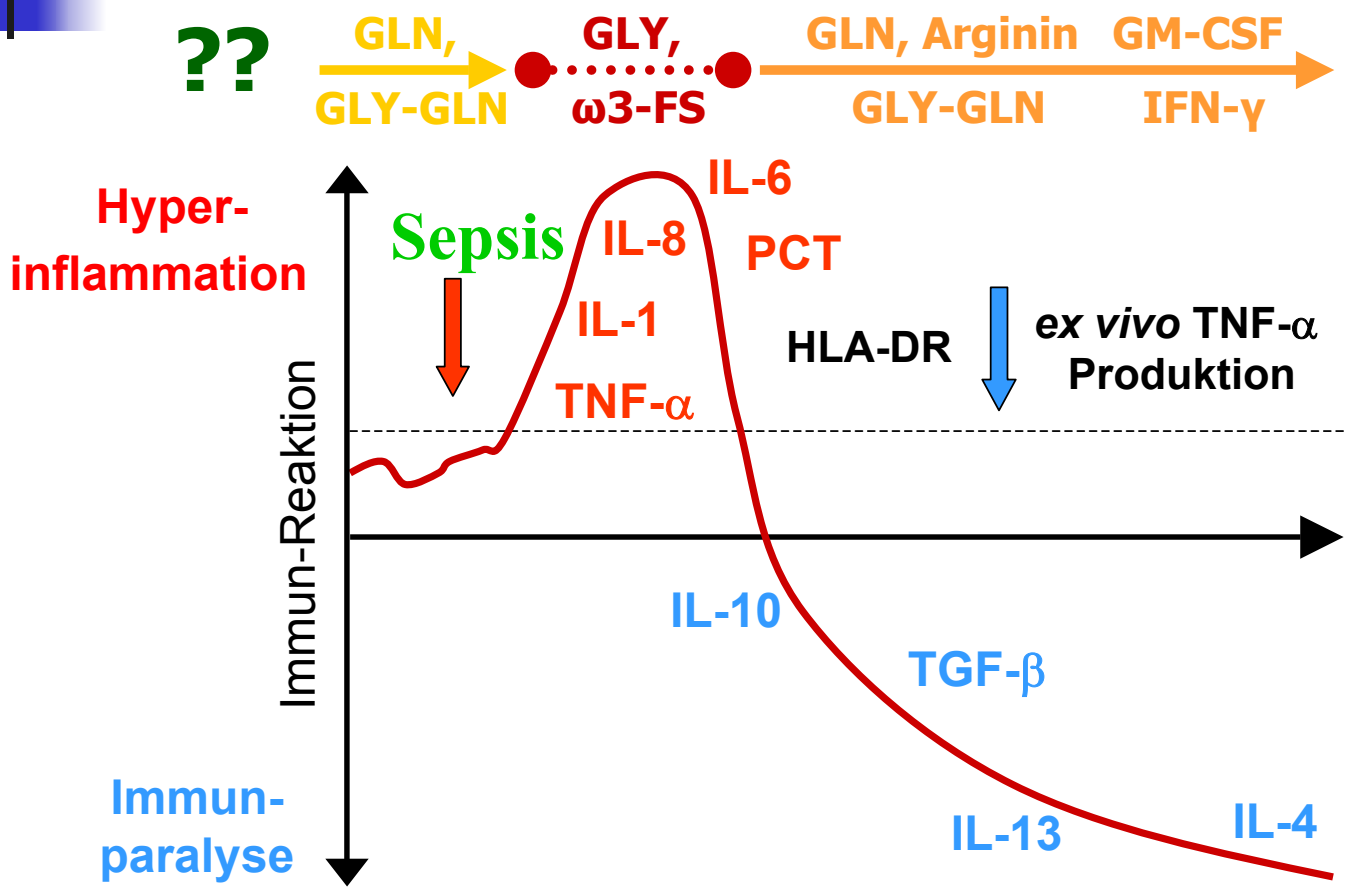
ω-3 Fettsäuren

- Reduziert proinflammatorische Zytokinsekretion (IL-1, IL-6, TNF-α)
- Vermindert Lymphozytenproliferation
- Reduziert Granulozytenadhäsion und Extravasation
- Reduziert die Produktion von toxischen Sauerstoffradikalen
- Reduziert die Gefäßpermeabilität

Unterschiedliche Sepsisphasen brauchen unterschiedliche therapeutische Interventionen



Unterschiedliche Sepsisphasen brauchen unterschiedliche therapeutische Interventionen



Ein neuer "Starting Point"

The New England
Journal of Medicine

VOLUME 345 NOVEMBER 8, 2001 NUMBER 19

INTENSIVE INSULIN THERAPY IN CRITICALLY ILL PATIENTS

GREET VAN DEN BERGHE, M.D., PH.D., PIETER WOUTERS, M.SC., FRANK WEEKERS, M.D., CHARLES VERWAEST, M.D., FRANS BRUYNINCKX, M.D., MIET SCHETZ, M.D., PH.D., DIRK VLASSELAERS, M.D., PATRICK FERDINANDE, M.D., PH.D., PETER LAUWERS, M.D., AND ROGER BOUILLON, M.D., PH.D.

Schlussfolgerungen der Autoren

- Reduktion der Mortalität auf der ICU von 8,0% auf 4,6%

- In conclusion, the use of exogenous insulin to maintain blood glucose at a level no higher than 110 mg per deciliter reduced morbidity and mortality among critically ill patients in the surgical intensive care unit, **regardless of whether they had a history of diabetes.**

Glukose und Immunbalance

Zytokine

The presence of glucose leads to a **higher resting cytokine** production; **after stimulation**, this **cytokine production is impaired** compared to the situation without glucose.

Morohoshi M. et al. *Ann NY Acad Sci* 1995; 748:562
Reinhold D. et al. *Horm Metab Res* 1996; 28:267

Phagozytose

Impairment of phagocytosis is found in PMNs isolated from poorly regulated patients and **better regulation** of the DM leads to an **improved function**.

Delamaire M. et al. *Diabetic Met* 1997; 14:29
Bagdade J.D. et al. *Diabetes Care* 1997; 20:392

Oxidative burst

Oxidative burst is higher or the same in PBMC of diabetic patients; **after stimulation, oxidative burst in PBMC is lower** than that of control patients.

Marhoffer W. et al. *Diabetes Care* 1992; 15:256
Shah S.V. et al. *J Clin Enodcrinol Metab* 1983; 57:740

Glukose und Immunbalance

Killing

The **killing capacity** of diabetic PMNs **is lower** than that of control PMNs.

Tater D. et al. *Horm Metab Res* 1987; 19:642
Tan J.S. et al. *J Lab Clin Med* 1975; 85:26
Gin H. et al. *J Clin Pathol* 1984; 37:1024

Monozyten

Impaired chemotaxis and **phagocytosis** - impaired function is possibly caused by an intrinsic defect in the monocytes themselves.

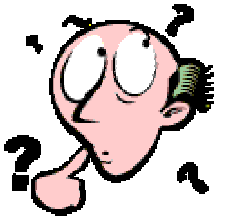
Deresinski S. et al. *Infect Dis Rep* 1995; 1:1
Katz S. et al. *Diabetes Care* 1983; 6:479

Virulenz

Some microorganisms become **more virulent** in a high glucose environment

Geerlings S.E. et al. *J Med Microbiol* 1999; 48:535
Hostetter M.K. *Diabetes* 1990; 39:271

Wie geht es weiter ?



Entwicklung und Etablierung eines Sepsis **Bedside**-Immunmonitoring

- ✓ Durchführung von Multicenter Studien mit standardisierten Protokollen und genauen Einschlusskriterien
- ✓ Immunologische Muster erkennen
- ✓ Erkennen von hyper-, hypo- und immunparalytischen Phasen
- ✓ Therapeutische Intervention entsprechend den Sepsisphasen

Vorteile des Immunmonitorings

- ✓ Frühe Identifikation von Patienten mit hohem Risiko
- ✓ Monitoring während der Therapie
- ✓ verbesserte Therapiemöglichkeiten
- ✓ verbessertes Patienten- und Kosten-Managment

Vielen Danke für Ihre Aufmerksamkeit