Madrid 17-19 noviembre 2011

9° Congreso de la Sociedad Espanola de Reumatologia Pediatrica

# Pathogenesis of Macrophage Activation Syndrome

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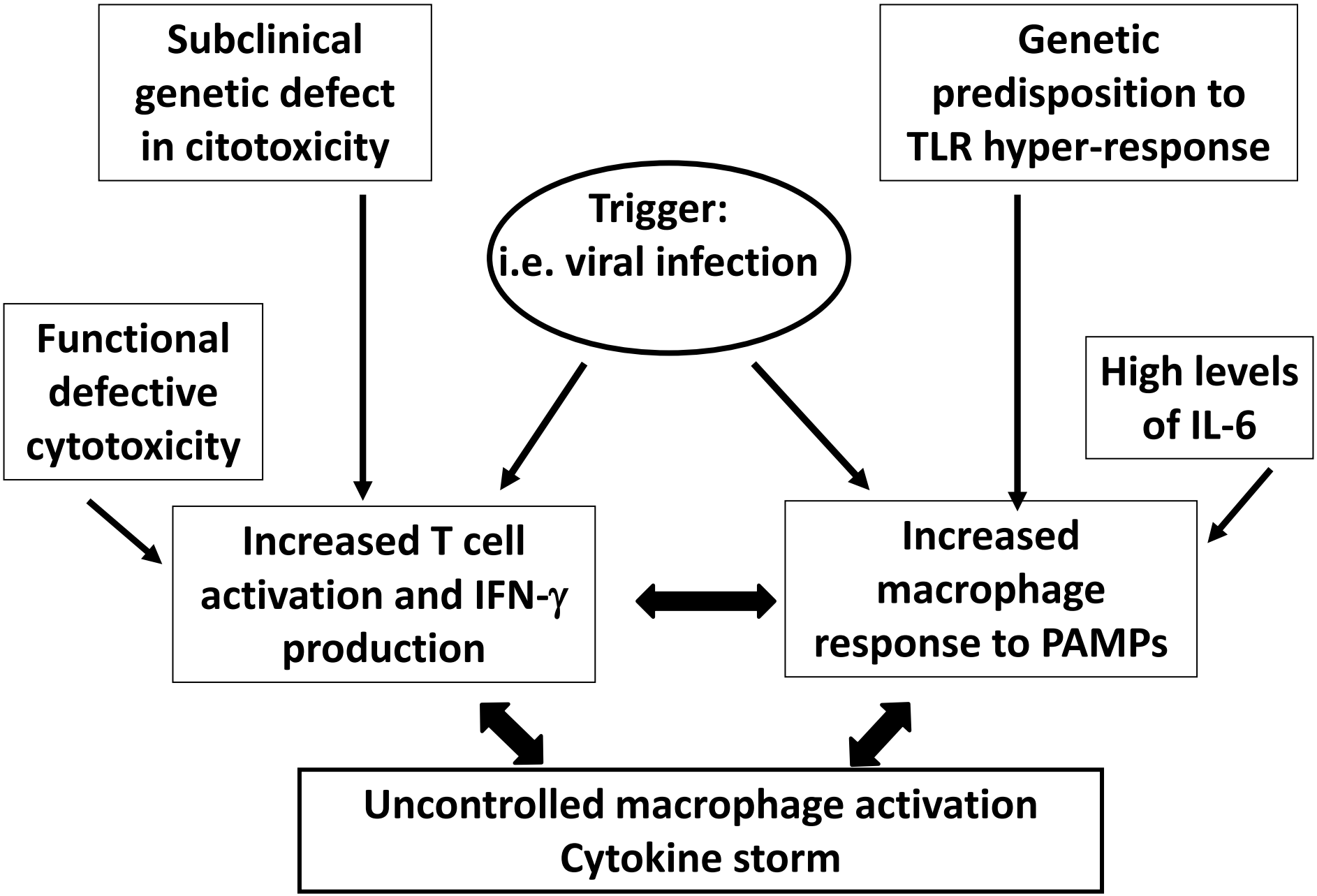
Bambino Gesù
OSPEDALE PEDIATRICO

# “L’Amore Dormiente” (Caravaggio) A sleeping cupid with systemic juvenile idiopathic arthritis (Espinel CH, Lancet 1994)

# Macrophage Activation Syndrome



- **Clinical presentation, classification and diagnosis**
  - **criteria, guidelines versus primary HLH**
- **Pathogenesis of MAS (s-JIA associated)**
  - **animal models, role of TLR stimulation, high background IL-6, cytotoxic defect**
- **Secondary (non-familial HLH)**
  - **frequency (?), severity**
- **Novel therapeutic target**
  - **IFN-γ in animal models**

# MAS: Clinical features

- **<u>Clinical presentation</u>:**

  typically acute, can be dramatic
- **<u>Clinical symptoms</u>:**

  nonremitting high fever

  hepatosplenomegaly

  lymphoadenopathy

  hemorrhagic manifestations **(purpura, easy bruising, mucosal bleeding)**

  CNS dysfunction **(lethargy, irritability, disorientation, headache, seizures, coma)**

  occasionally renal and cardiac failure

# MAS: Laboratory features

- **Cytopenia (leukopenia, anemia, thrombocytopenia)**
- **Elevated serum liver enzymes (ALT, AST, GGT, bilirubin)**
- **Abnormal coagulation profile (prolonged PT and PTT, hypofibrinogenemia, increased FDP and D-dimer)**
- **Elevated triglycerides, LDH**
- **Reduced Na and albumin**
- **Decreased ESR**
- **Hyperferritinemia**

# Preliminary diagnostic guidelines for MAS complicating systemic JIA

## Laboratory criteria

- **Decreased PLT** (≤ 262 x $10^{9)}$
- **Elevated GOT/AST** (> 59 mU/L)
- **Hypofibrinogenemia** (≤ 2.5 g/L)
- **Decreased WBC** (≤ 4.0 x $10^{9}$/L)

## Clinical criteria

- **Hemorrhages** (purpura, easy bruising, mucosal bleeding)
- **CNS dysfunction** (irritability, disorientation, lethargy, headache, seizures, coma)
- **Hepatomegaly** (≥ 3 cm below the costal arch)

## Histopathologic criterion

**Hemophagocytosis in the bone marrow**

**Diagnostic rule: the diagnosis of MAS requires the presence of any 2 of the 4 laboratory criteria or at least 1 laboratory and 1 clinical criteria. A BM aspirate for the demonstration of macrophage hemophagocytosis may be required only in doubtful cases**

**Ravelli A et al. J Pediatr 2005**

# Classification of HLH

| **Primary/Genetic** | |
|---|---|
| Familial | FHL1 |
| | FHL2 |
| | FHL3 |
| | FHL4 |
| | FHL5 |
| Sporadic onset associated with immune deficiencies | Chediak Higashi syndrome |
| | Griscelli syndrome type 2 |
| | Hermansky-Pudlak syndrome Type II (HPS II) |
| | X linked lymphoproliferative disorder type 1 |
| | X linked lymphoproliferative disorder type 2 |
| **Secondary/acquired** | |
| | Infections |
| | Autoinflammatory/macrophage activation syndrome |
| | Maligancy |
| | Immunosuppression |
| | Metabolic |

# HLH diagnostic guidelines

1. Fever
2. Splenomegaly
3. Cytopenia (at least 2 of the 3):
   - HB < 90 g/l
   - PLT <100 x 10 9 /l
   - Neutrophils <1,0 x 10 9 /l
4. Hypertriglyceridmia and/or hypofibrinogenemia:
   - TG ≥ 265 mg/dl
   - Fibrinogen ≤ 1.5 g/l
5. Hemophagocytosis in BM, spleen or LN (no evidence of malignancy)
6. Low or absent NK cell activity
7. Ferritin ≥ 500 ng/ml
8. Soluble CD 25 ≥ 2 400 U/ml

**Diagnostic rule: HLH is diagnosed when at least 5 criteria are met**

Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al.
HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis.
Pediatr Blood Cancer 2007;48:124-31

- **Clinical presentation, classification and diagnosis**
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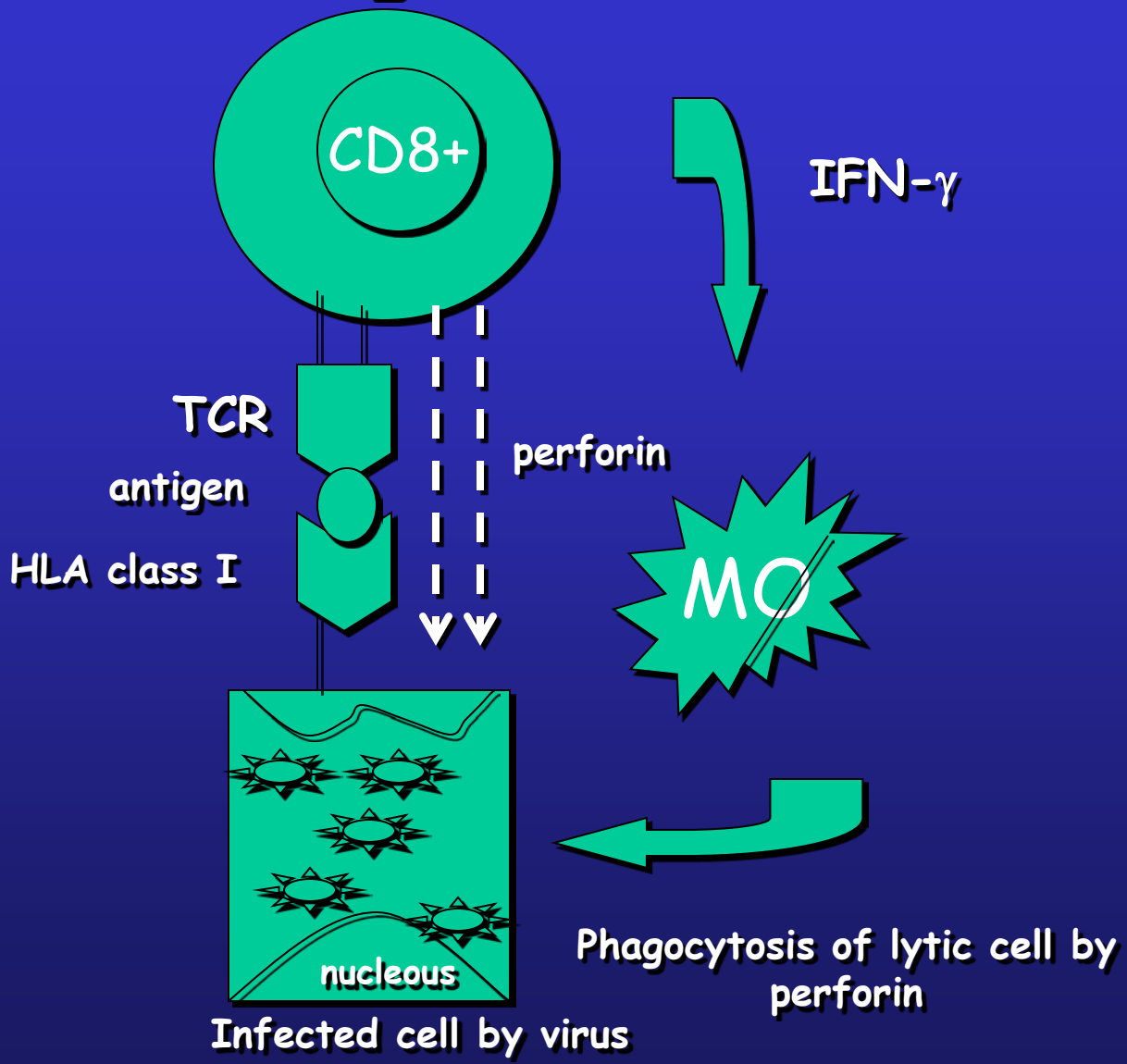
| | Infections |
| | Autoinflammatory/macrophage activation syndrome |
| | Maligancy |
| | Immunosuppression |
| | Metabolic |

# Genetics of Familial HLH

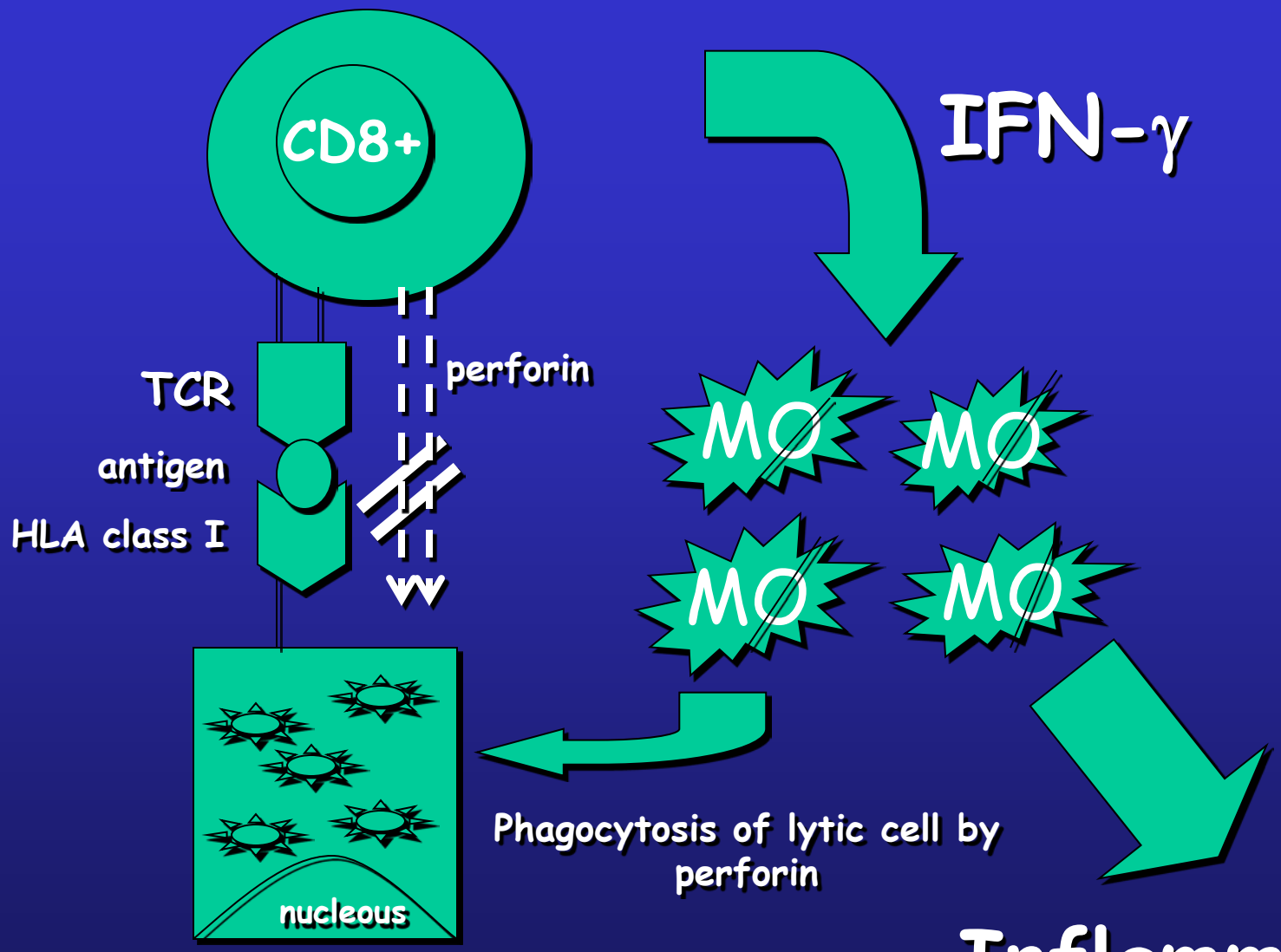
- **Genes in familial HLH**
  - **FHL2: mutations in the gene encoding perforin (*PRF1*)***
  - **FHL3: mutations in the gene encoding Munc13-4 (*UNC13D*)***
  - **FHL4: mutations in sintaxin 11 (*STX11*)***

***Involved in controlling granule exocytosis in cytotoxic lymphocytes**

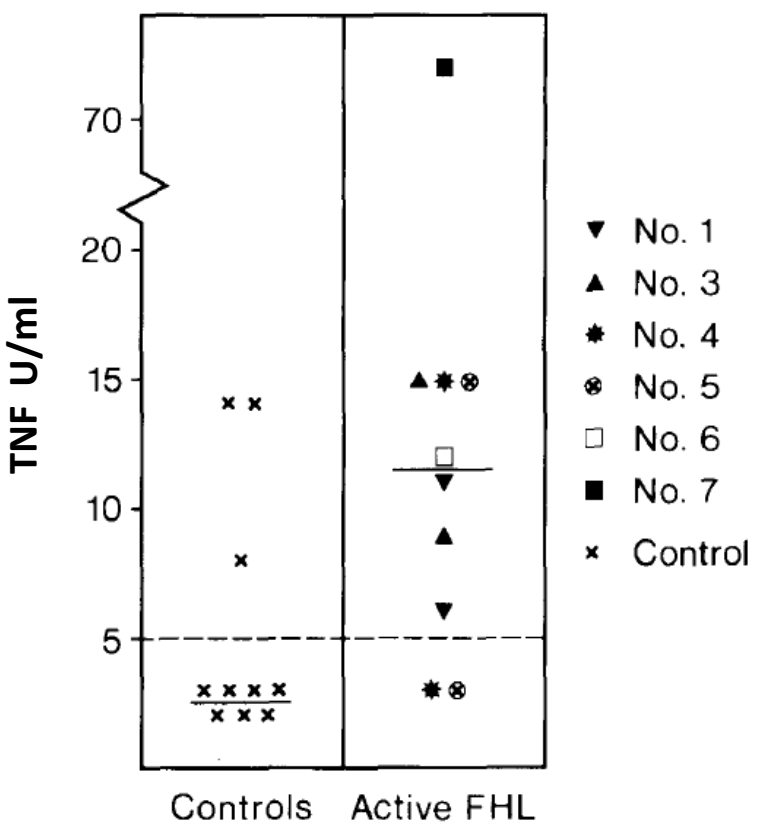
# Killing Mechanisms

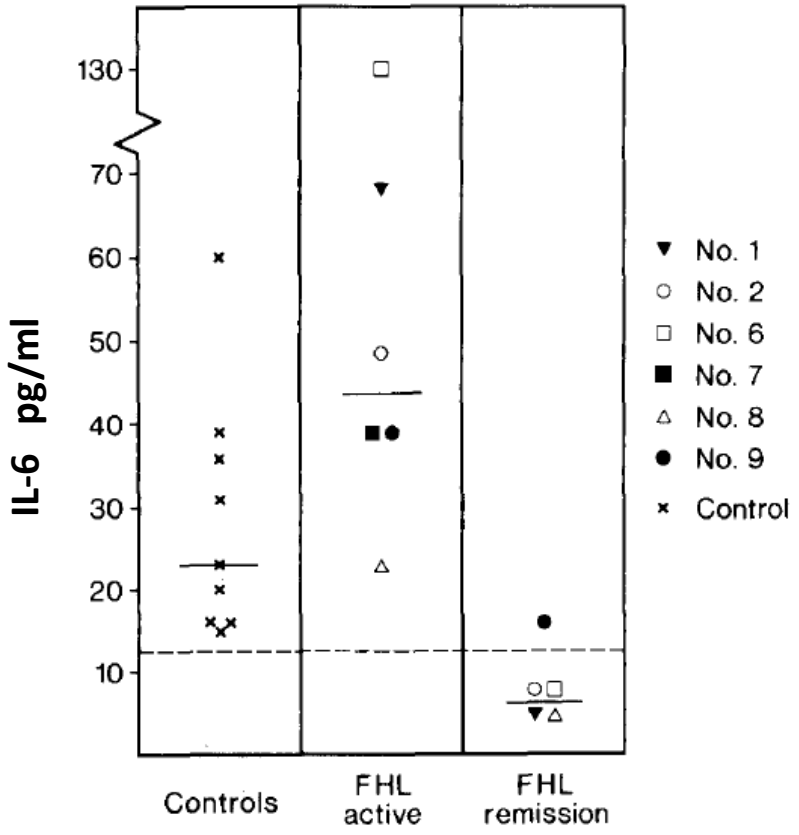


# Perforin Defect



# High circulating levels of inflammatory cytokines in familial HLH

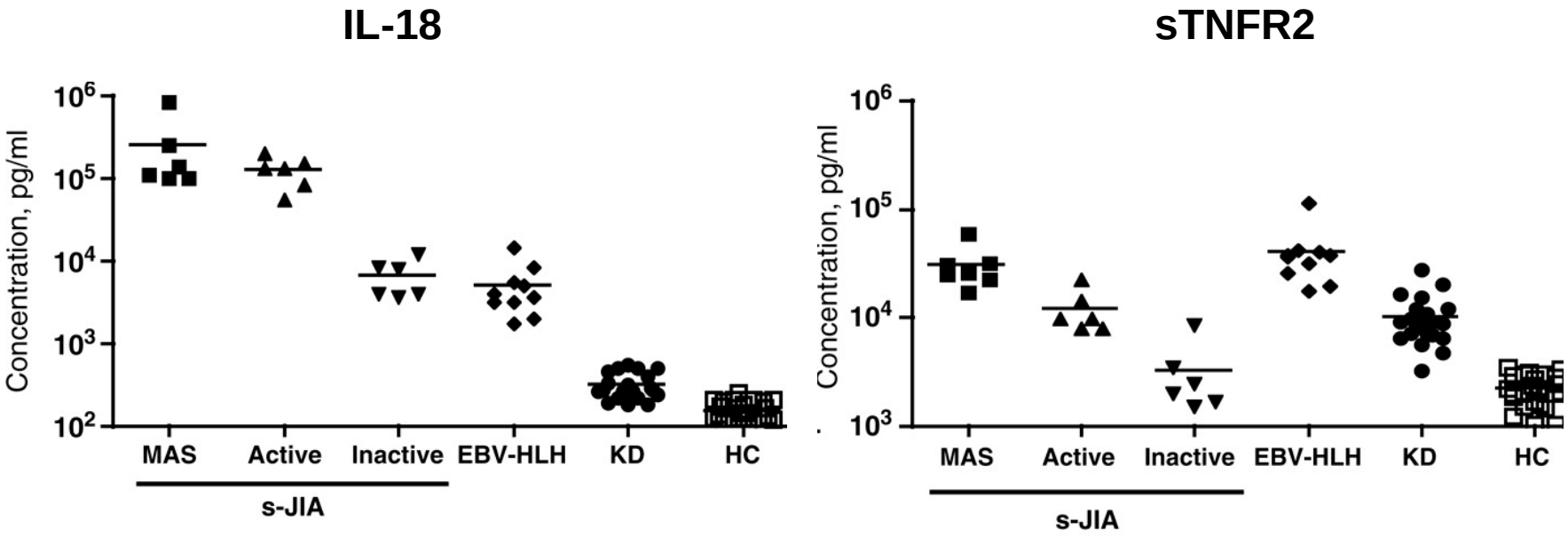



Henter et al, Blood 1991

# High circulating levels of inflammatory cytokines in s-JIA associated MAS

- **Increased levels of TNF-α (and of soluble TNF Receptors 1 and 2), IL-6 and IL-18**




Stephan CER 1994
De Benedetti Br J Rheumatol 1997
Shimizu Rheumatology 2010

# HLH: Pathogenesis

**Non-Familial**

**Familial**

**MAS**

**Genetic defect in the cytotoxic pathway**

**Activation of Mo and T cells**

# HLH

## Non-Familial

## Familial

Genetic defect in the cytotoxic pathway

Activation of Mo and T cells

## MAS

Background of Rheum Dis

No known genetic defect

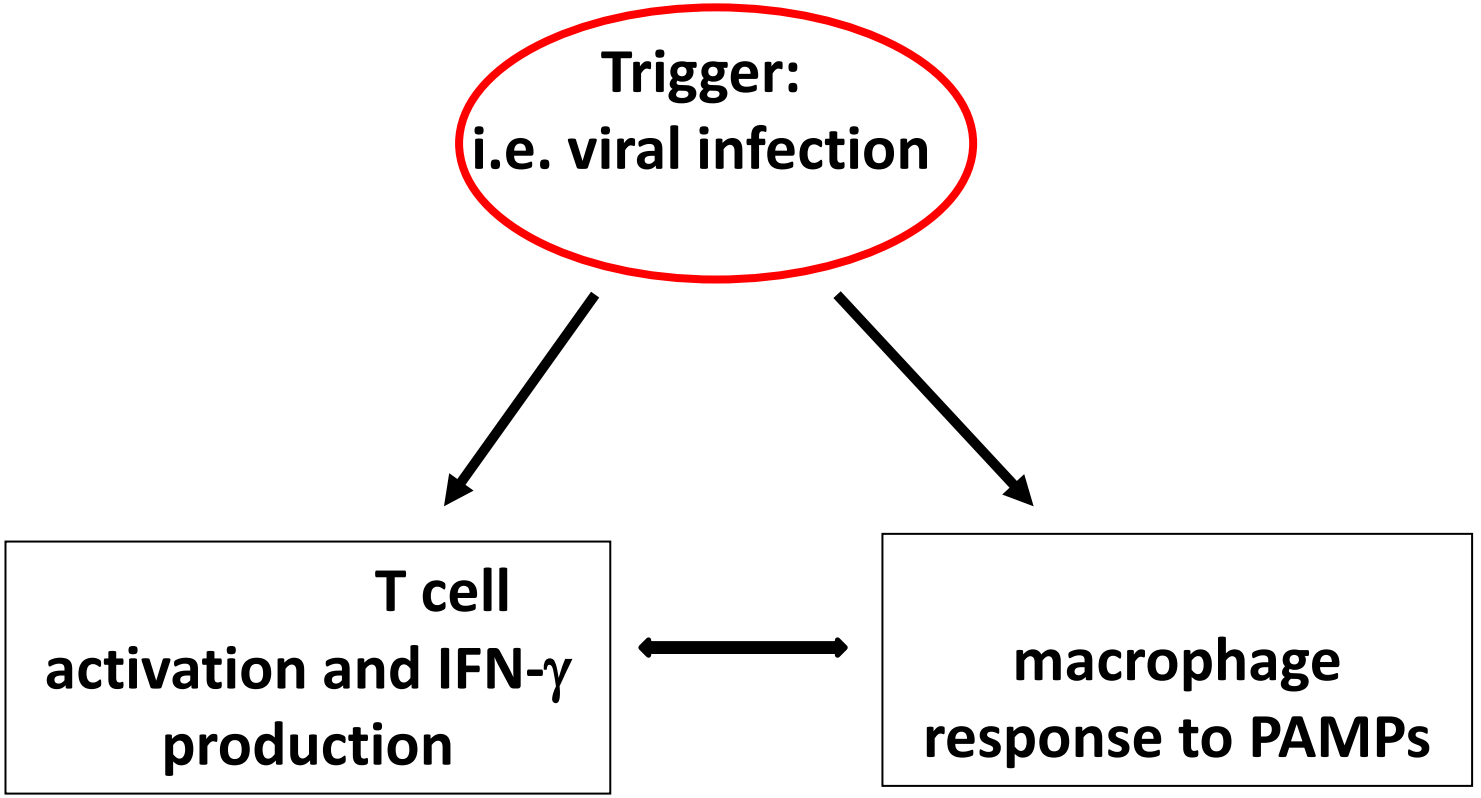
No clear pathogenesis

**Unique clinical syndrome**

# MAS: Triggering factors

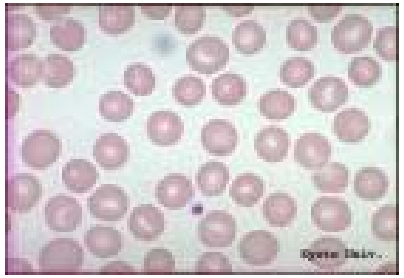
- **No triggers**
- **Flare-up of the underlying disease**
- **Infections (viral, often EBV, other infections)**
- **Changes in Treatment (Aspirin or other NSAIDs, gold salts, sulfasalazine, methotrexate, biologics)**

# MAS


Trigger:
i.e. viral infection
T cell
activation and IFN-γ
production
macrophage
response to PAMPs

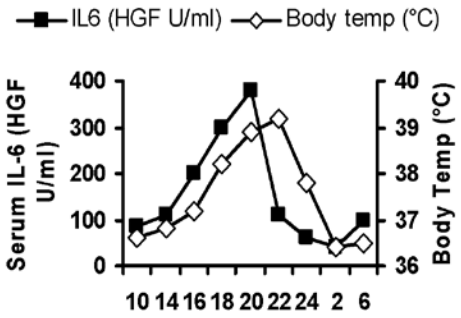
# Prominent Interleukin-6 production in systemic JIA

## Anemia



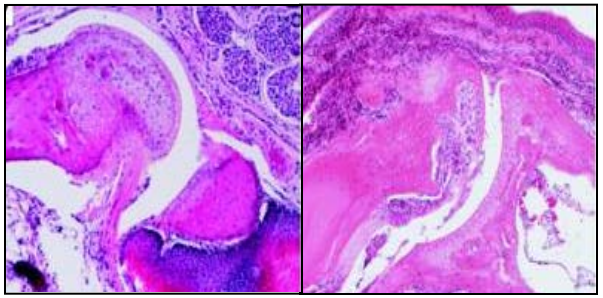
Lancet 1995
Blood 1996

## Fever



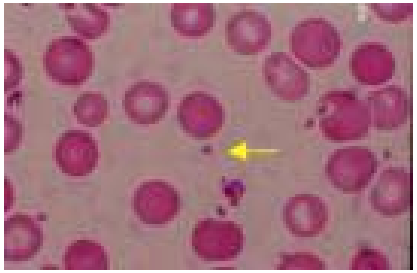

A&R 1991

## Joint Inflammation



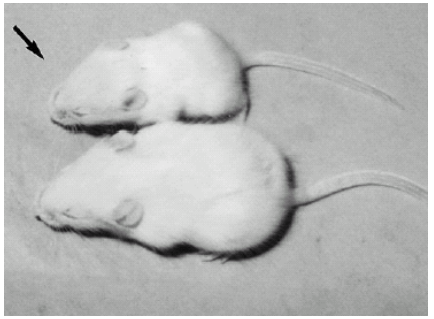
J Exp Med 1998

## Thrombocytosis



A&R 1991

## Growth Impairment

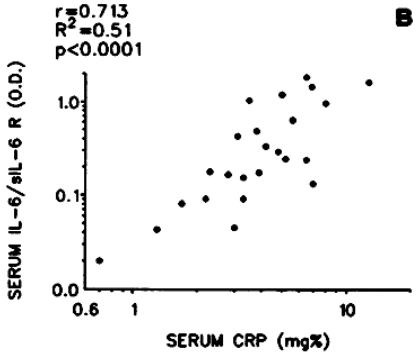


J Clin Invest 1997
Endocrinol 2001

## Osteoporosis

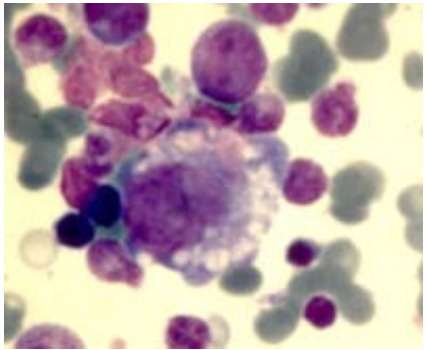
A&R 2006

## IL6/soluble IL6R and CRP



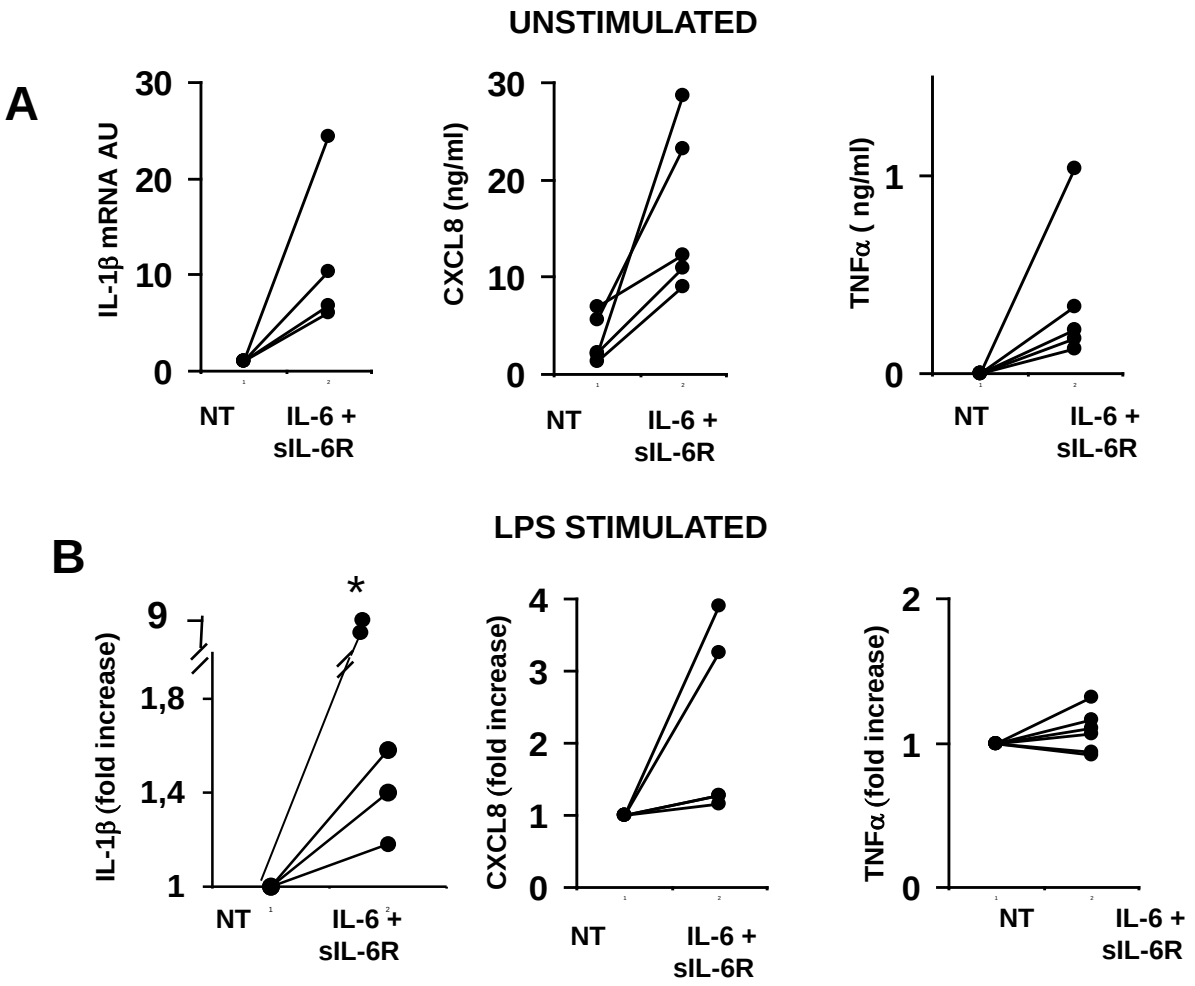

J Clin Invest 1994

## MAS



# IL-6 and cytokine production

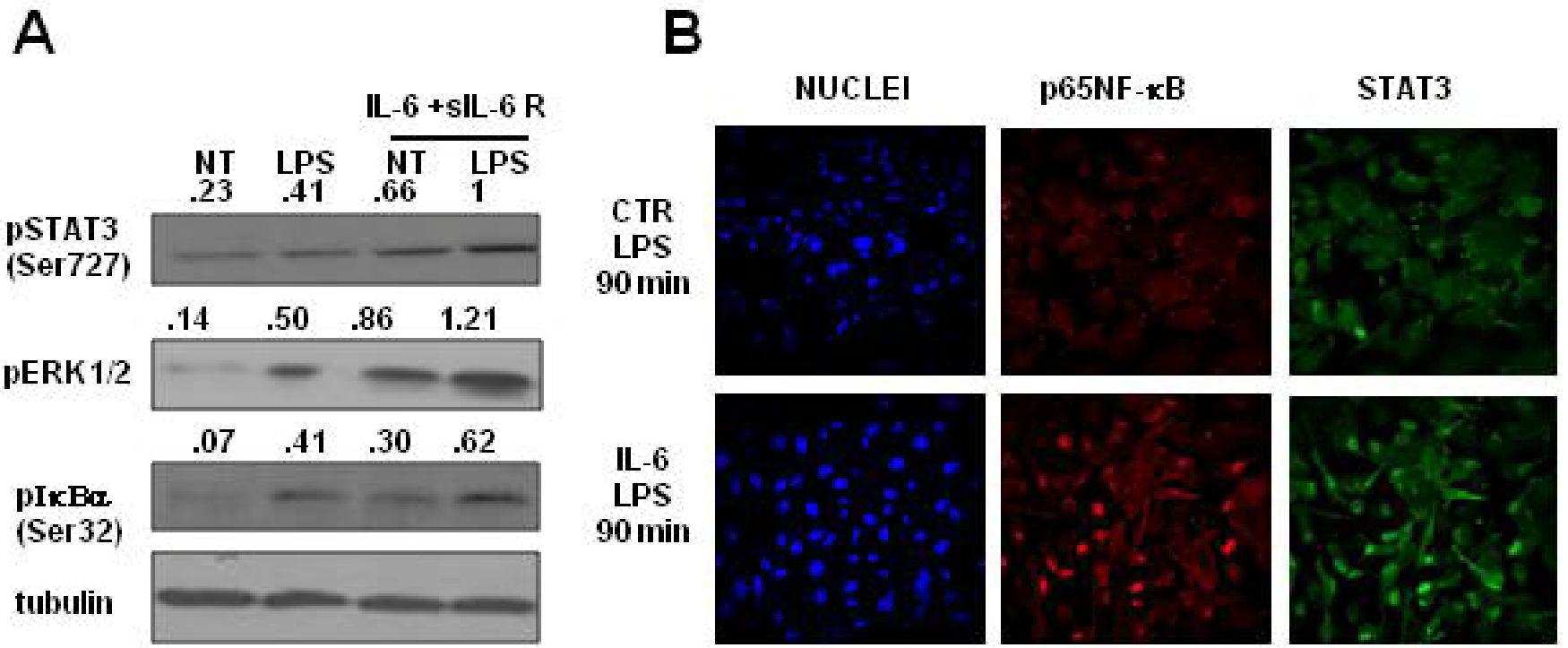
## Preincubation with IL-6 + sIL-6R of human macrophages




Strippoli R et al, A&R 2011 in press

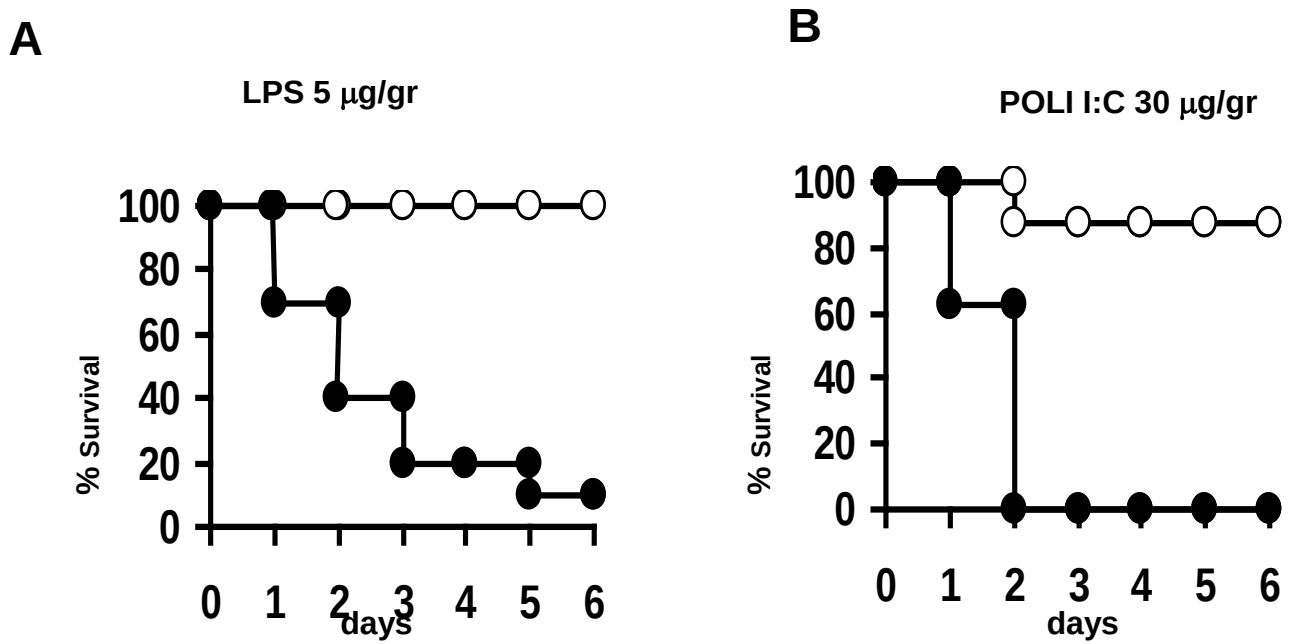
# IL-6 and TLR responses

## Preincubation with IL-6 + sIL-6R in human macrophages

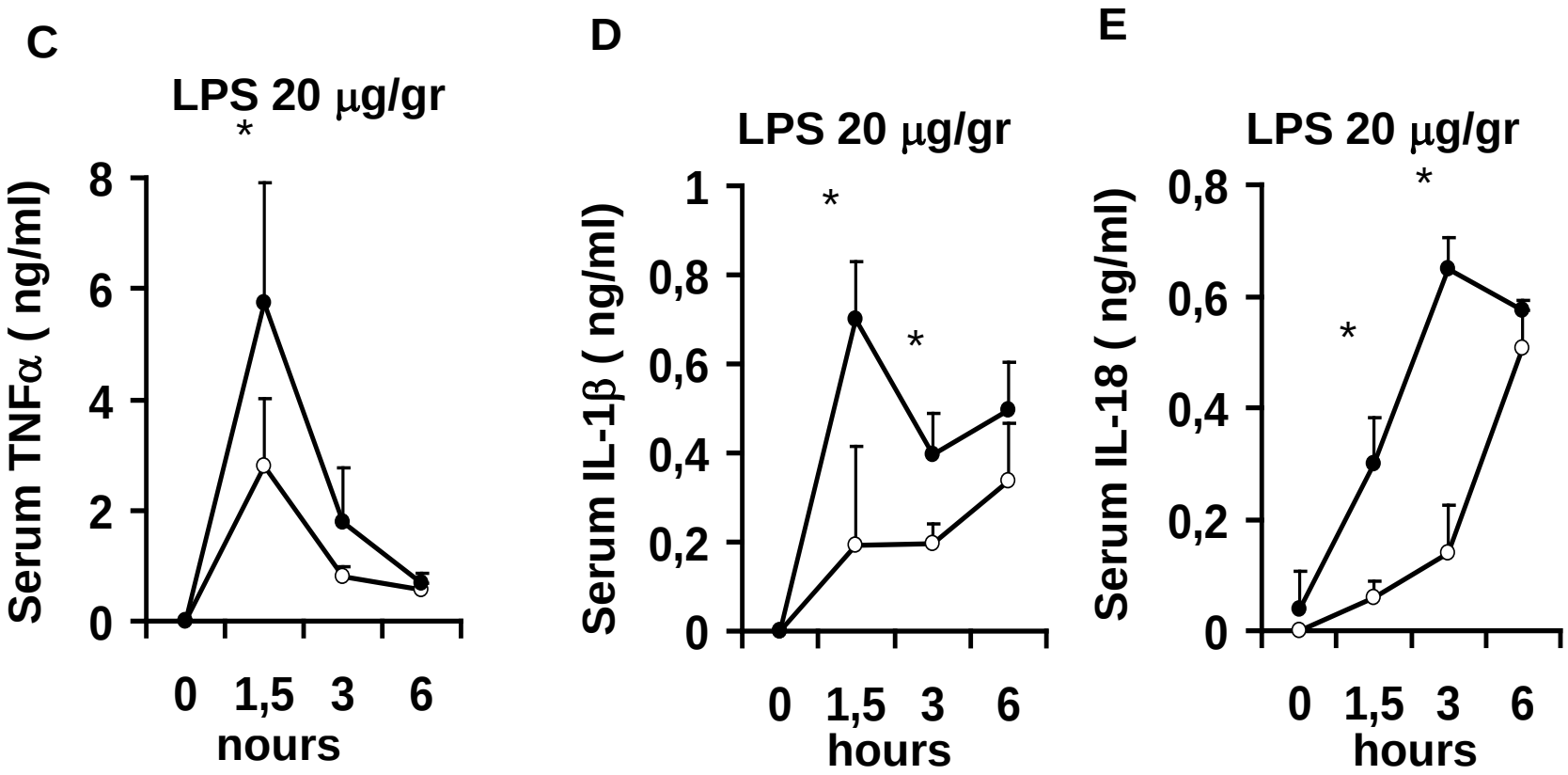



Strippoli R et al, A&R 2011 in press

# IL-6 transgenic mice
# Increased lethality following TLR ligands administration



# IL-6 transgenic mice
# High IL-6 in vivo lead to hyperresponse to TLR ligands



# Exposure to high levels of IL-6 in vivo leads to increased lethality to TLR ligands in IL-6 transgenic mice

| | LPS | WT Mean SEM | TG Mean SEM |
|---|---|---|---|
| Neutrophil count ($x10^3/mm^3$) | 0 h | 0.67 0.27 | 0.63 0,14 |
| | 12 h | 0.91 0.025 | 0.71 0,18 |
| | 96 h | 0.67 0.175 | 0.28 0,12* |
| Hemoglobin levels (gr/dl) | 0 h | 14.6 0.69 | 13.7 1,04 |
| | 12 h | 15,6 1.05 | 15.6 0,49 |
| | 96 h | 14,9 0.15 | 10.1 0.3* |
| Ferritin levels (ng/ml) | 0 h | 265 96.70 | 298 138.8 |
| | 24 h | 530 75.1 | 1358 445.9* |
| LHD (U/L) | 24 h | 3864 444 | 6343 984* |

Strippoli R , submitted

# High levels of IL-6 induce hyperesponse to TLR ligands mimicking infections

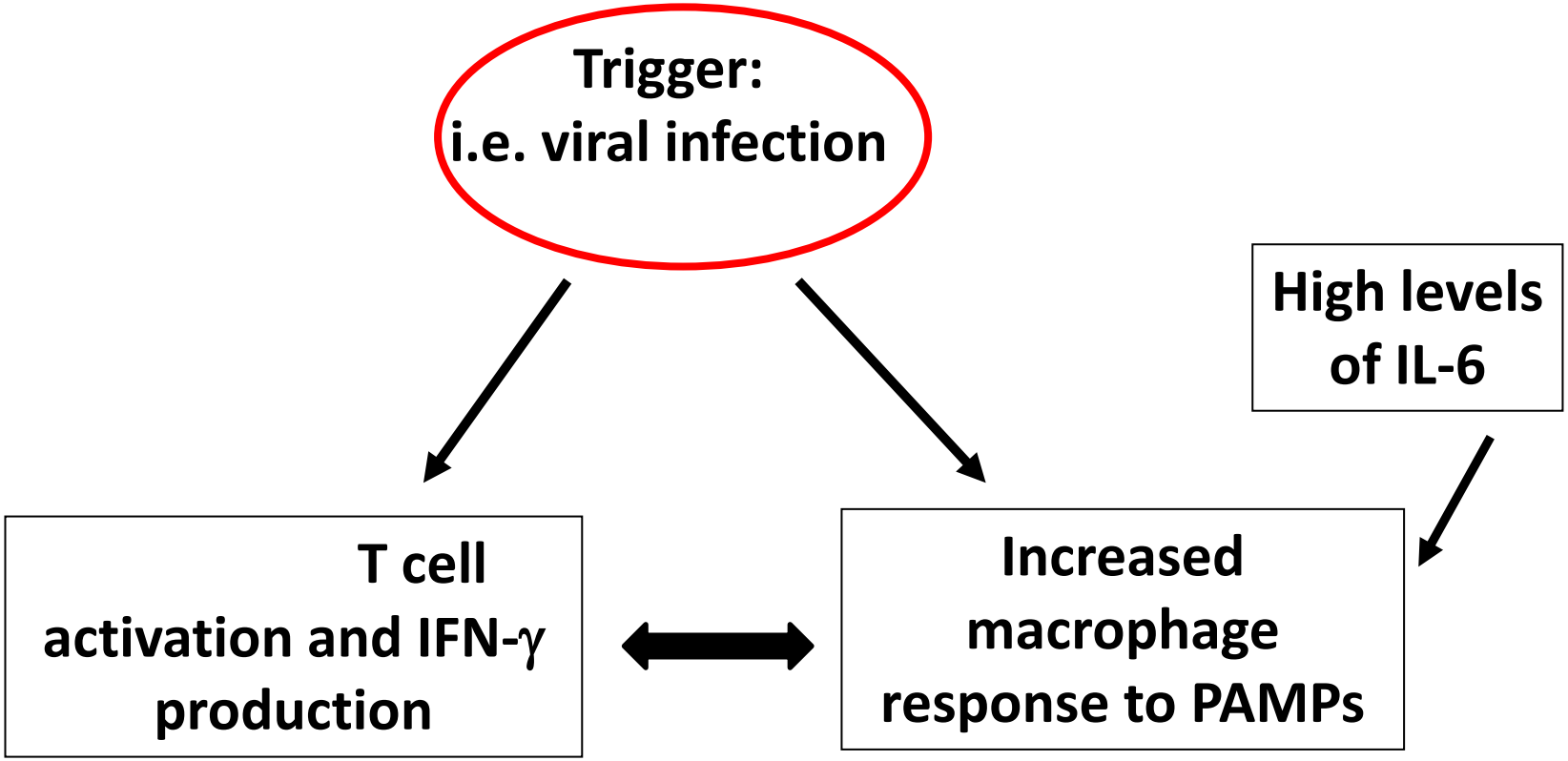
## Human macrophages

- Increased IL-1 and chemokine expression
- Increased CD68 expression
- Increased NFκB nuclear translocation and MAP kinases activation following TLR activation

## IL-6 transgenic mice

- Increased production of IL-1β, IL-18, TNF-a, IFN-γ
- Increased lethality following TLR ligands
- Increased ferritin, sCD25
- Decrease in platelet and white blood cell counts

# MAS


Trigger:
i.e. viral infection
High levels of IL-6
T cell activation and IFN-γ production
Increased macrophage response to PAMPs

# NATURAL KILLER CELL DYSFUNCTION IN PATIENTS WITH SYSTEMIC-ONSET JUVENILE RHEUMATOID ARTHRITIS AND MACROPHAGE ACTIVATION SYNDROME

ALEXEI A. GROM, MD, JOYCE VILLANUEVA, BS, SUSAN LEE, BS, ELLEN A. GOLDMUNTZ, MD, MURRAY H. PASSO, MD, AND ALEXANDRA FILIPOVICH, MD

*(J Pediatr 2003;142:292-6)*

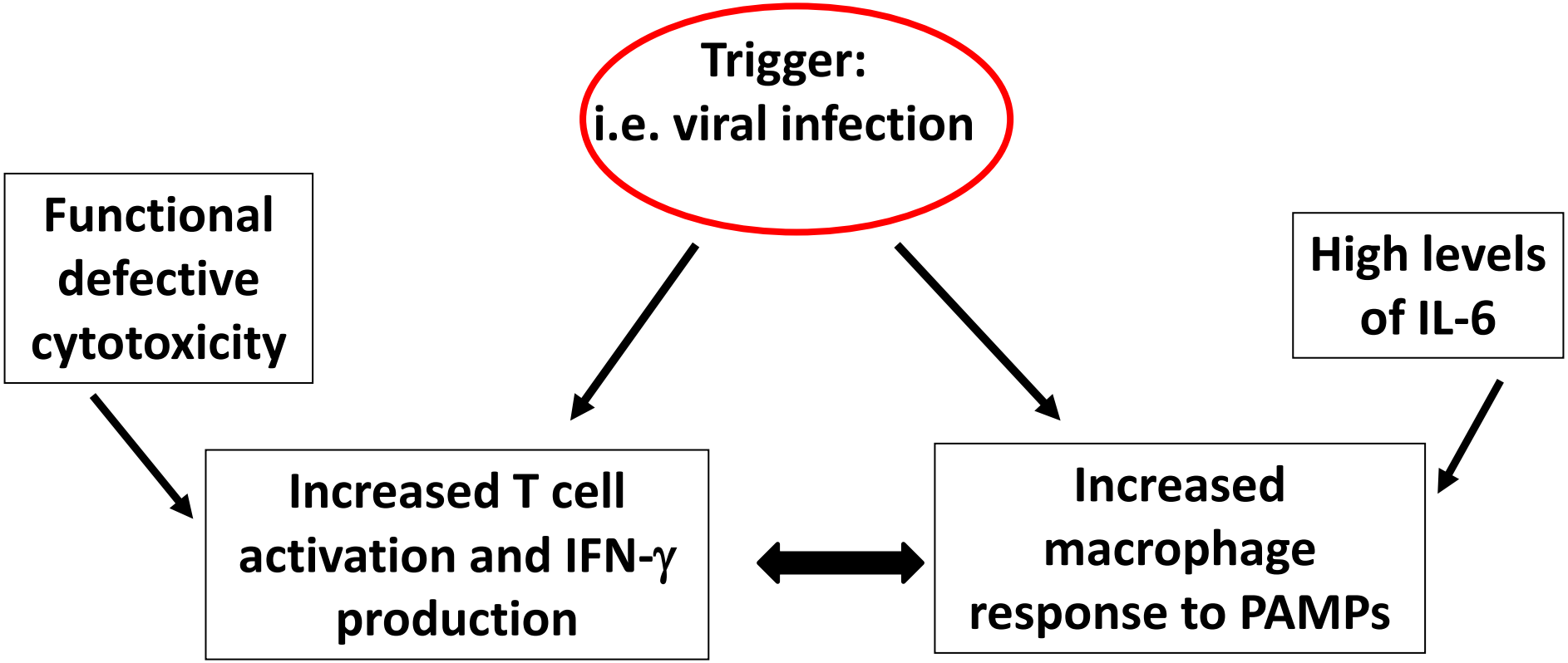
**Table II. Patterns of NK activity and perforin expression in cytotoxic CD8+ cells, NK cells, and NK T cells**

| Patient | Age (y) /Sex | NK activity (LU) | NK cells (%) | Perf+NK cells (%) | Perf+CD8+ cells (%) | Perf+NK T cells (%) |
|---|---|---|---|---|---|---|
| 1 | 17 y/F | 0↓ | 0↓ | 0↓ | 42↑ | 44 |
| 2 | 7 y/M | 0.3↓ | 0.2↓ | 98↑ | 17↑ | 44↑ |
| 3 | 5 y/M | 0.3↓ | 4 | 93↑ | 16↑ | 25 |
| 4 | 16 y/M | 0↓ | 1↓ | 99↑ | 56↑ | 97↑ |
| 5 | 20 y/M | 0↓ | 1↓ | 81↓ | 6↓ | 3↓ |
| 6 | 22 y/F | 4.6↓ | 5 | 82↓ | 2↓ | 7↓ |
| 7 | 7 y/F | 0.6↓ | N/D | 64↓ | 4↓ | 0↓ |
| Controls | 1-15 y (n = 41) | 11.3 ± 4.9* | 13 ± 9 | 86 ± 5 | 7 ± 5 | 23 ± 11 |
| | 15-50 y (n= 39) | | 19 ± 12 | 92 ± 6 | 18 ± 10 | 54 ± 24 |

*The difference between the patients and controls is statistically significant at $P < .001$.
*N/D*, Not done.

**Grom, J Pediatrics 2003**
**Wulffraat, Rheuimatology 2003**

# MAS


Trigger:
i.e. viral infection
Functional defective cytotoxicity
High levels of IL-6
Increased T cell activation and IFN-γ production
Increased macrophage response to PAMPs

# Cytotoxic pathways in s-JIA

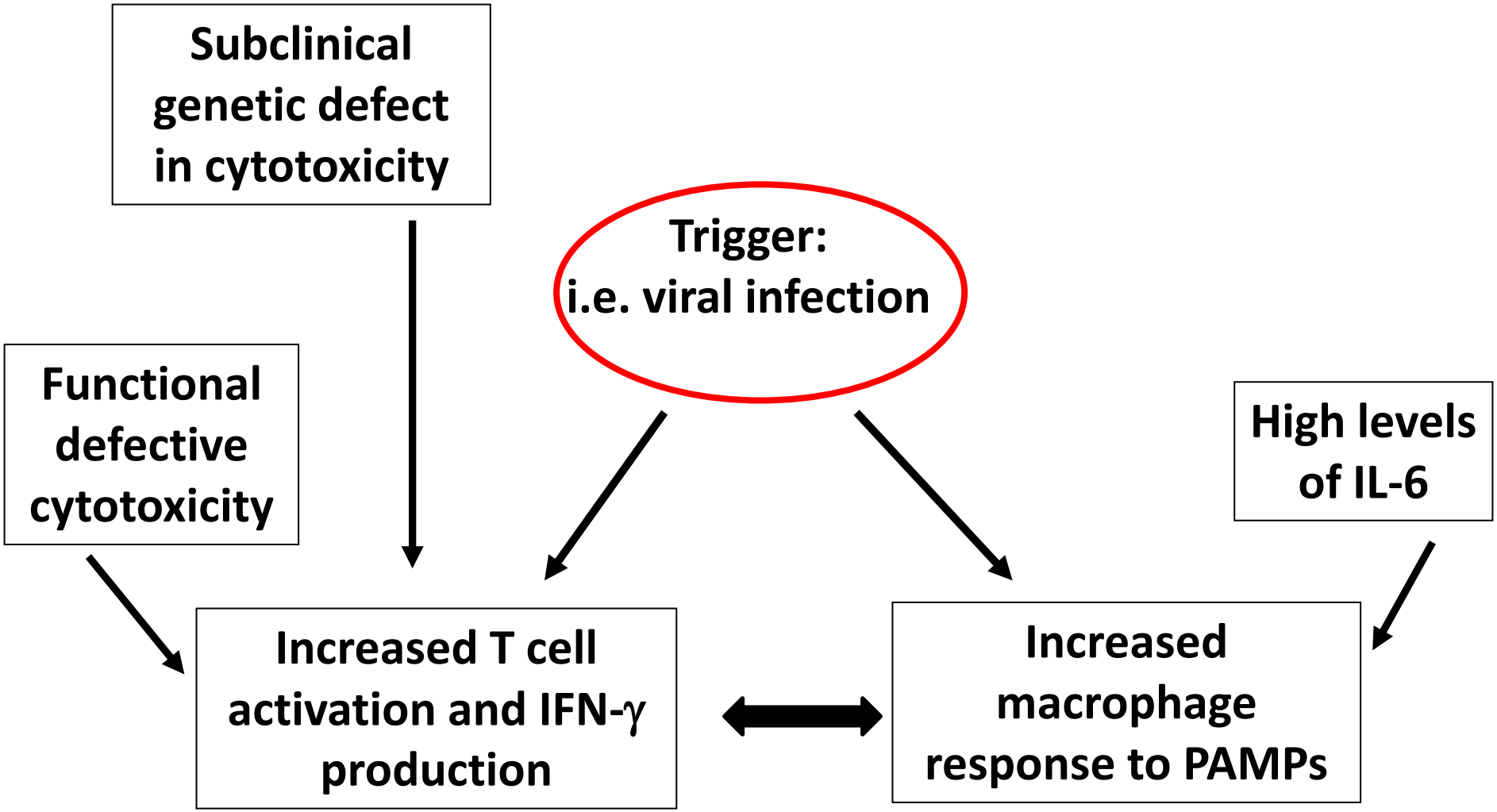
- **Heterozygosity of low penetrance perforin mutations**

  **(Vastert, Rheumatology 2010)**

- **Potentially functionally relevant MUNC haplotype and MUNC mutations**

  **(Zhang, A&R 2008; Hazen, A&R 2008)**

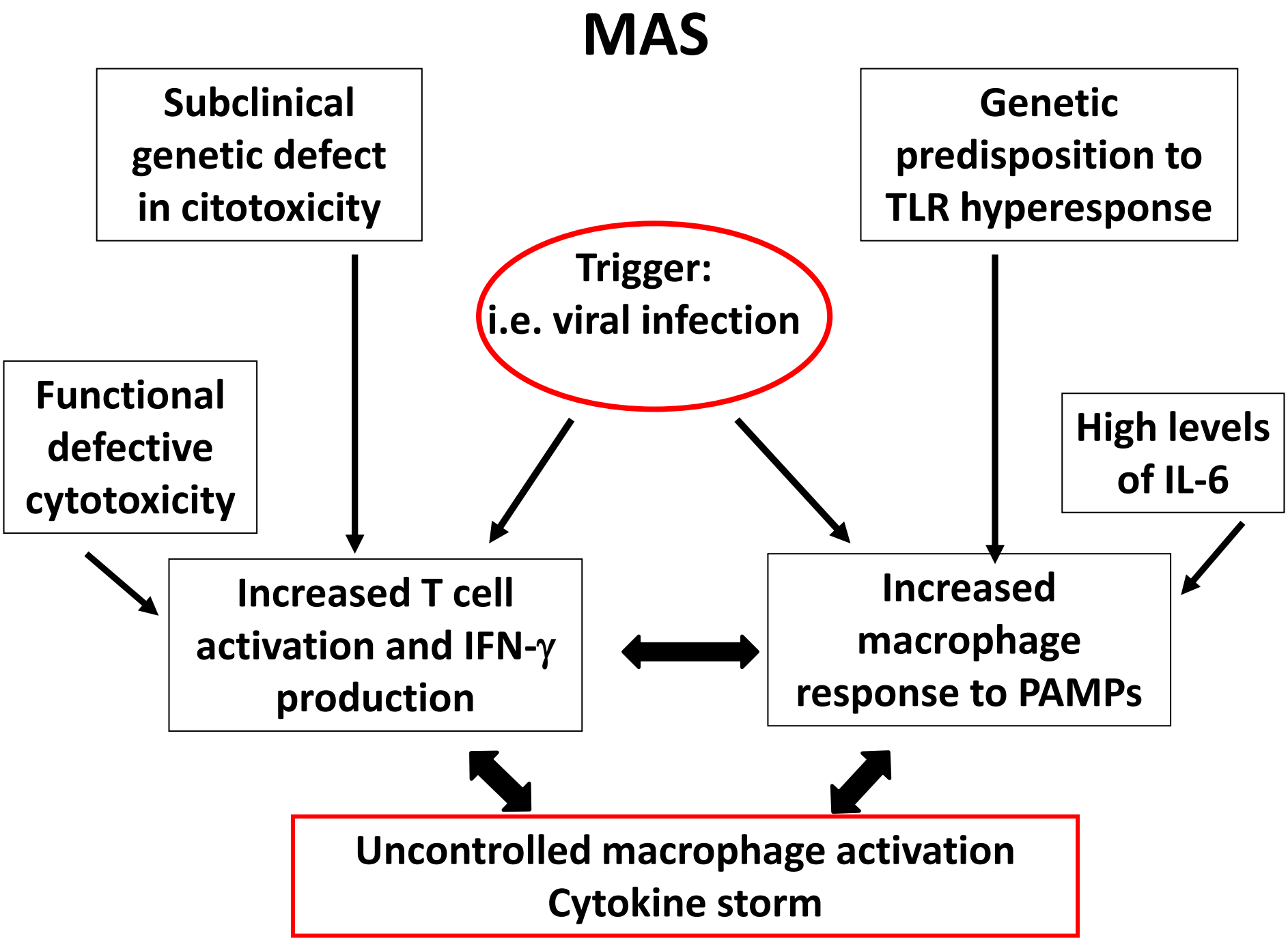
# MAS


Subclinical genetic defect in cytotoxicity
Trigger: i.e. viral infection
Functional defective cytotoxicity
High levels of IL-6
Increased T cell activation and IFN-γ production
Increased macrophage response to PAMPs

# Toll-like receptors signalling in s-JIA

**Yanagimachi M, et al. (J Rheumatol 2010)**

**Association of IRF5 Polymorphisms with Susceptibility to Macrophage Activation Syndrome in Patients with systemic Juvenile Idiopathic Arthritis**


MAS
Subclinical genetic defect in citotoxicity
Genetic predisposition to TLR hyperesponse
Trigger: i.e. viral infection
Functional defective cytotoxicity
High levels of IL-6
Increased T cell activation and IFN-γ production
Increased macrophage response to PAMPs
Uncontrolled macrophage activation
Cytokine storm

- **Clinical presentation, classification and diagnosis**
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# HLH

| | Familial | Non-Familial | MAS |
|---|---|---|---|
| 1) | 3 | 8 | 10 |
| 2) | 3 | 6 | 12 |
| 3) | 16 | 19 | NR |


1) OBG 2008-2010
2) Ishii, Blood 2005
3) Ibarra, Clin Vaccine Immunol 2011

# HLH Non-Familial

## Major organ involvement - Treatments

| | RicCel | RawAll | JosPer | NoeDof | GiuUba | MicLup | NicBia | LudDiC |
|---|---|---|---|---|---|---|---|---|
| CNS | Y | Y | | | | | Y | |
| Cardiac Failure | | Y | Y | | | Y | | Y |
| Renal Failure | | Y | Y | Y | | | Y | Y |
| GC High Dose | Y | Y | Y | Y | Y | Y | Y | Y |
| Cy-A | Y | Y | Y | Y | Y | Y | Y | Y |
| Cyclophosphamide | Y(**) | | Y(*) | | | Y | | Y |
| Anakinra | | | Y | | | Y | | Y |
| Plasmapheresis/UF | | Y | | | | | | Y |
| Length of stay(days) | 61 | 60 | 172 | 19 | 23 | 56 | 42 | 65 |

(*) 9 PULSES OVER 3 MONTHS
(**) 6 PULSES OVER 2 MONTHS

# HLH Non-Familial

## Complications

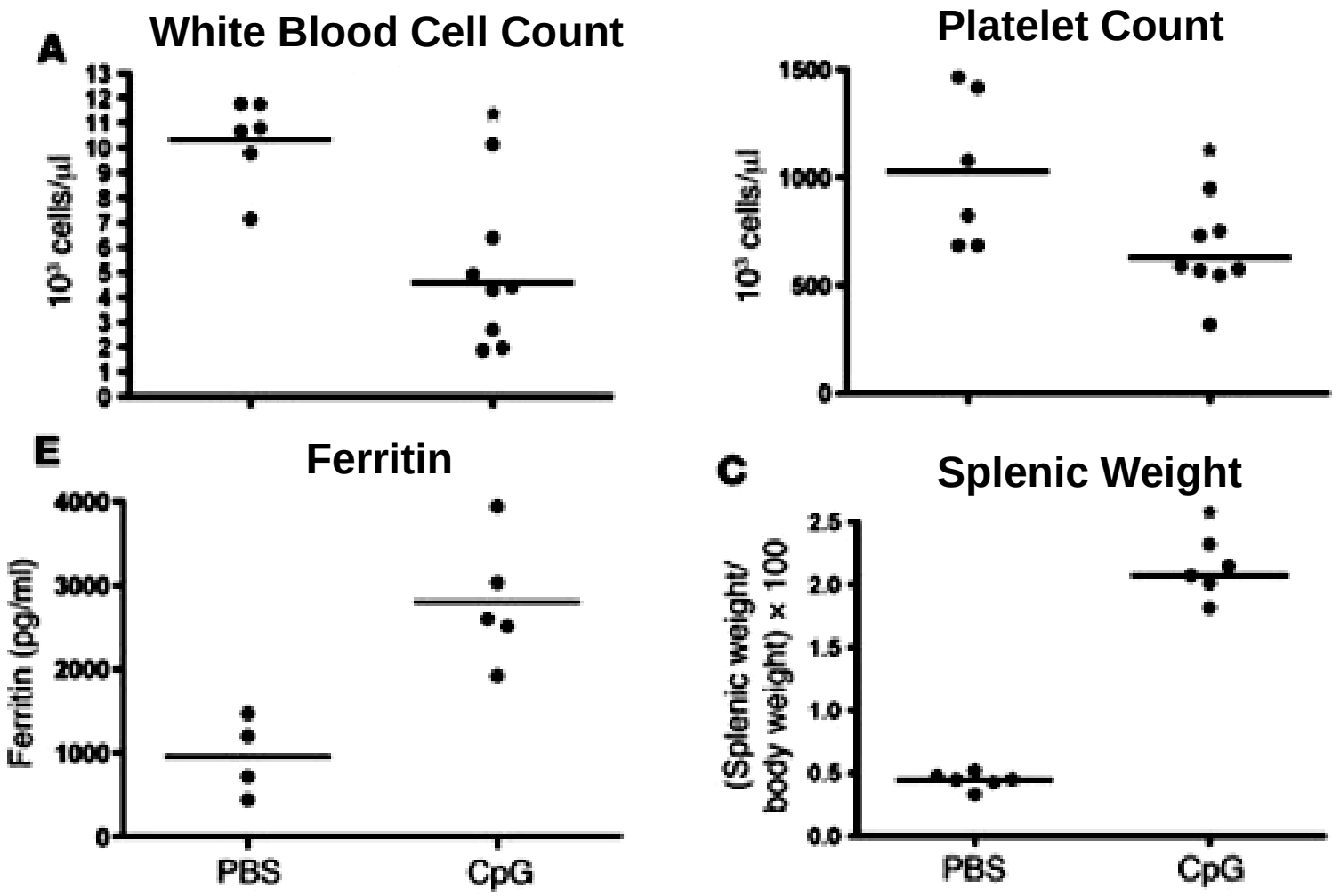
| | RicCel | RawAll | JosPer | NoeDof | GiuUba | MicLup | NicBia | LudDiC |
|---|---|---|---|---|---|---|---|---|
| Hypertension | | Y | Y | | | Y | Y | Y |
| Osteoporosis (-3SD) | Y | | Y | | | Y | | |
| Crash fracture | | | Y | | | Y | | |
| Aspergillosis | | | | | | Y | | |
| Sepsis | | Y | | | | | | |
| Chronic course | Y | | Y | | | Y | Y | Y |
| Length of Stay(days) | 61 | 60 | 172 | 19 | 23 | 56 | 42 | 65 |

# HLH

**Familial** **Non-Familial** **MAS**

- **Defect in cytotoxic activity (NK)**
- **Increased neopterin (Ibarra, 2011)**
- **Tissue IFN-$\gamma$ producing T cells and IL-6 and TNF-$\alpha$ producing macrophages (Biliau, 2005)**

# Repeated administration of CpG (TLR9 ligand) induces MAS in mice



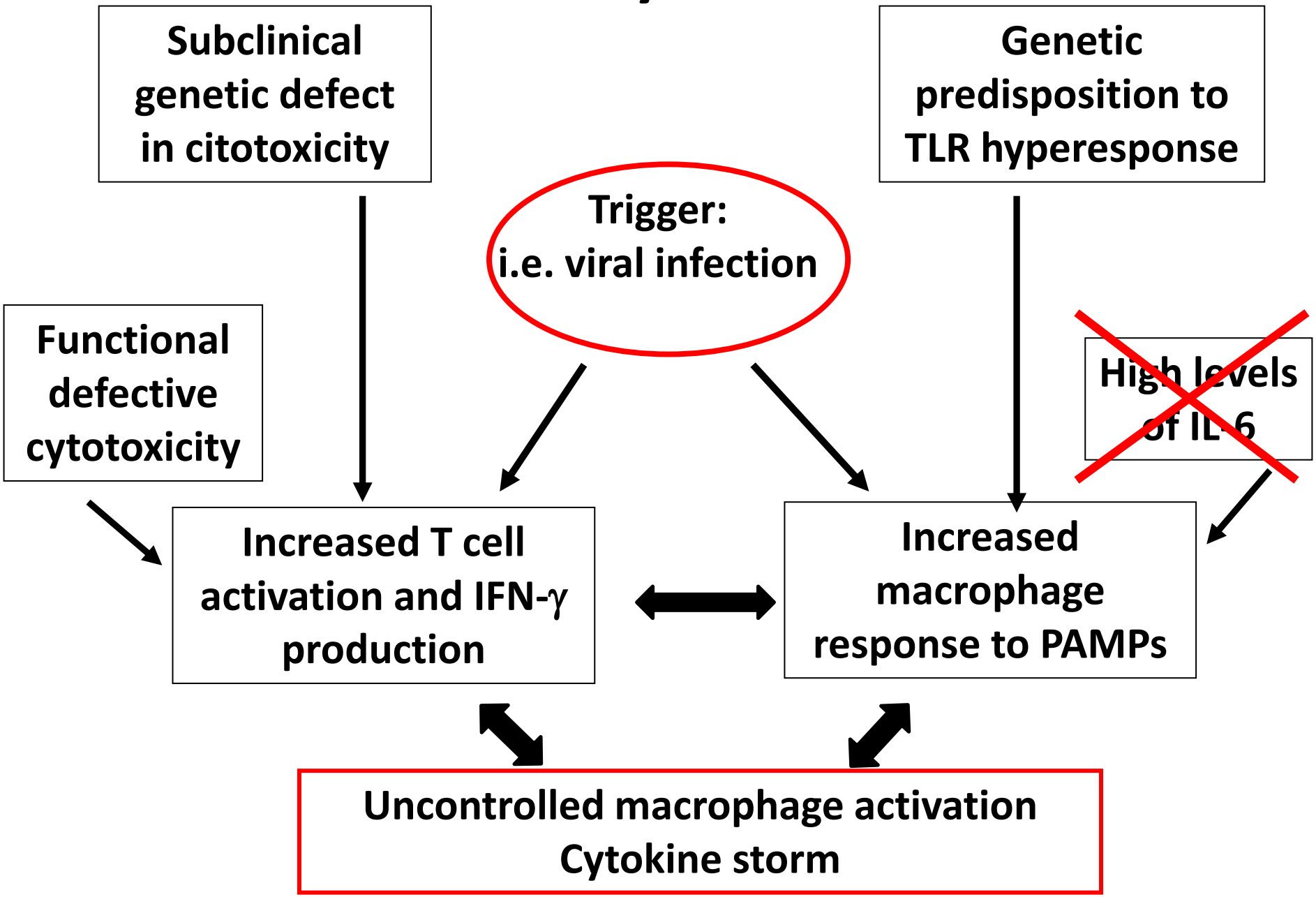

Behrens E et al, JCI 2011

# Repeated administration of CpG (TLR9 ligand) induces MAS in mice
# A model for secondary non familial HLH?

- **Decrease in white blood cells, platelet and hemoglobin**
- **Increase in ferritin and LDH**
- **Hypercytokinemia (IL-1, IL-12, IL-6, IFN-$\gamma$)**
- **Not lethal**

**Behrens E et al, JCI 2011**

# MAS – secondary non familial HLH



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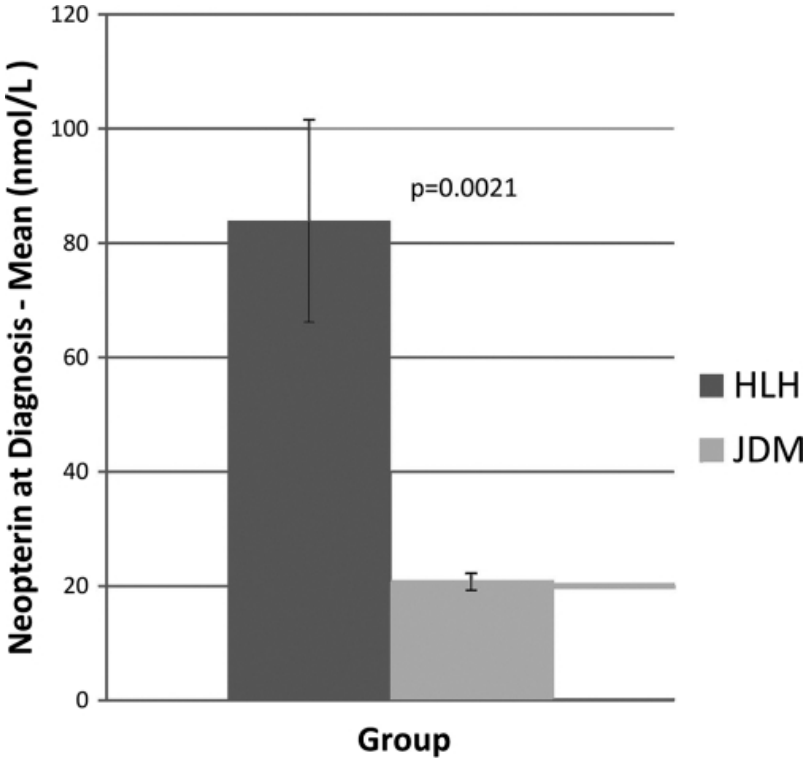
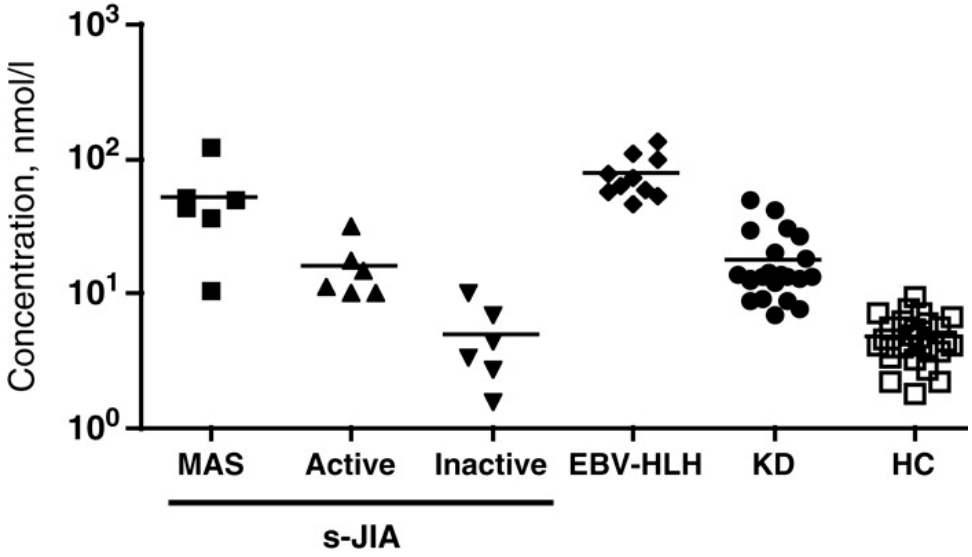
# Current HLH treatment

- **Current HLH treatment consists of:**
  - **Immuno-chemotherapy**
  - **Haematopoietic stem cell transplantation (familial only)**
- **The immuno-chemotherapeutic agents mostly used are:**
  - **Corticosteroids**
  - **Cyclosporin A**
  - **Etoposide (VP-16)**
  - **Iv-Ig, Anakinra, Tocilizumab, Etanercept**

# Neopterin levels are increased in patients with familial/non familial HLH and in MAS




Ibarra, Clin Vaccine Immunol 2011




Shimizu, Rheumatology 2010

# An animal model of primary HLH
# Perforin KO mice infected with LCMV

Jordan, Blood 2004

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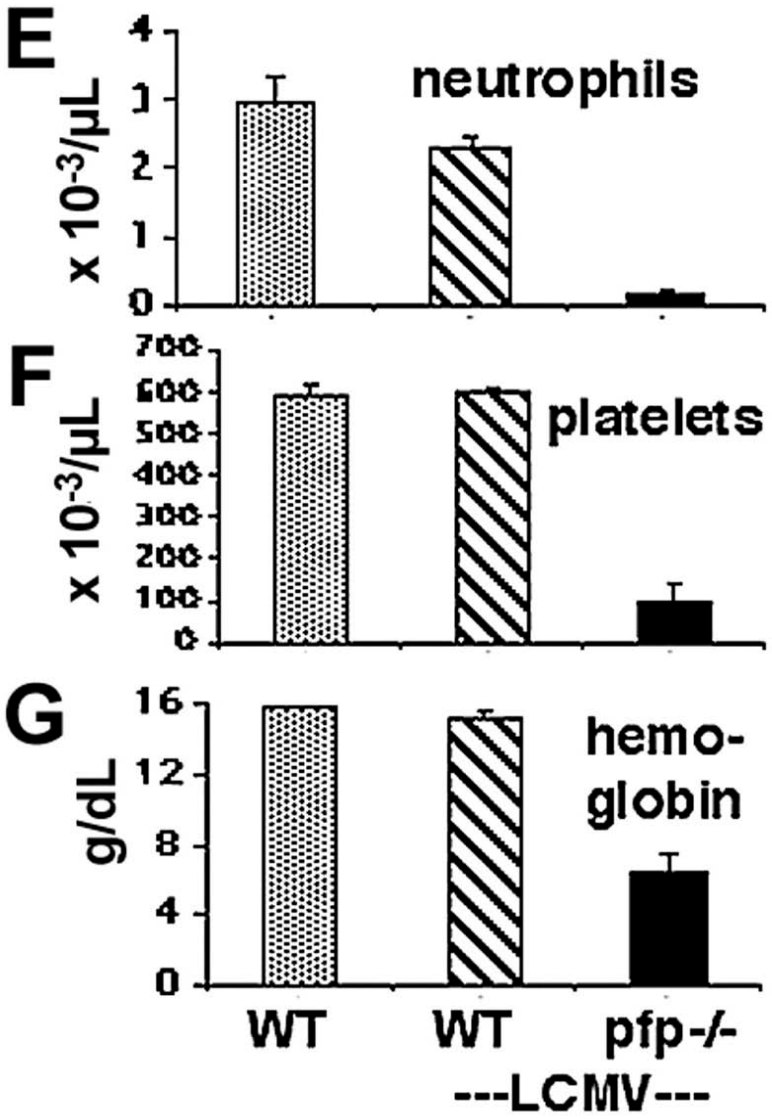
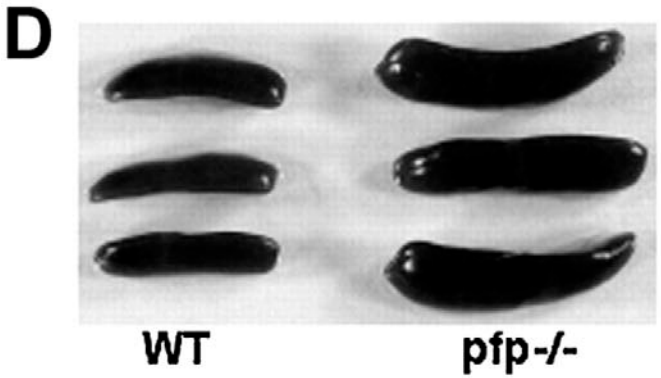
# Perforin KO mice infected with LCMV

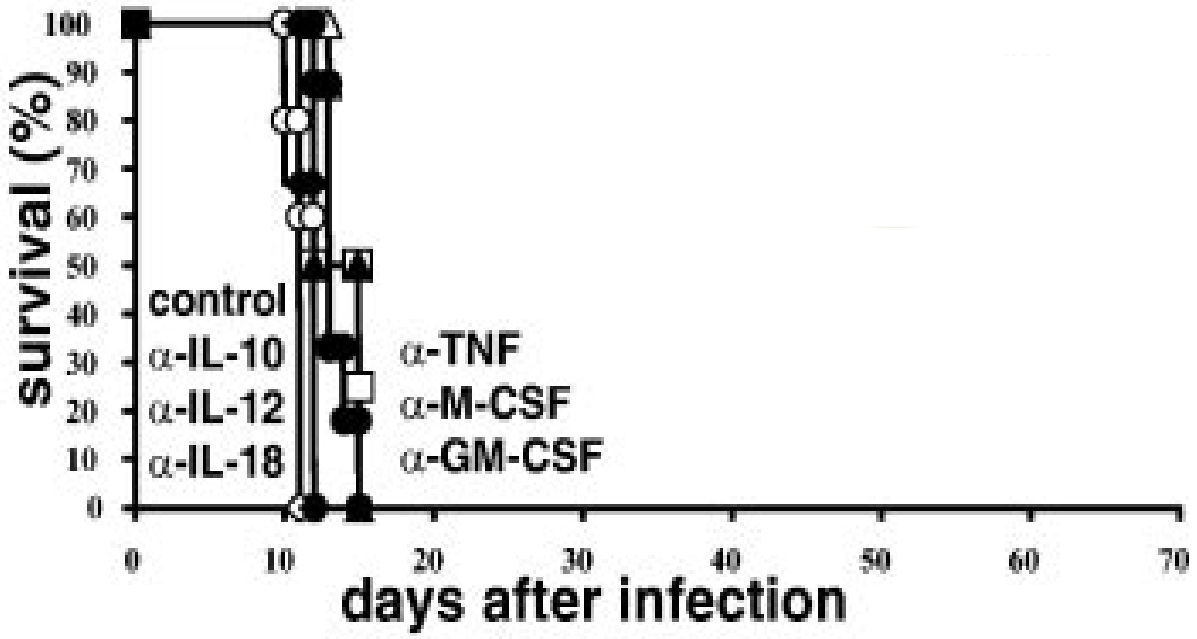



- 100% lethality
- Cytopenia
- Splenomegaly
- Increased ferritin
- Decreased fibrinogen




Jordan, Blood 2004

# Treatment with anti-IFNγ protects from death in LCMV-infected perforin-deficient mice




Jordan, Blood 2004

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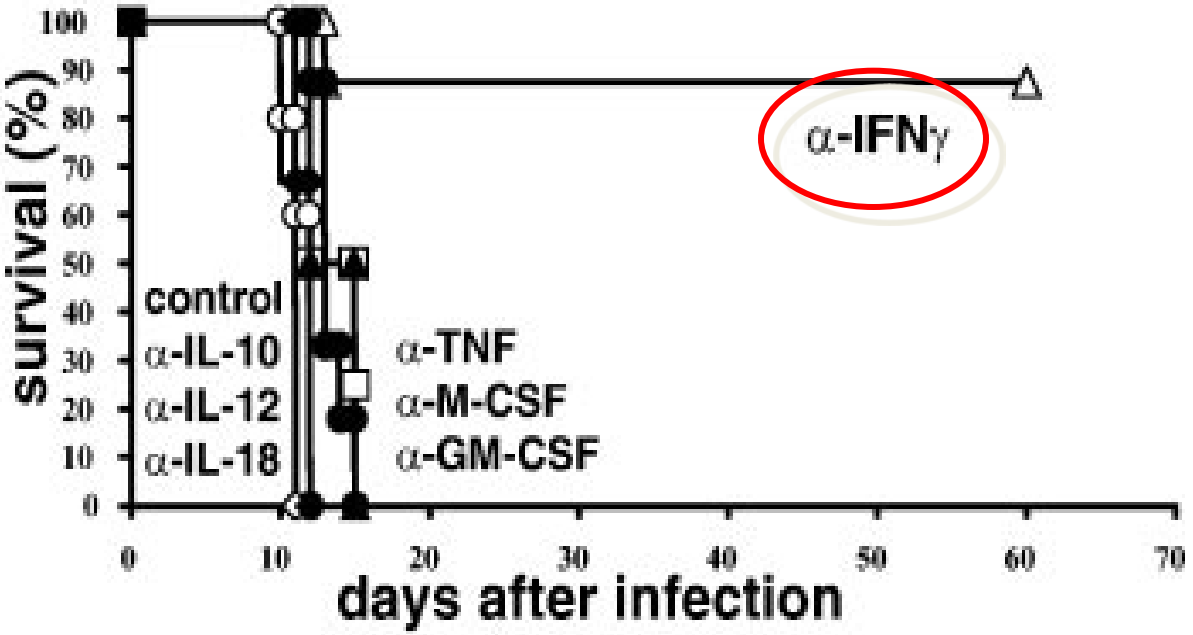
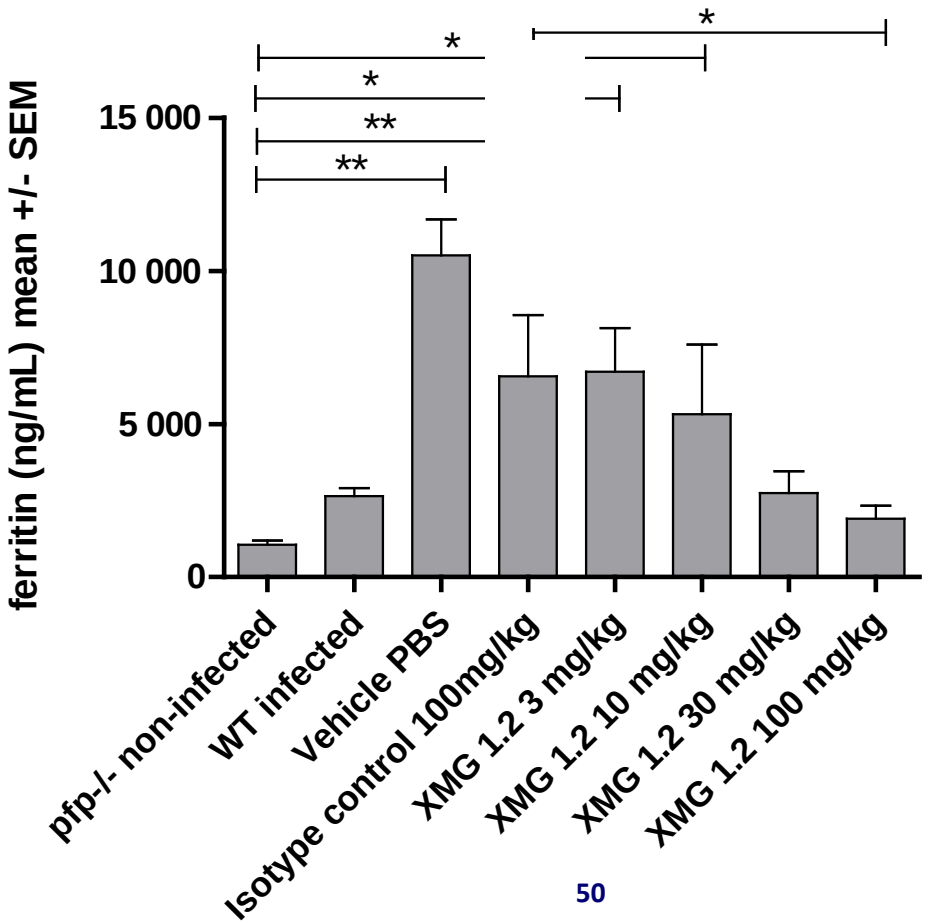



Jordan, Blood 2004

# Anti-IFN-γ reverses the laboratory features of HLH in a dose dependent manner

## LCMV infected pfp-/- mice

### Ferritin

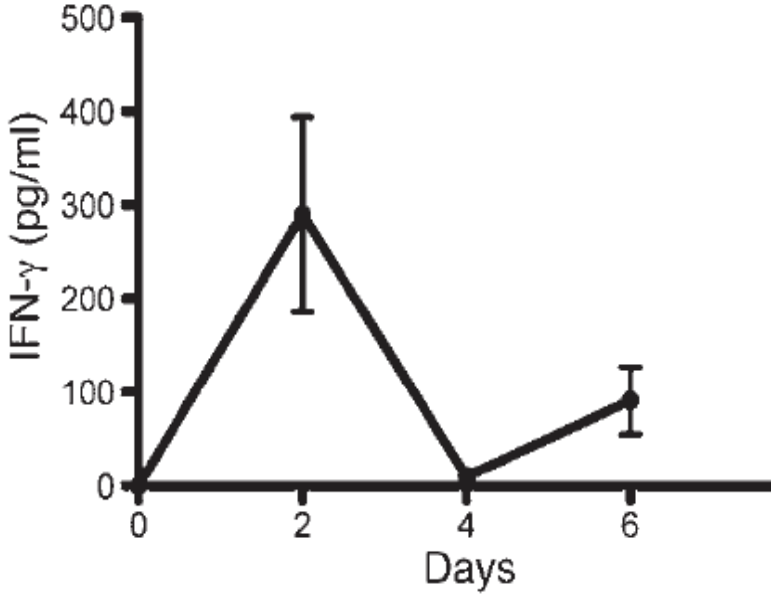


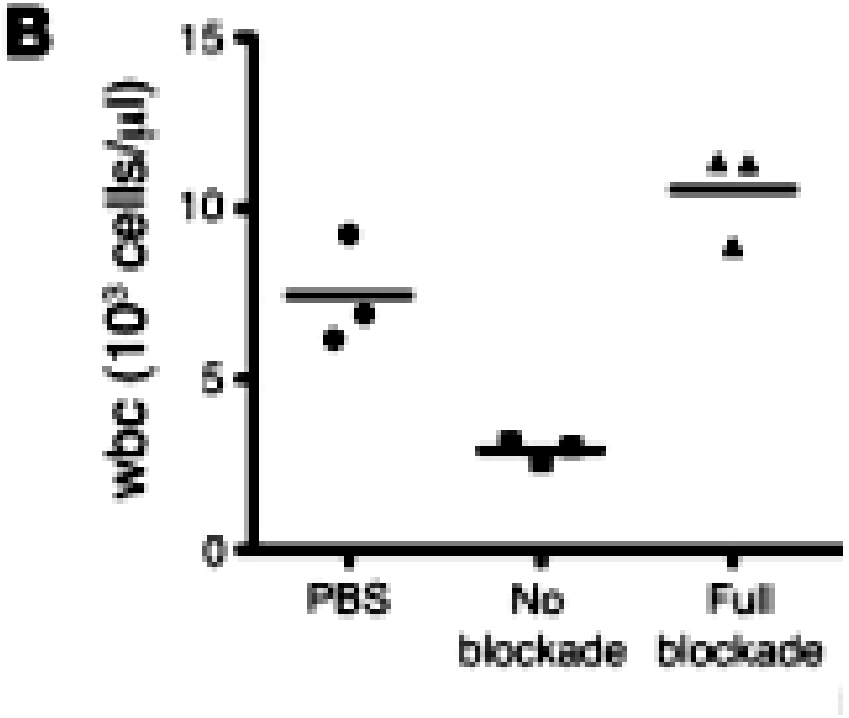

Courtesy of

NovImmune
TURNING SCIENCE INTO MEDICINE

# Anti-IFN-γ reverses the laboratory features of non familiar HLH in mice

## CpG (TLR9 ligand) repeated administrations




Behrens, JCI 2011

- **Clinical presentation, classification and diagnosis**
  - **criteria, guidelines versus primary HLH**
- **Pathogenesis of MAS (s-JIA associated)**
  - **animal models, role of TLR stimulation, high background IL-6, cytotoxic defect**
- **Secondary (non-familial HLH)**
  - **frequency (?), severity, animal model**
- **Novel therapeutic target**
  - **IFN-γ in animal models**