



**BAD KREUZNACH**



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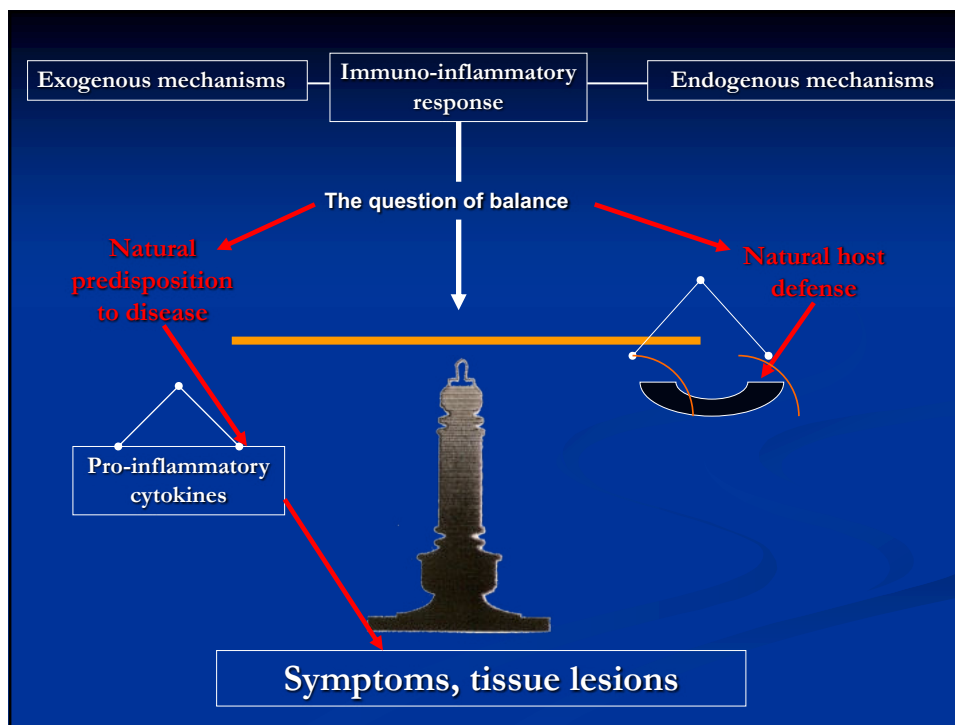
# Immunological Aspects of Ozone Treatment in Rheumatoid Arthritis

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1. Effect of Ozone of the immune system
2. The Immunsystem in old patients
3. Correlation of plasma interleukin-1 levels with disease activity in rheumatoid arthritis with and without ozone

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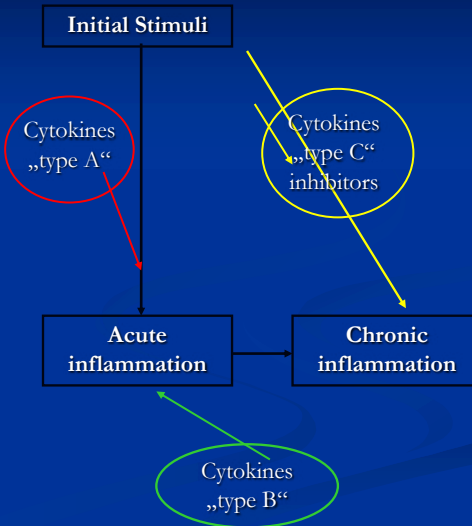
## Conceptual classification of some cytokines

From the functional point of view, Cytokines may be classified as follows:

**„A“ Type:** Induction of acute inflammation including cytokines as IL-8, IL-9, MIP, TNF, IL-1

**„B“ Type:** persistence of inflammation with tissue destruction and cell proliferation including cytokines as IL-1, TNF, PDGF, FGF, EGF, TGFb, IL-6

**„C“ Type:** Inhibitory action including cytokines as IL-4, IL-10, IL-6, TGFb, IFN $\gamma$  or antagonists including cytokines such as IL-1 $\alpha$ , TNF-R-BP



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## Some general considerations about cytokines

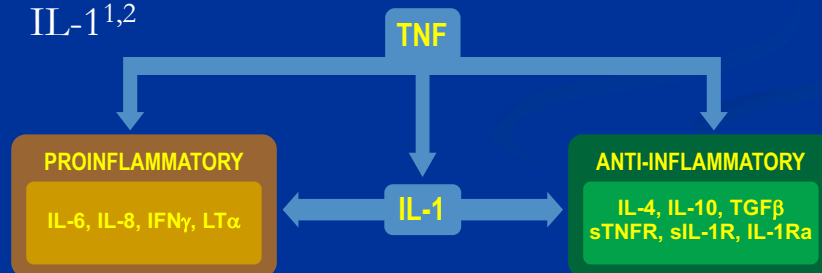
The balance between the numerous pathological and related downmodulatory diseases mechanisms in RA are mediated mainly by **cytokines**. Cytokines are soluble proteins and peptides **without enzymatic activity** and function in small quantities as mediators of information between cells. The cytokine network consists of molecules such as **interleukines (IL)(IL-13)**, **growth factors** and **interferons**. Cytokines bind to the correspondent **receptor** of target cells. After internalization of the cytokine-receptor complex various signals are triggered, which most often lead to the **induction of new proteins**.

The down modulation of the cytokine action can be effected by **soluble receptor molecules** which often consist of an enzymatically cleaved extracellular part of a receptor molecule. This process is referred to as receptor shedding. In RA a lot of different cytokines work in concert. Some of them are well known today but the **fine tuning** of their action and disease modulation still remains unclear. The cytokine network with its characteristics makes a lot of sense if we look at the main **communication systems** of our body. **TNF and IL-1** are major players in the pro-inflammatory process of RA of which some conceptual aspects will be discussed now.

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## The central role of TNF

- Drives events in the inflammatory cascade<sup>1,2</sup>
- Triggers production of other cytokines, including IL-1<sup>1,2</sup>

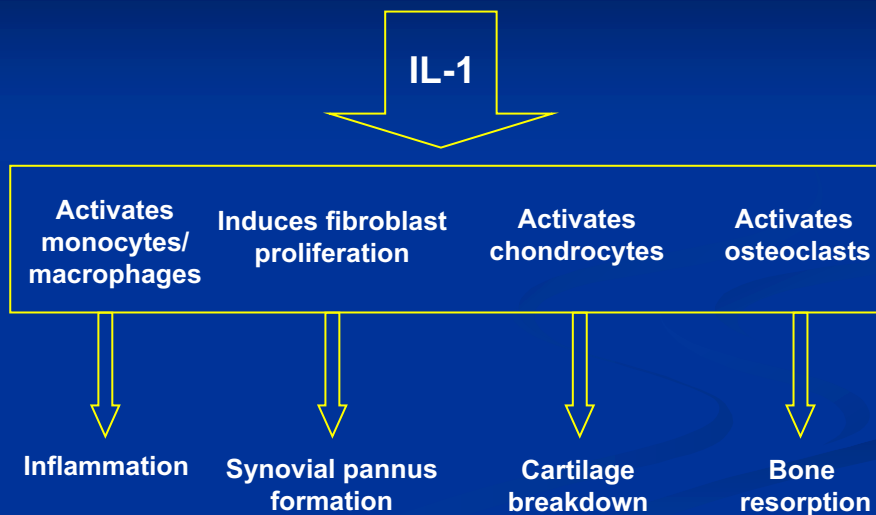


Adapted from Feldmann et al, 1996.<sup>1</sup>

References: 1. Feldmann M, Brennan FM, Maini RN. *Cell* 85:307-310, 1996. 2. Baumgartner SW. *South Med J* 93:753-759, 2000.

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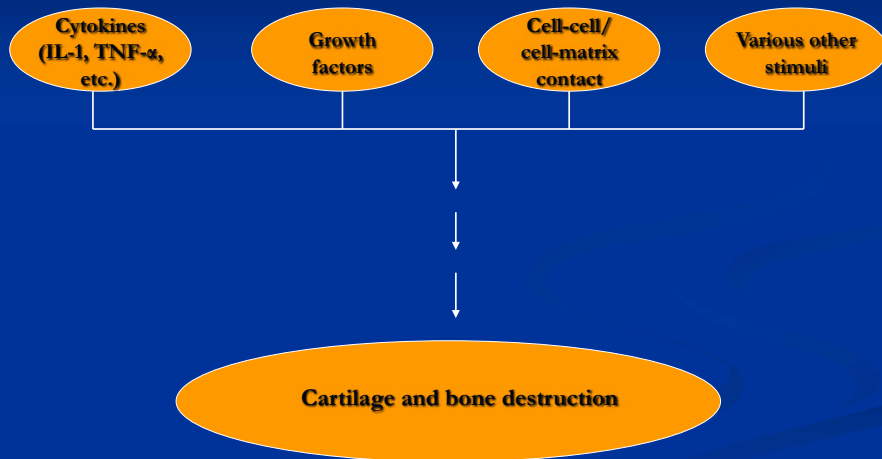
## IL-1 Plays a Pivotal Role in the Inflammatory and Destructive Processes of RA



Dinarello C. *N Engl J Med*. 2000;343:732-734; Arend W, Dayer J-M. *Arthritis Rheum*. 1990;33:305-315; van den Berg W. *Ann Rheum Dis*. 2000;59(suppl 1):i81-i84.

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## Inflammation and Joint Destruction



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## Characteristics of the 22-kd interleucin-1 (IL1) inhibitor

Sources:	Human monocytes cultured on adherent immune complexes or IgG.
	Urine of patients with fever.
Mechanism:	Binds to the IL-1 receptor on target cells.
Effects:	Prevents IL-1 augmentation of thymocyte proliferation. Prevents IL-1 induction of prostaglandin E2 and collagenase production by synovial fibroblasts and chondrocytes. Does not block tumor necrosis factor alpha stimulation of target cells.

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## Biologic effects of interleucine-1 that may occur in rheumatoid arthritis

Systemic:	<p>Decreased appetite</p> <p>Increased granulocyte-macrophage colony-stimulation factor production-</p> <p>Synthesis of acute-phase proteins</p>
Local:	<p>Chemotaxis of polymorphonuclear cells, lymphocytes, and monocytes</p> <p>Adherence of white blood cells to endothelial cells</p> <p>Fibroblast proliferation, Prostaglandin E2, Collagenase, and neutral protease production by fibroblasts and chondrocytes</p> <p>Increased production of collagen and an inhibitor of neural proteases, Stimulation of T and B</p>

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## Interleukin -1

<u>Metabolic</u>	<u>Physiologic</u>	<u>Hematologic</u>	<u>Immunologic</u>
↑ACTH	Fever	↑ACTH	T-cell Activation
↑Corticosteroids	Sleep	↑GM-CSF	B-cell Activation
↑↓Insulin	Shock	↑IFN	↑NK-binding
↓Plasma Zn/Fe	↑Na Excretion	↑Phagocytosis	↑Ab
↑Hepatic Protein			↑Lymphokines
↓Albumin			

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# Biologie

<u>Property</u>	<u>IL1</u>	<u>TNF</u>
Endogenous pyrogen fever	+	+
Slow-wave sleep	+	+
Hemodynamic shock	+	+
Increased hepatic acute phase	+	+
Protein synthesis	+	+
Decrease albumin synthesis	+	+
Activation of endothelium	+	+
Decreased lipoprotein lipase	+	+
Decreased cytochrome P450	+	+
Decreased plasma F.e/Zn	+	+
Increased fibroblast proliferation	+	+
Increased synovial cell collagenase	+	+
And PGE2 inductions of IL-1	+	+
T/B cell activation	+	-

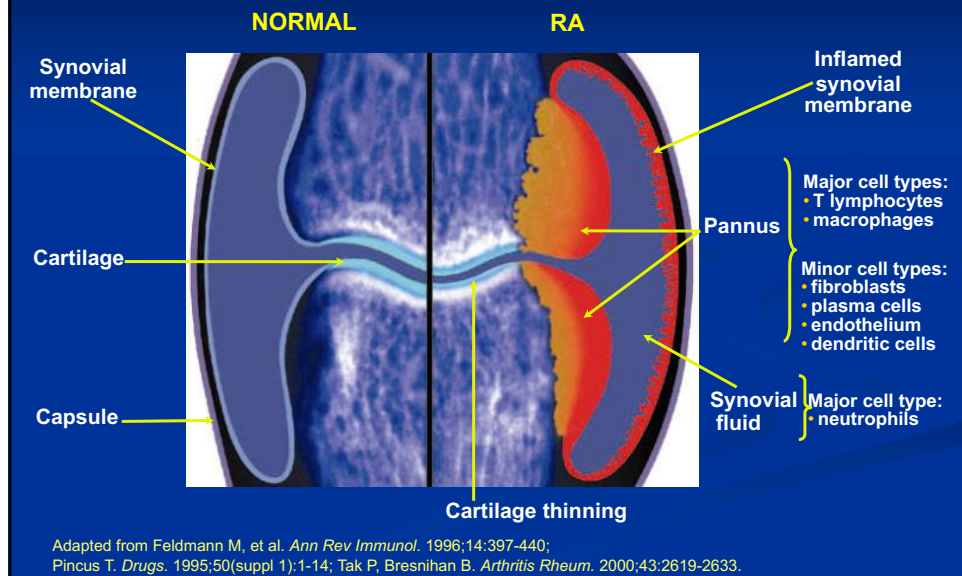
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## Evidence suggesting the role of interleukin-1 (IL-1) in rheumatoid arthritis

<u>Location</u>	<u>Features</u>
Synovial fluid	Variable levels of IL-1 bioactivity  Elevated levels of IL-1 alpha and IL1 beta proteins Cells may not spontaneously produce IL-1
Synovial tissue	Spontaneous in vitro production of IL-1 alpha and IL-1 beta, High levels of IL-1 alpha and IL-1 beta messenger RNA
Blood	Presence of IL-1 beta in levels that correlate with disease activity

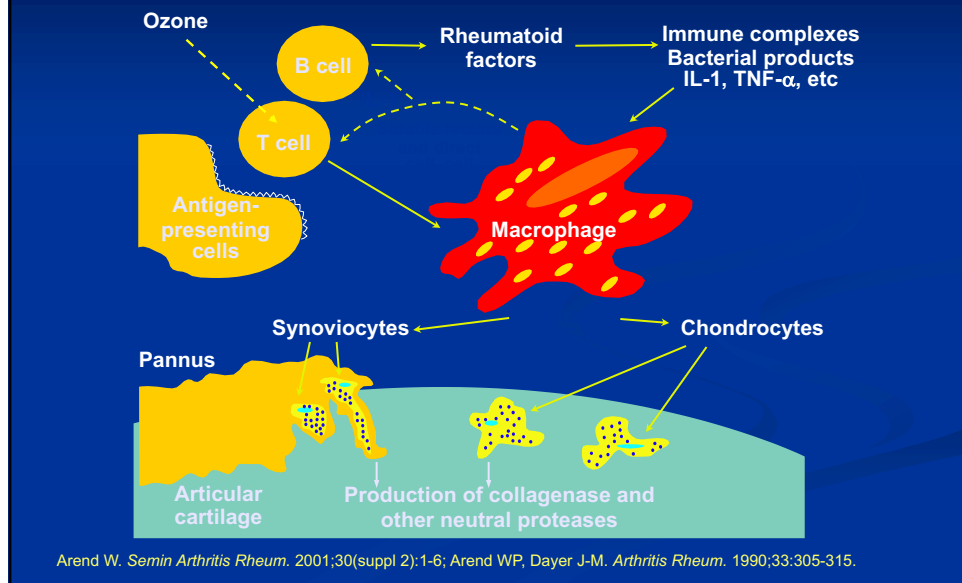
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## RA is Characterised by Synovitis and Joint Destruction



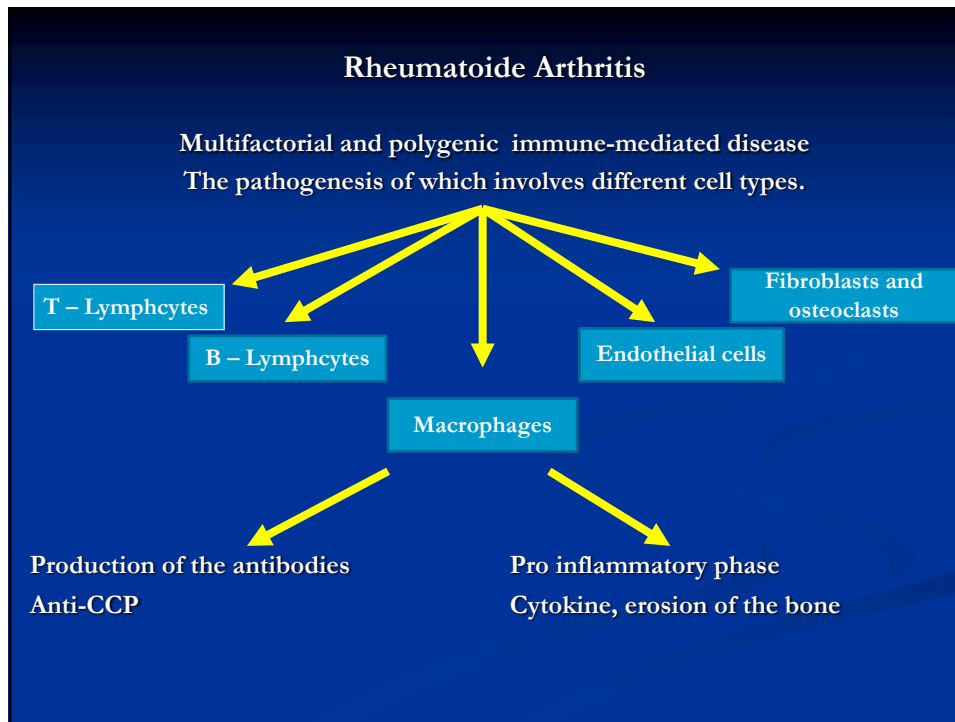
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## Numerous Cellular Interactions Drive the RA Process

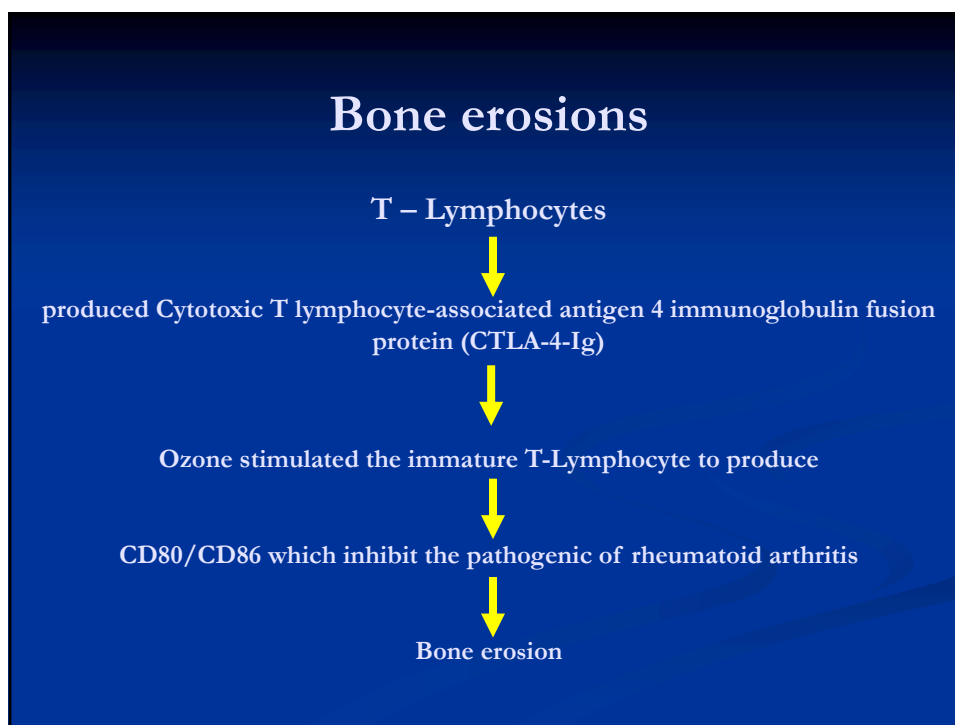


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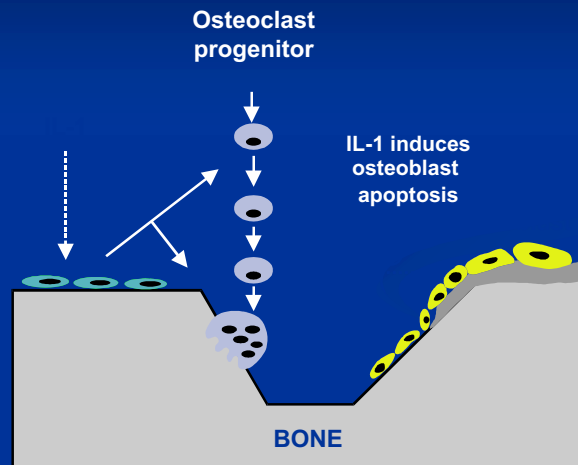


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## IL-1 Regulates Osteoclast Differentiation and Activity



OPGL = osteoprotegerin ligand

Adapted from Tsuboi M, et al. *J Lab Clin Med.* 1999;134:222-231; Gravallesse E, Goldring S. *Arthritis Rheum.* 2000;43:2143-2151; Gravallesse E, et al. *Arthritis Rheum.* 2000;43:250-258.

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## Activation of the T-cells

### Migration – Infiltration (Macrophages, Monocytes, Fibroblasts)

Activation of the pro inflammatory (TNF-Alpha, IL1, IL 6)  
Presents of the cytotoxic and vascular growth factor

Infiltration of the endothelial cells

Activation of macrophages and osteoclasts Metalloproteinase

Adhesion of the molecules - recruitment of inflammatory cells – in the joint

Induce angiogenesis – increased production of pro inflammatory cytokines –

activated macrophages and osteoclasts

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# Correlation of plasma interleukin-1 levels with disease activity in rheumatoid arthritis with and without ozone

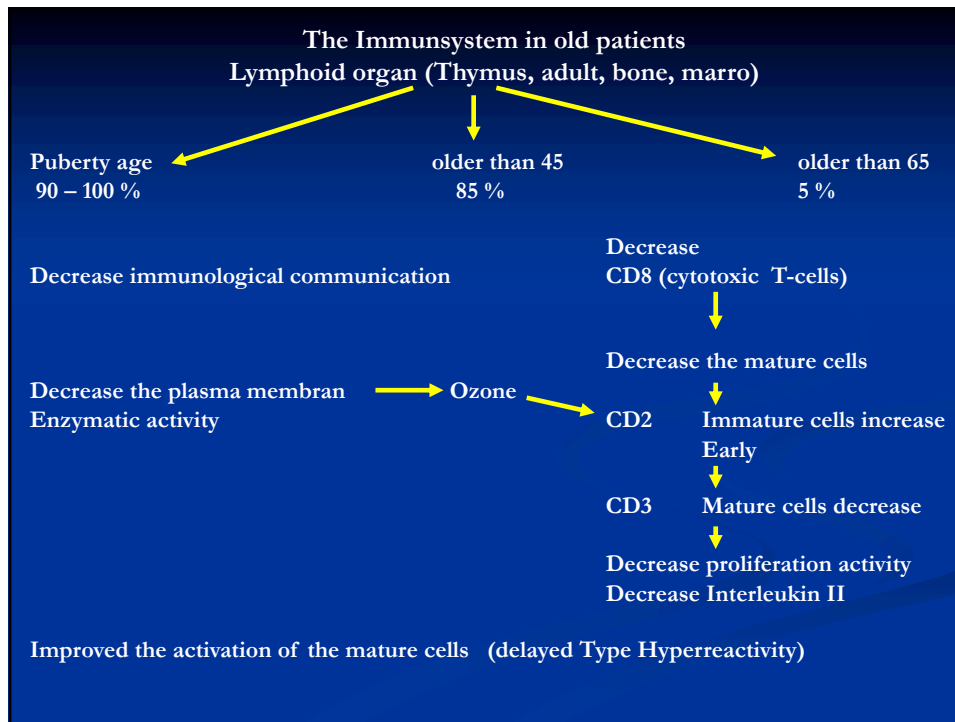
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## The Aim of the Study

1. Ozone: Direct or indirect effect of the cytokines?
2. The reaction of the Ozone of the cytokines especially of Interleukin I and TNF Alpha
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Changes the immune system in old age	
Cellular Immunity	Decrease the B-cells proliferation Decrease the signal process the T-cells Decrease the cytokine secretion (Interleukin II, III, IV etc.) Increased the memory-cells Decreased the T-Suppressor function Decreased the DTH (delayed Type Hyper-Reactivity)
Humoral Immunity	Decrease the B-cells Diminish B-cell proliferation of Mitogene Decrease the antibodies Increase the autoimmunity organ (not specific antibody building) Diminish the B-cells mature (through Decrease binding affinity
Unspecific immunity	Reduce the antibody presentation Reinforcement inflammatory mediators (PGE2, IL-6,CRP)

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## Study identification and inclusion criteria

### Groups of patients with Rheumatoid Arthritis each group n = 25 patients

- 1. Group:
  - NSAIDs + Biological agent (Enebrel 50mg weekly)
- 2. Group:
  - Ozone 3 x weekly(15-20  $\gamma$  / ml, Major Haemotherapy) + NSAIDs + Enebrel 50mg weekly

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## Methods

50 patients with RA admitted in our study with following criteria:

DAS 28

Morning stiffness

Visual analog pain score

25 RA patients treated with 02/03 + NSAID +DMARDs

(25 patients)

Biochemical examinations

Haemoglobin

Leucocytes

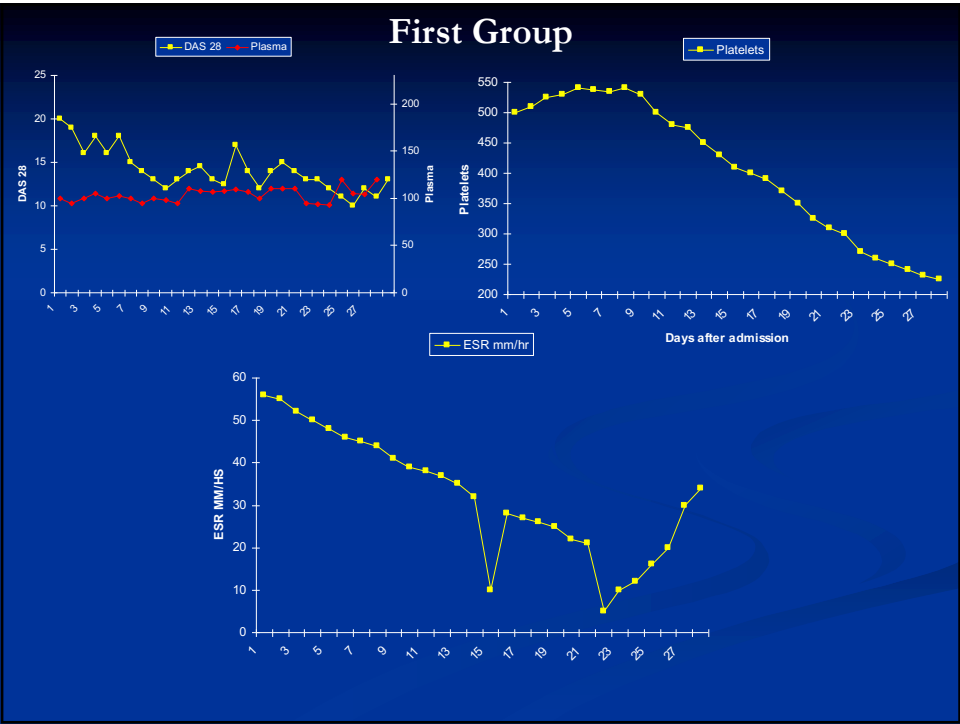
Platelets counts

Erythrocytes count

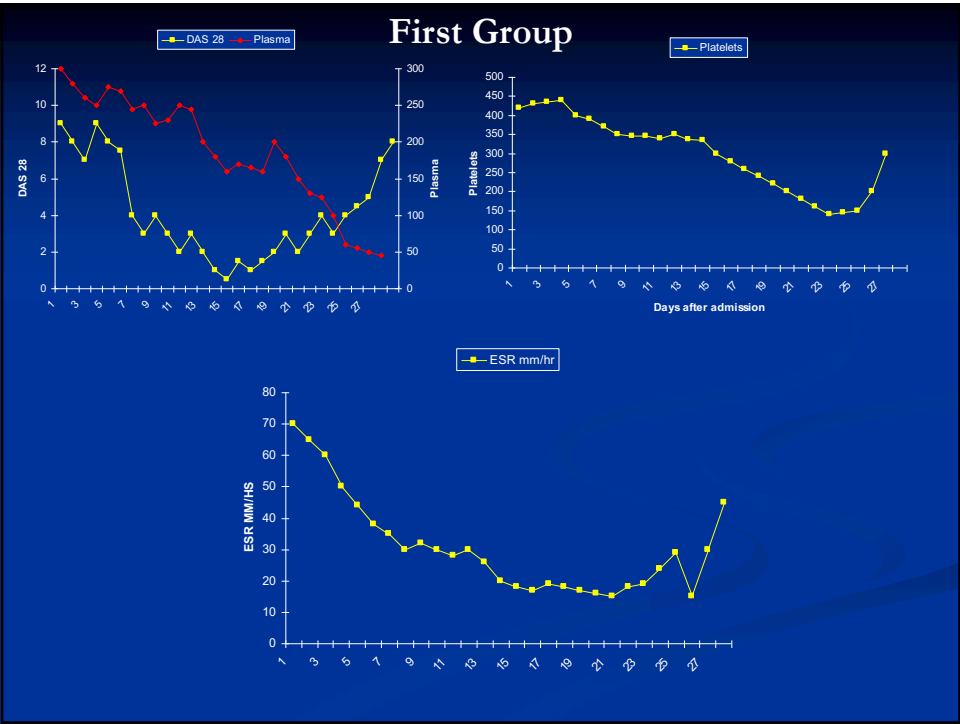
ESR

Rheumatoid factor

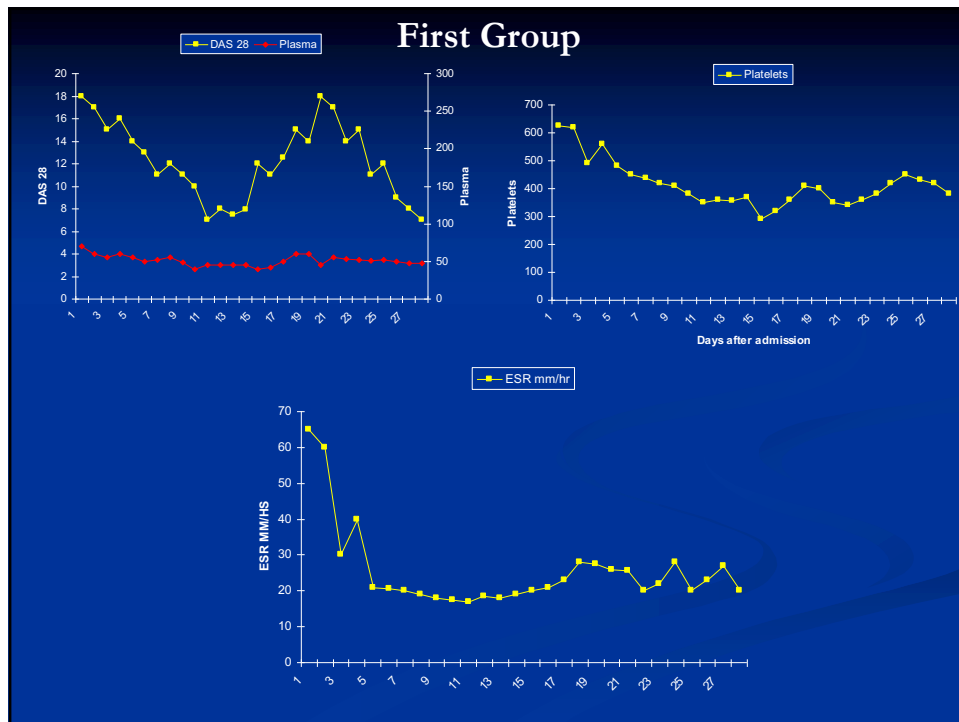
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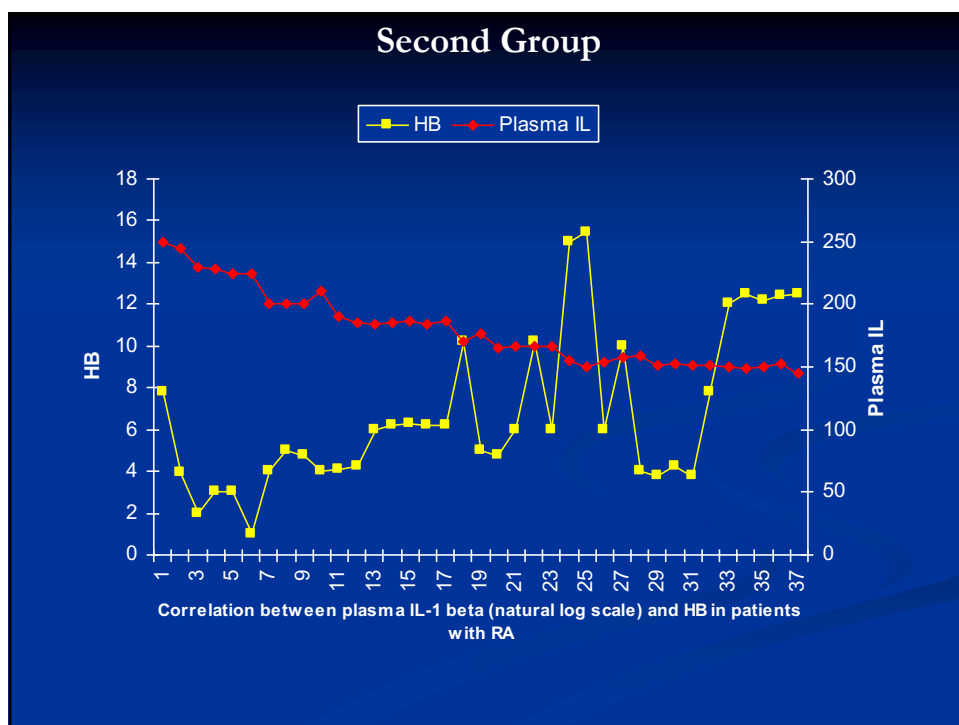
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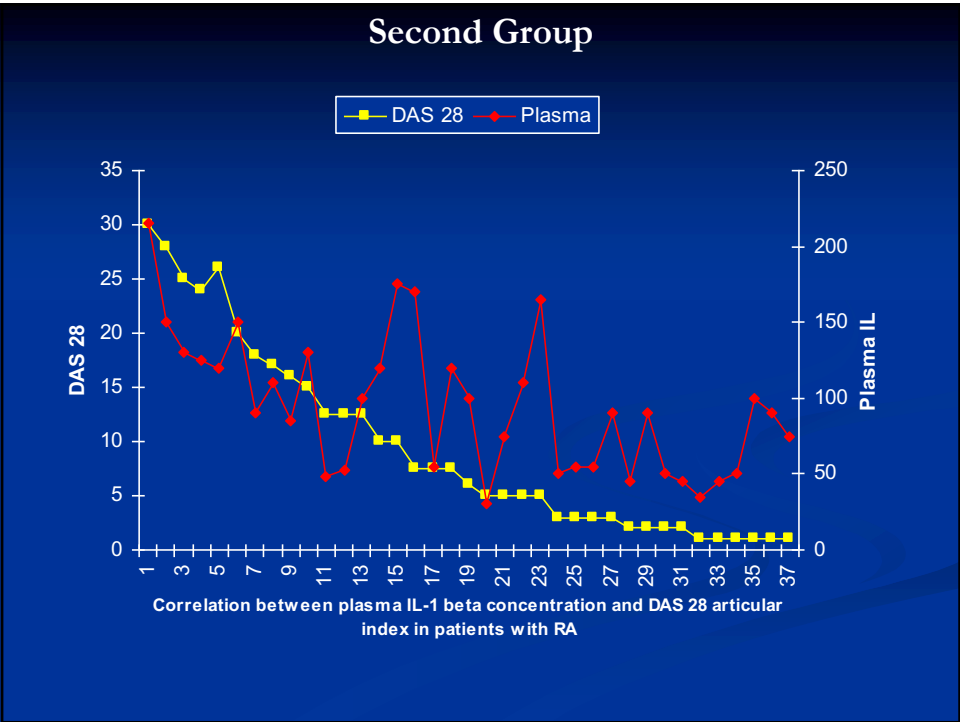
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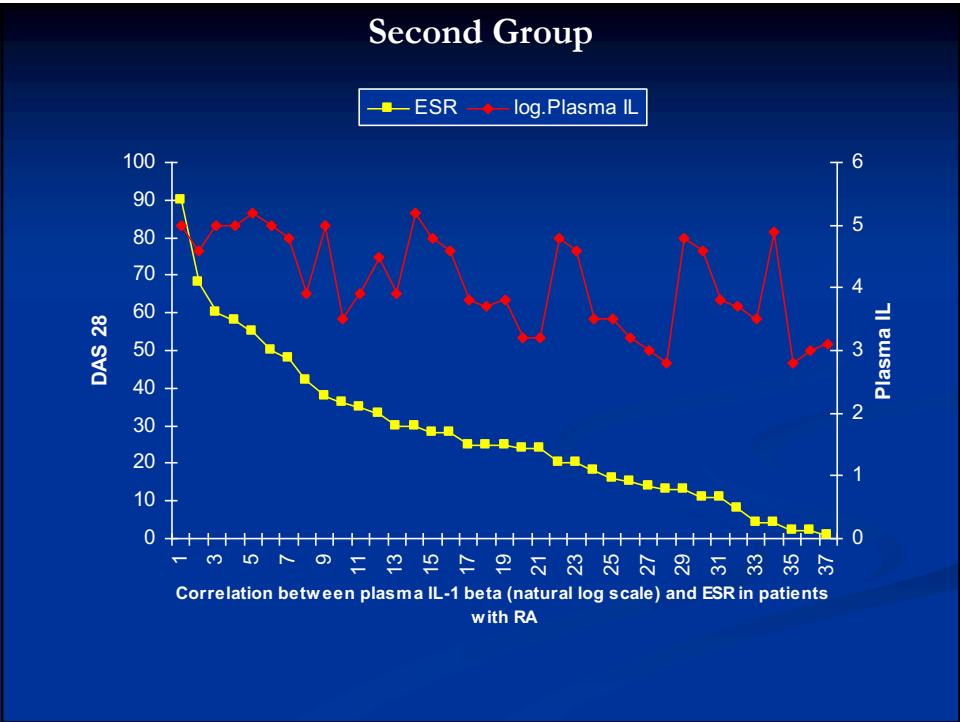
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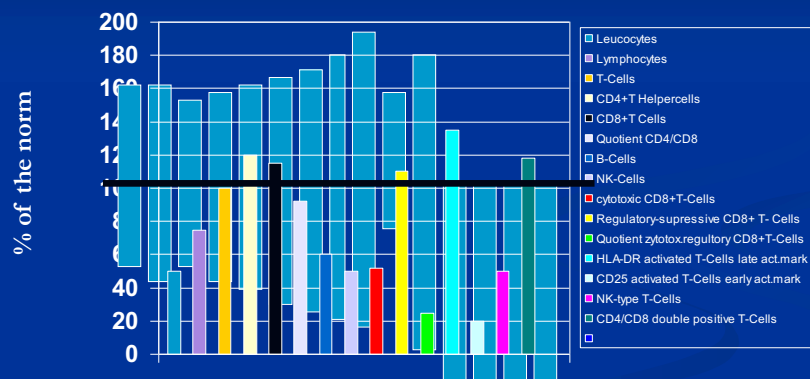


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# Cellular Immune Status Plus

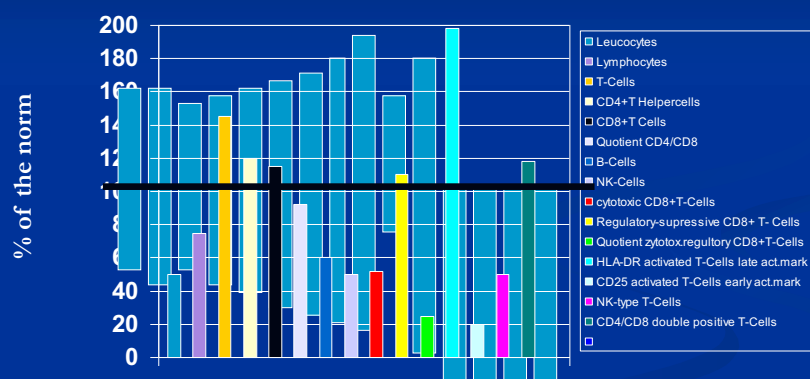
First group



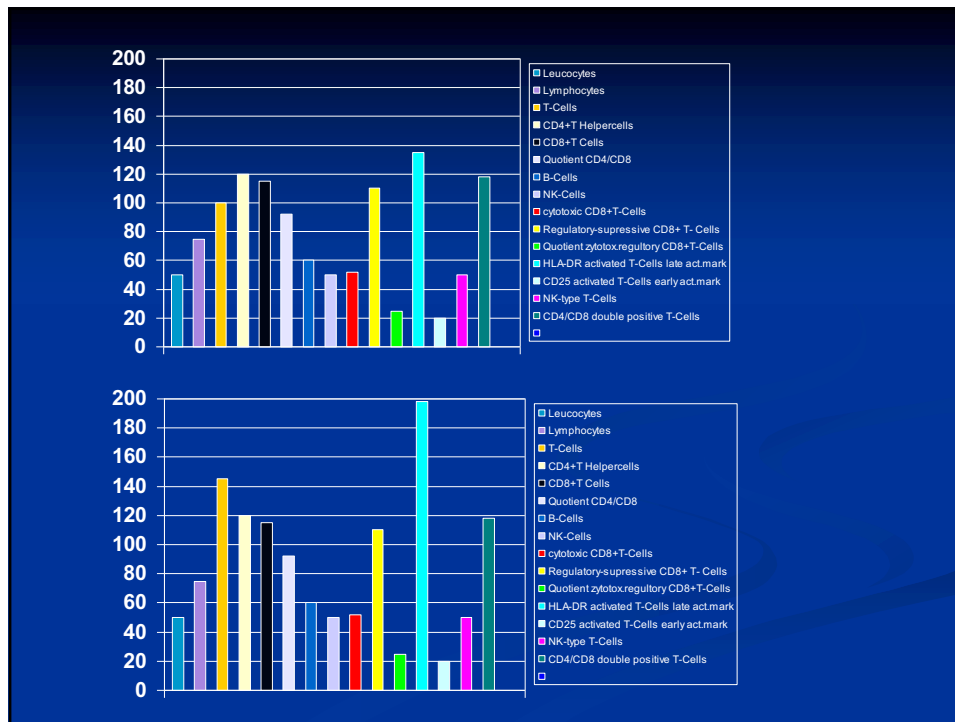
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# Cellular Immune Status Plus

Second group



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## Summary

- 1. Ozone is more effective in combined form than single form in the treatment of RA
- 2. Rapid significant decrease in CRP and ESR
- 3. The incidence of clinically significant treatment in relation laboratory abnormalities was similar among treatment groups with NSAIDs
- 4. The ability of ozone to reduce level of acute phase Proteins in the blood was significant greater than in the other group
- 5. The ozone effect of the cytokines could be due to either inhibition of the production of cytokines through the effect of the Lymphocytes or inhibition of response to cytokines
- 6. Ozone in clinically and biochemical superior in combined form than single form in treatment of RA

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Thank you for  
your attention

Vielen Dank für  
Ihre  
Aufmerksamkeit



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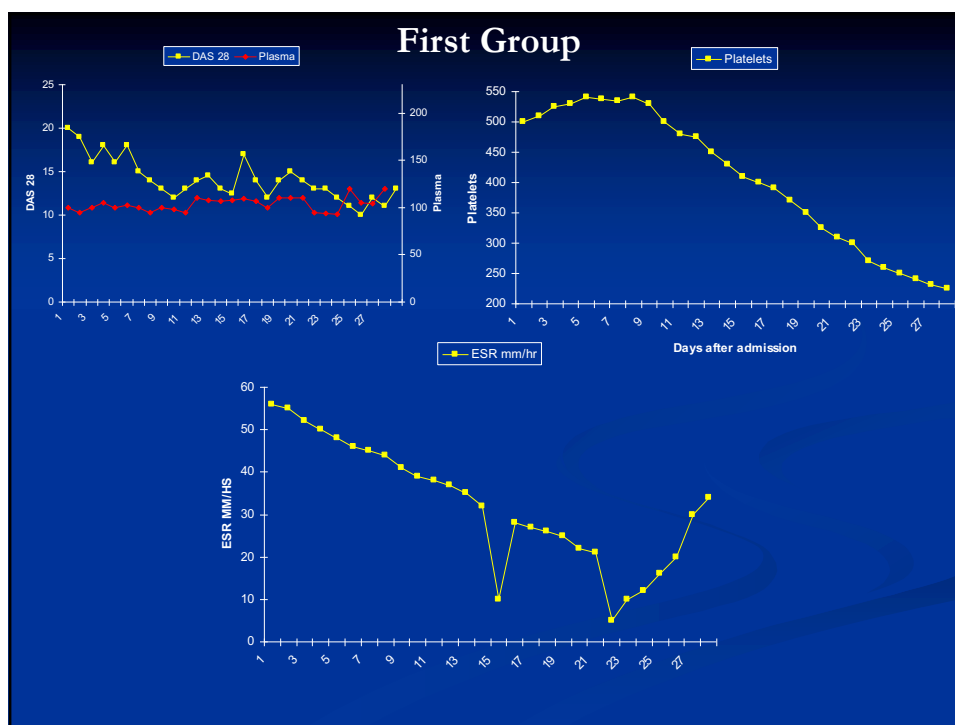
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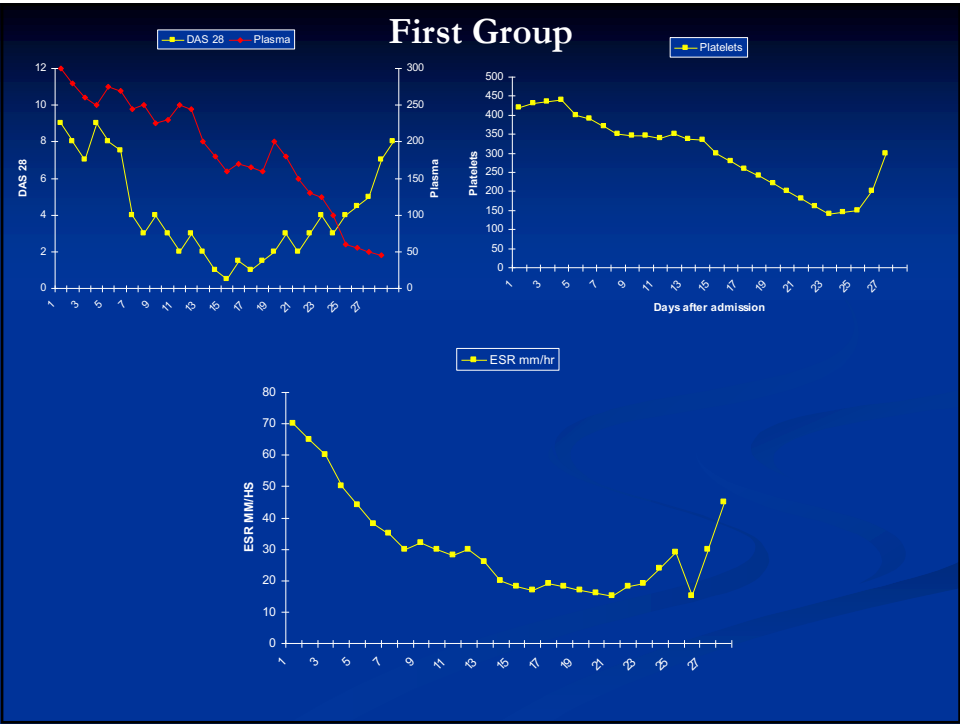
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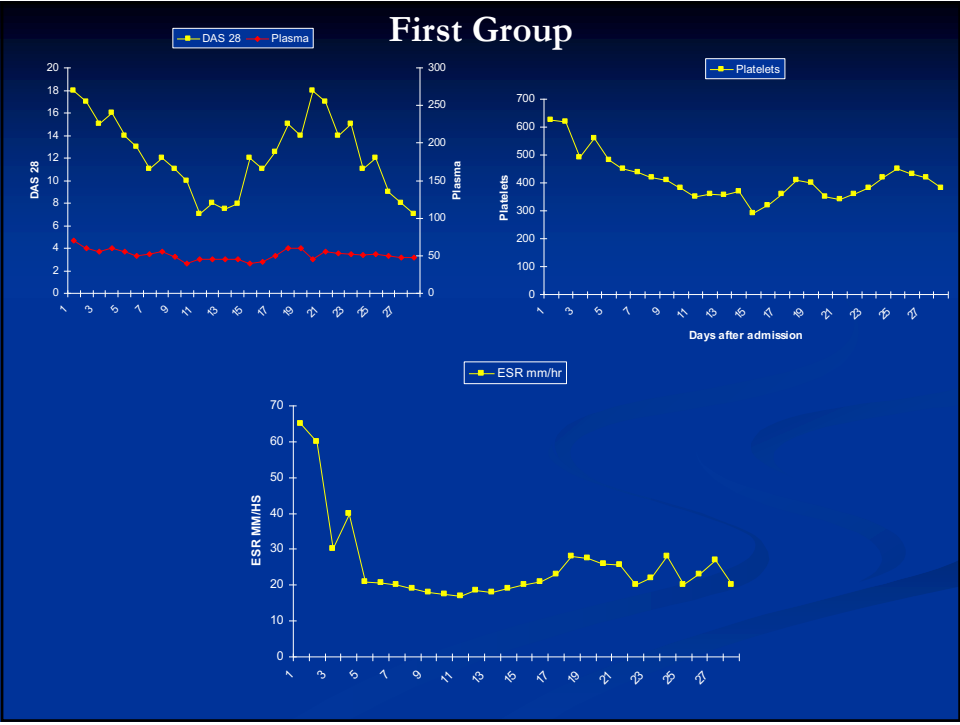
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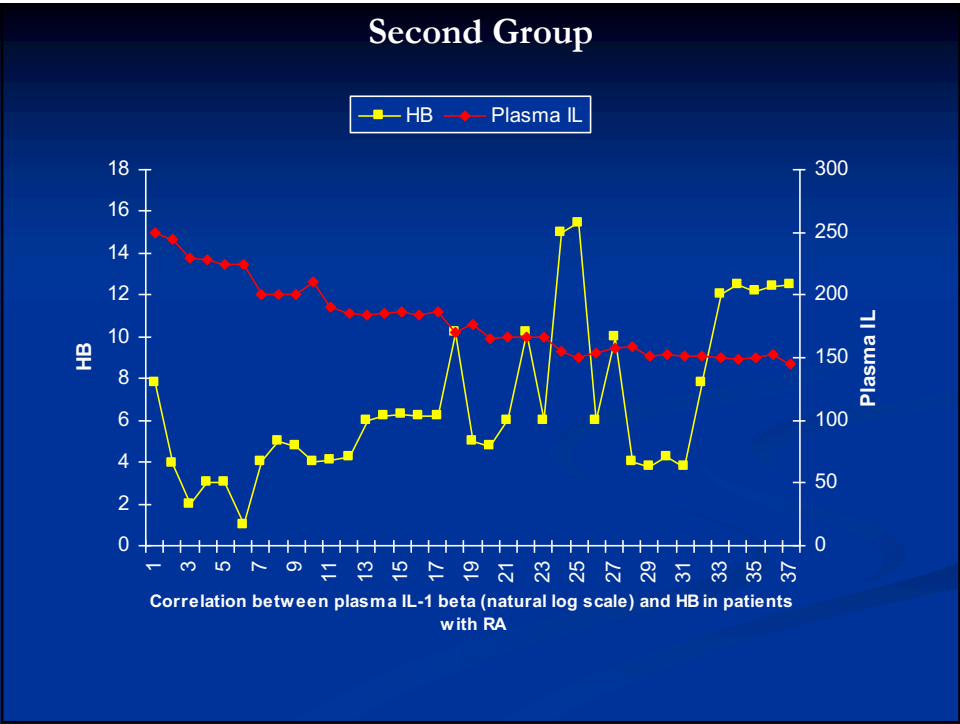
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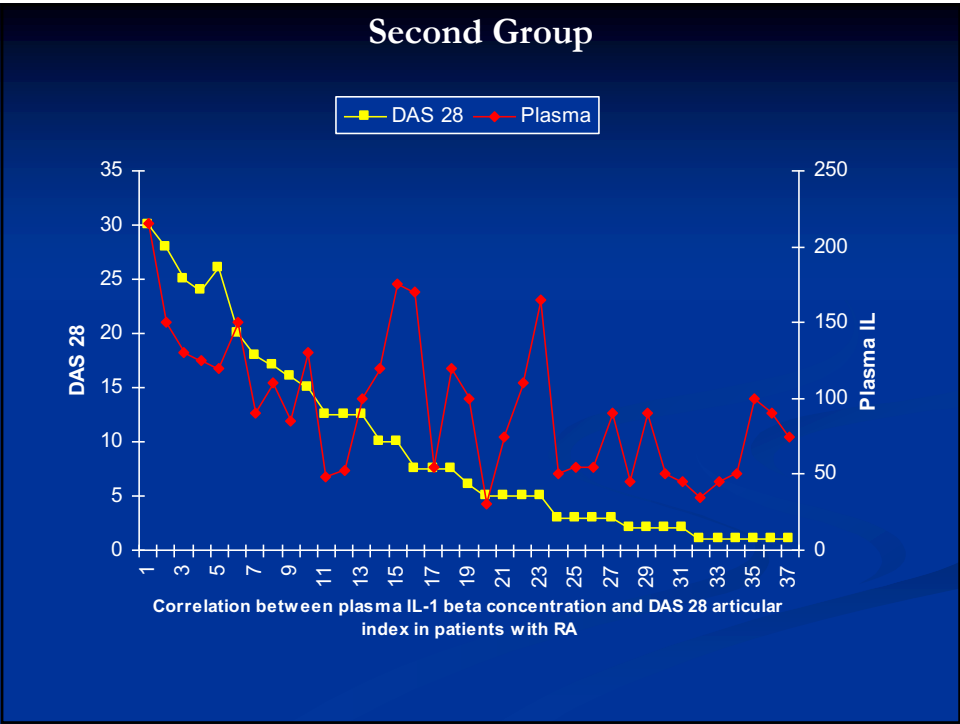
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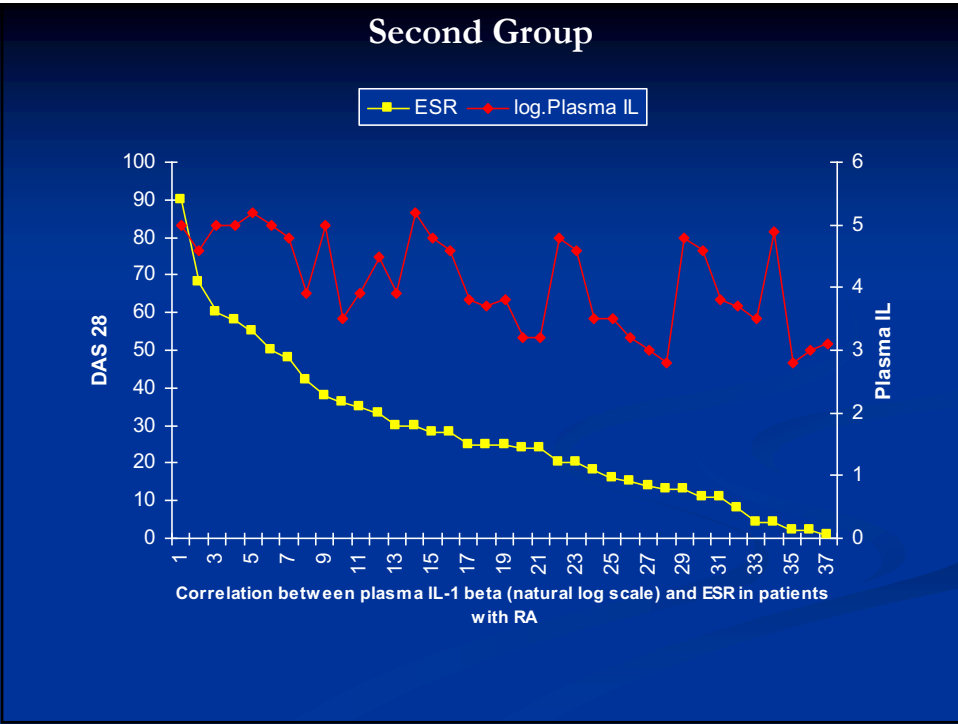
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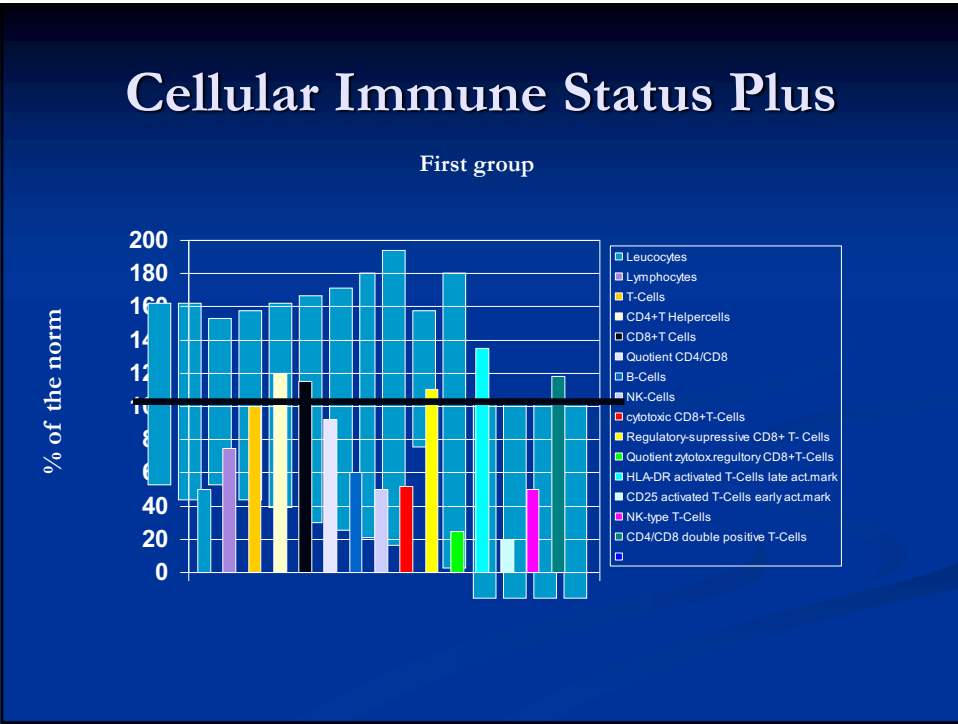
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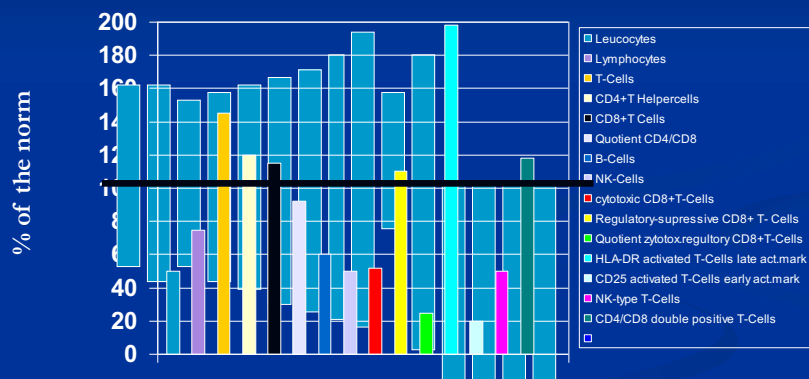


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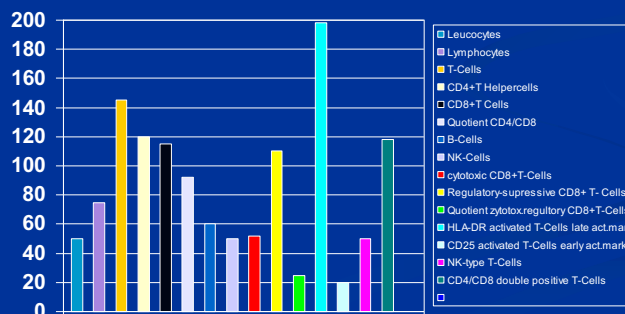
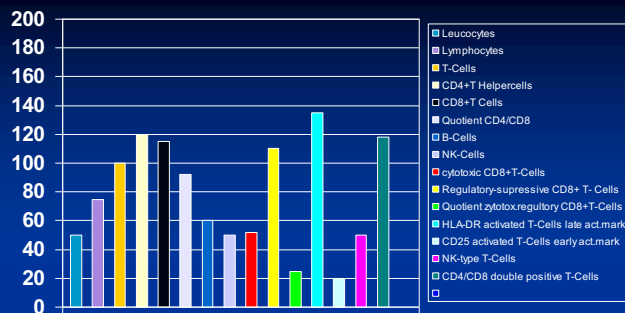


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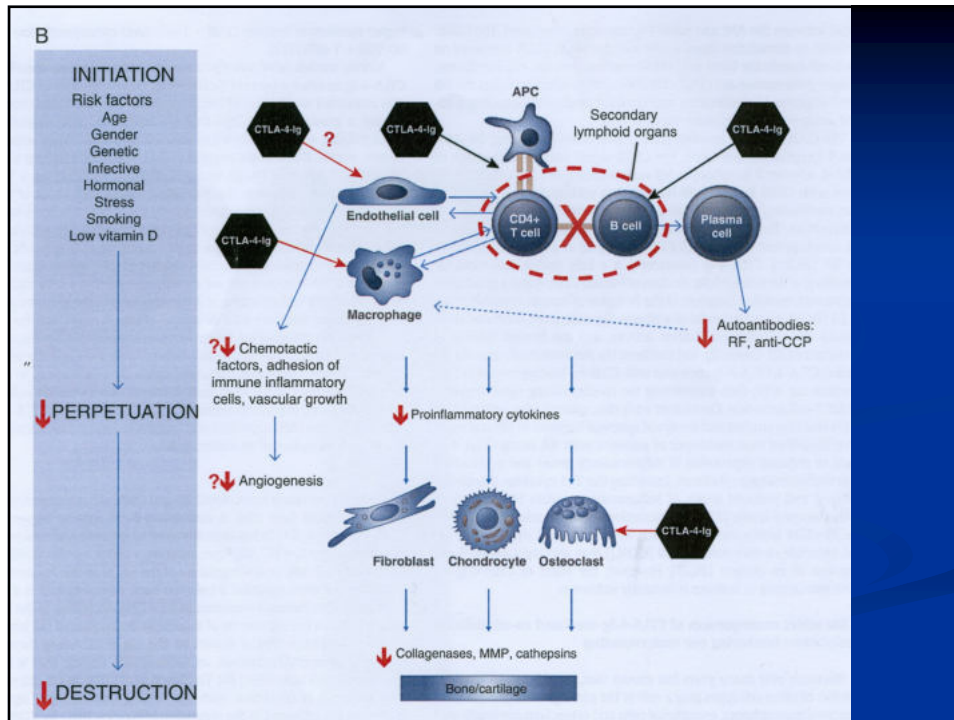
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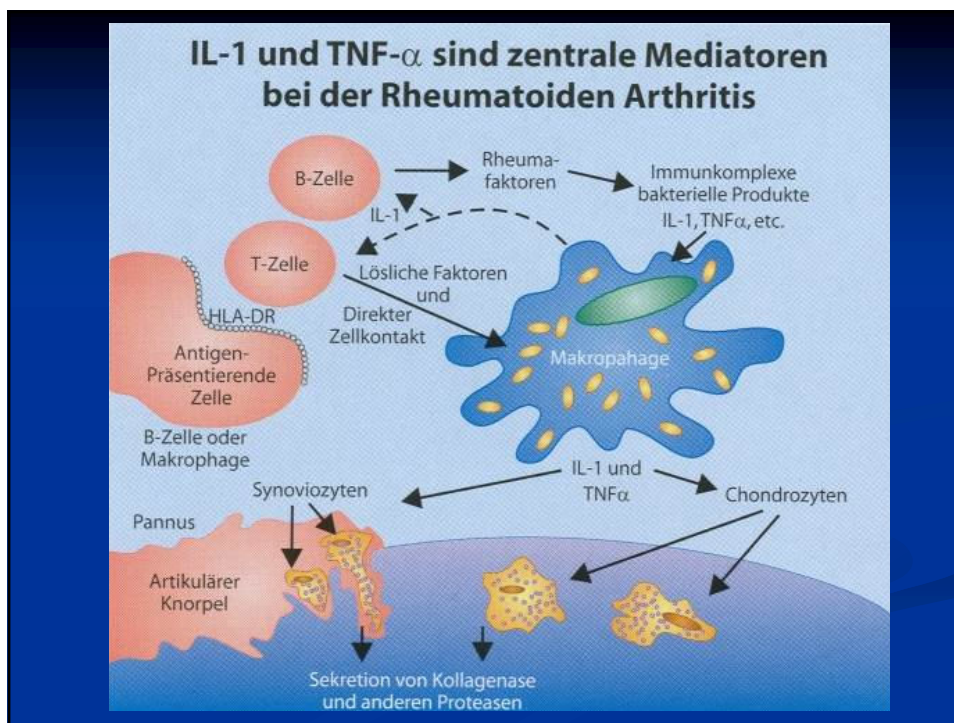
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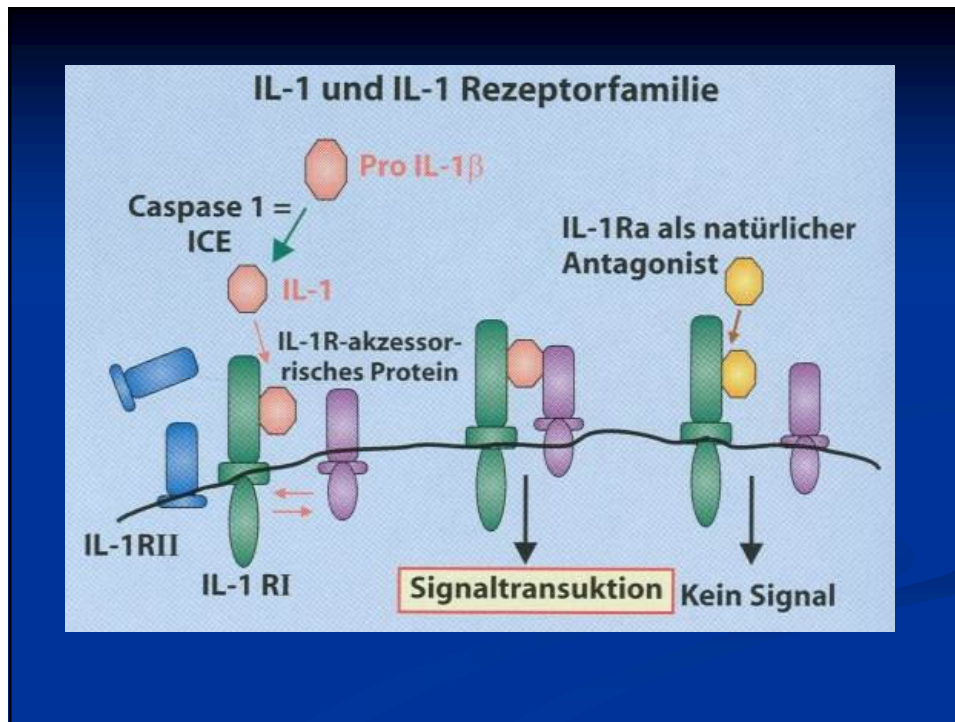
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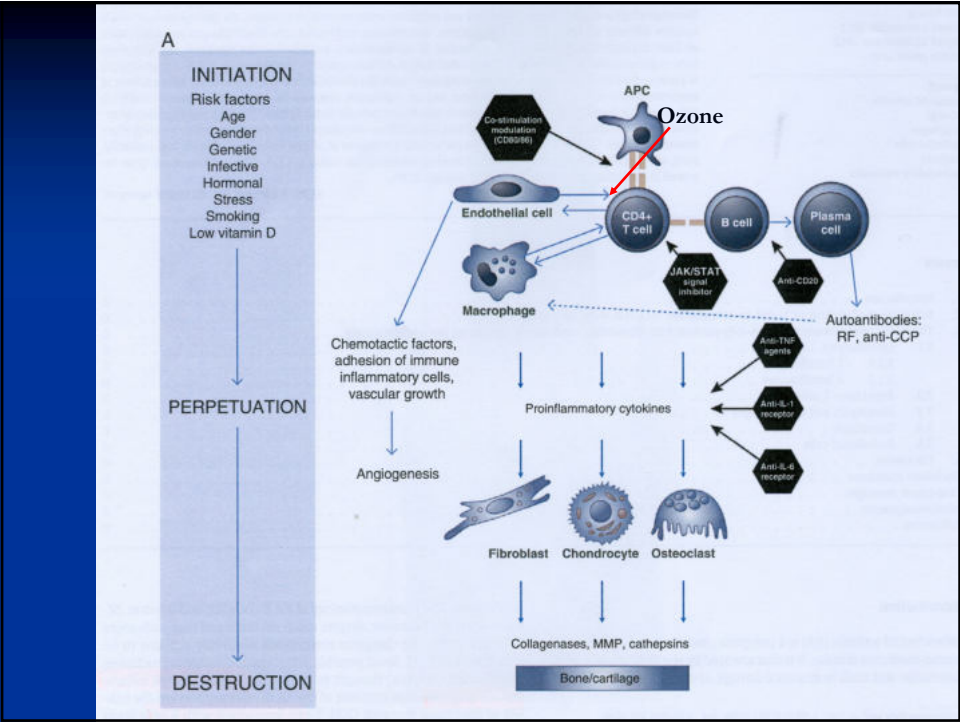
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## The factor which effect the NK cell

1. Chemo therapy
2. Auto radical oygene radical
3. Psychological stress
4. Smoker
5. Anxiety
6. Grief
7. Sorrow
8. Insomnia

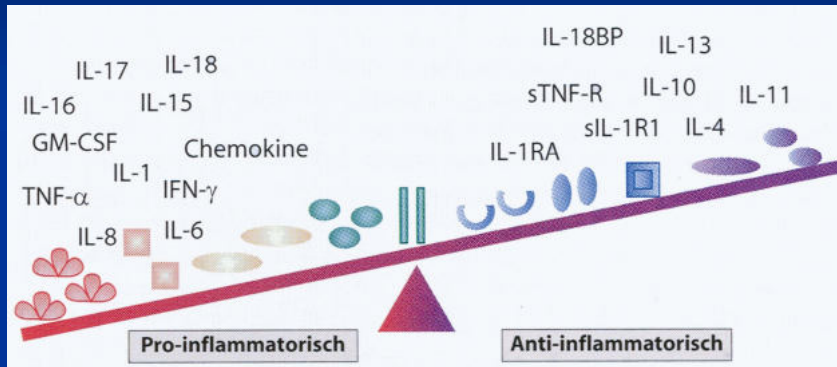
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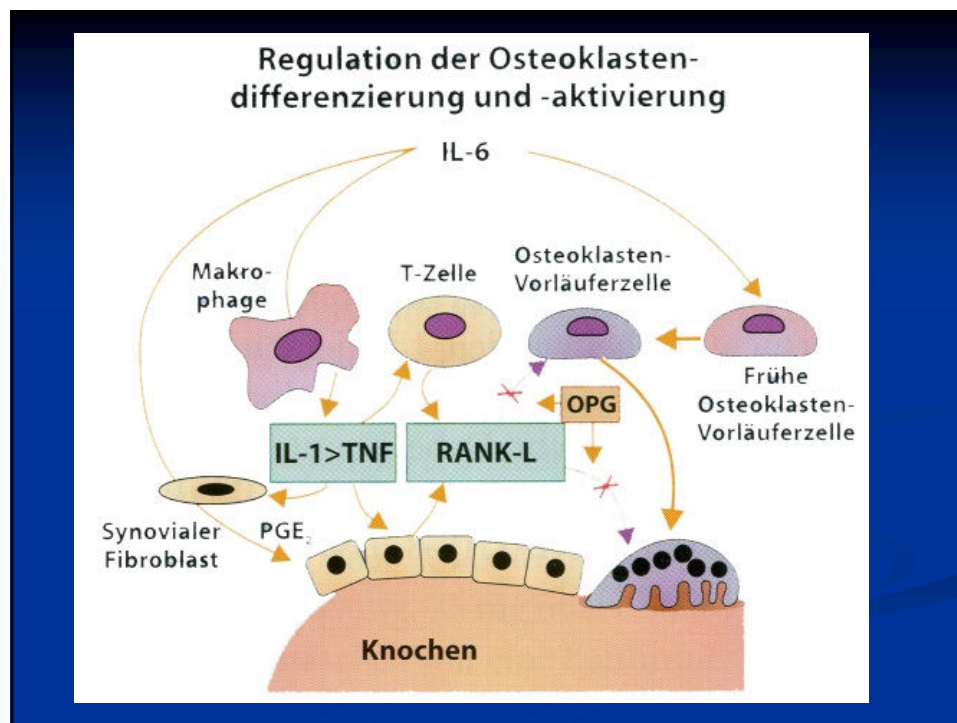


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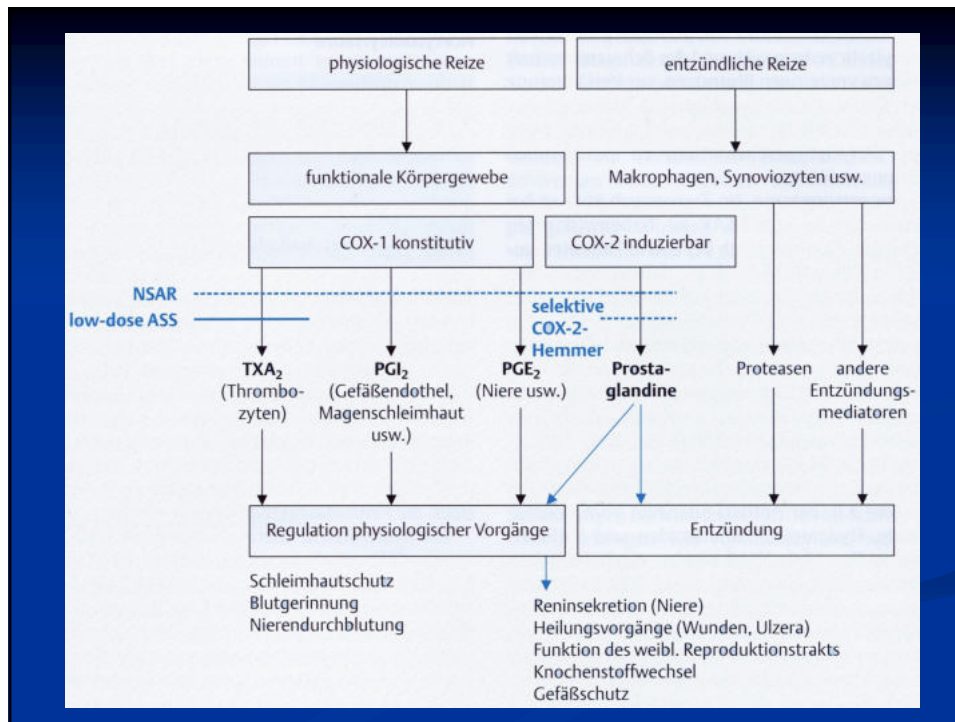




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## The Role of NK Cells in old patients

### Property of the nuclear cells

- NK cells not change in old patients
- NK cells not dependence of the MHC complex
- NK as immune modulation therapy with Ozone
- NK with CD 8+ the most effective in immune therapy

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