Neutropaenia with biologics

First Excellence in Rheumatology Meeting
Istanbul. 19th February, 2011

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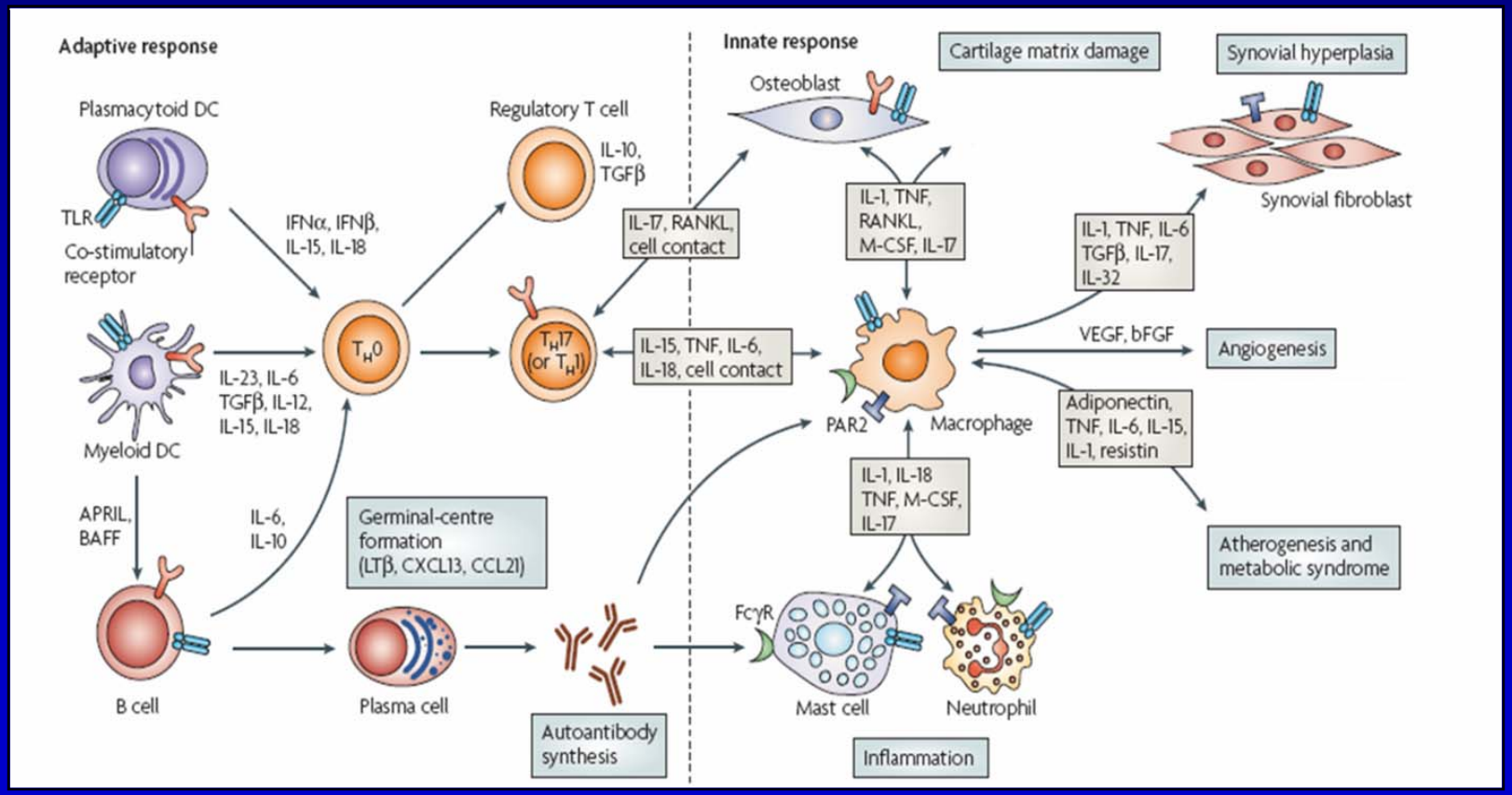
Overview and learning objectives

- The role of the neutrophil in health and in rheumatoid arthritis
- Physiological regulation of neutrophil number and function
- What effect do biologic anti-cytokine therapies have on neutrophils in RA?
- Are there clinical implications of these effects of anti-cytokine therapies?
- Are there differences between different cytokines inhibited?
- What are the management implications?

Cytokines Are Important Regulators of Rheumatoid Arthritis Pathogenesis

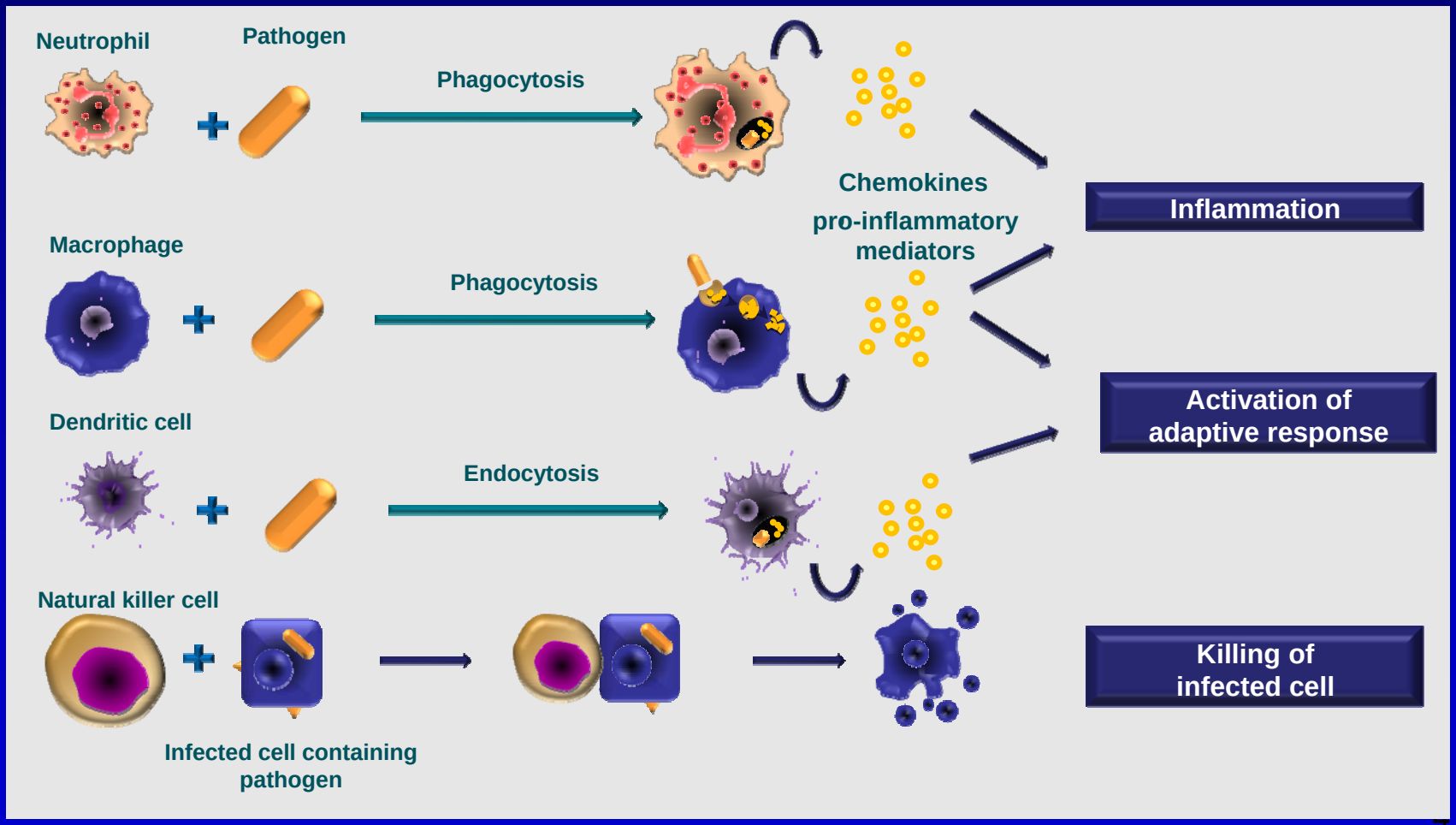
- Cytokines regulate many biological processes, including immune and inflammatory responses[1]
- Cytokine-driven immune and inflammatory responses mediate target tissue pathology and systemic disease in rheumatoid arthritis[2]



1. O'Shea JJ. *Ann Rheum Dis.* 2004;63(suppl 2):ii67-ii71; 2. McInnes IB, Schett G. *Nat Rev Immunol*. 2007;7(6):429-442.

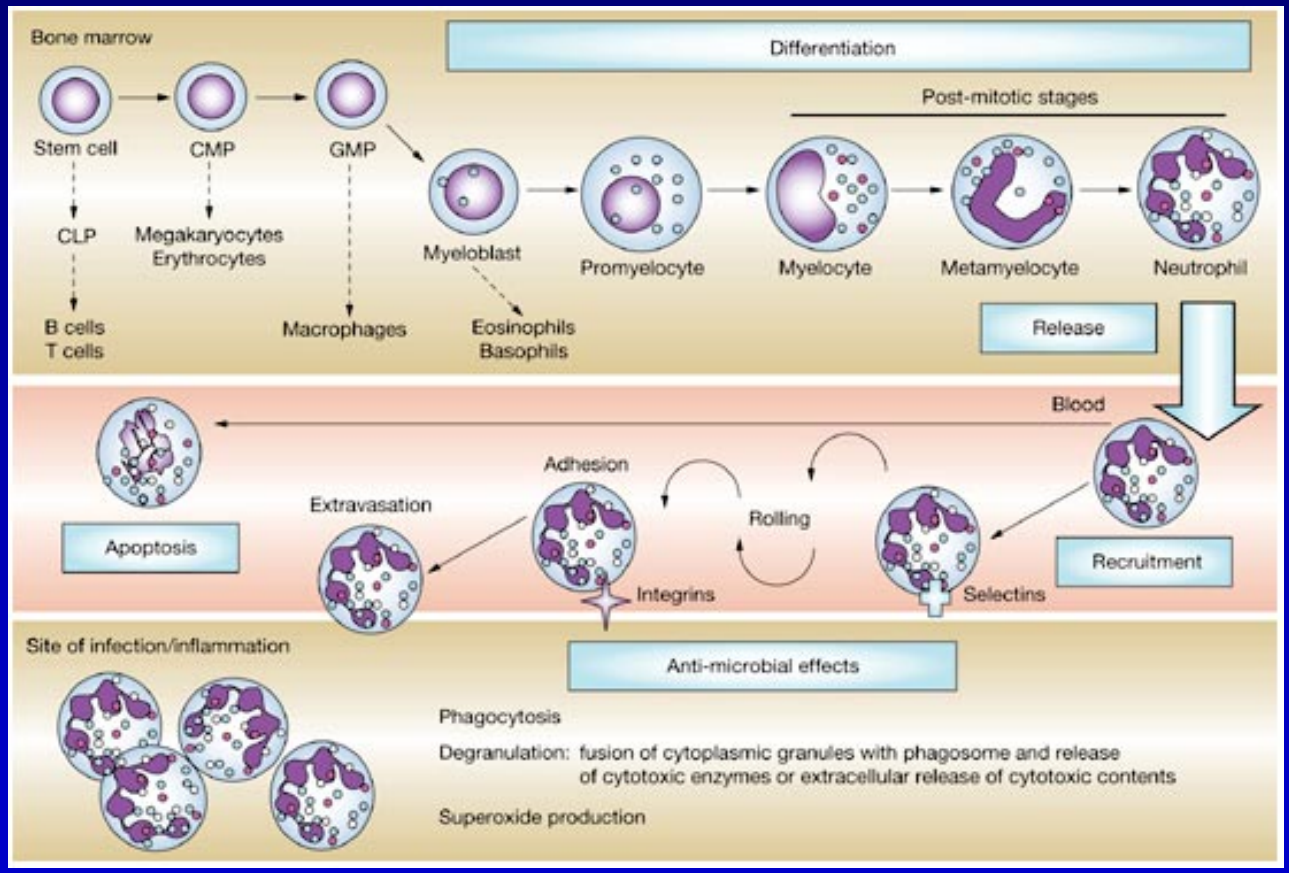
Innate Immunity

- First line defense; consists of non-specific, rapidly occurring responses that are mediated by phagocytic and natural killer cells as well as soluble molecules.



Murphy K, et al. Basic concepts in immunology; Innate immunity. In: *Janeway's Immunobiology*. 7th ed. New York, NY: Garland; 2008:1-38, 39-108.

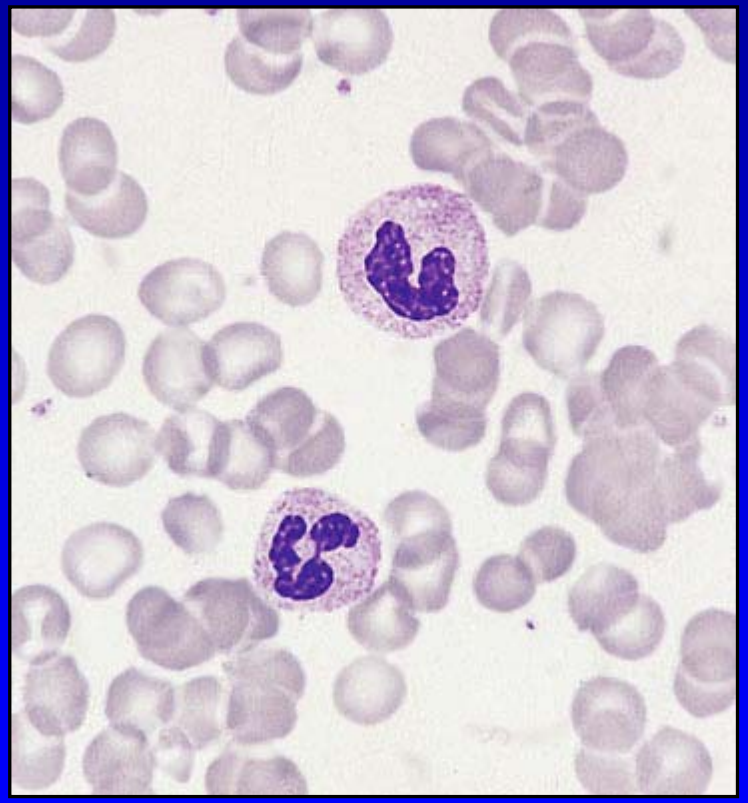
Differentiation, release, recruitment and activation of neutrophils



Eyles JL *et al.* (2006) *Nat Clin Pract Rheumatol* 2: 500–510

Neutrophils

- 13 microns diameter
- Segmented nucleus
- Pink granules on H+E
- Primary granules
  - Peroxidase +ve
- Secondary granules
  - Peroxidase -ve

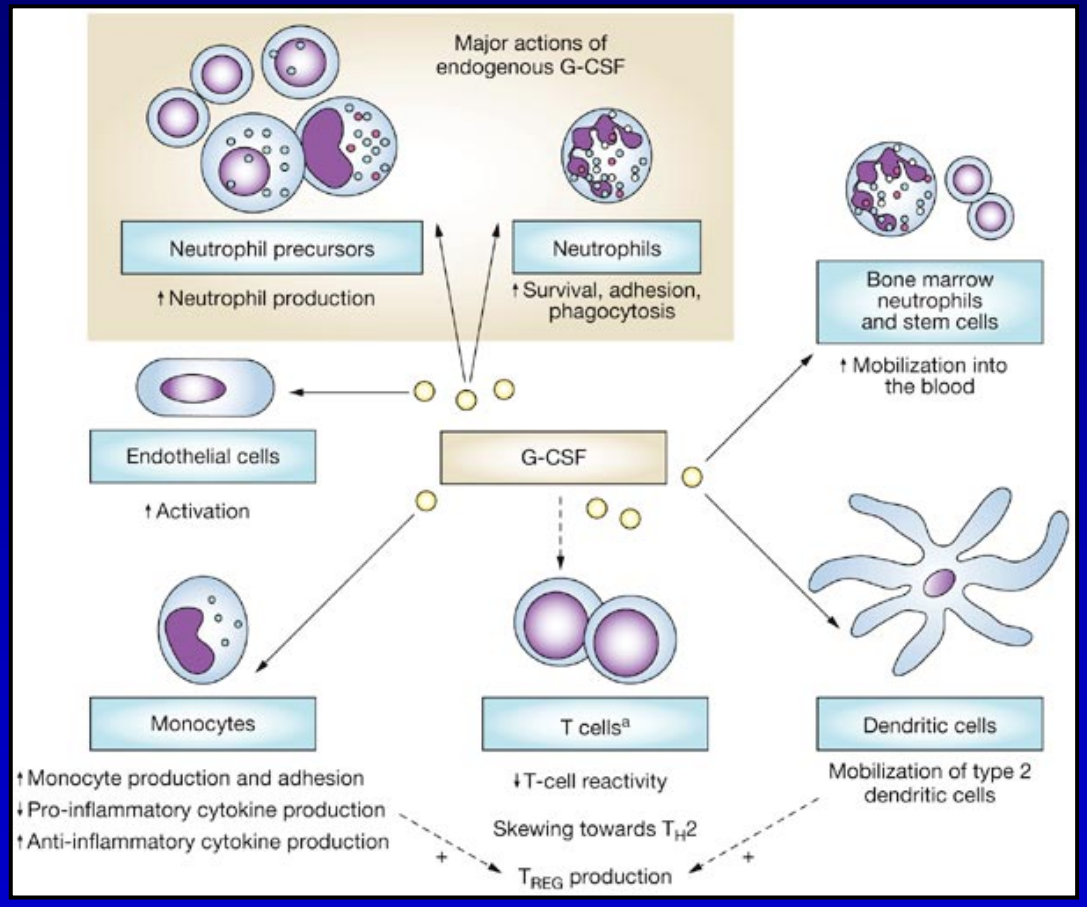


Granulocyte Production

- Maturation sequence in marrow
  - Myeloblasts 15 hrs
  - Promyelocytes 24 hrs
  - Myelocytes 104 hrs
  - Metamyelocytes 40 hrs
  - Band forms 66 hrs
  - Neutrophils 95 hrs

- Circulation half-life 6.7 hrs

The major biologic actions of G-CSF



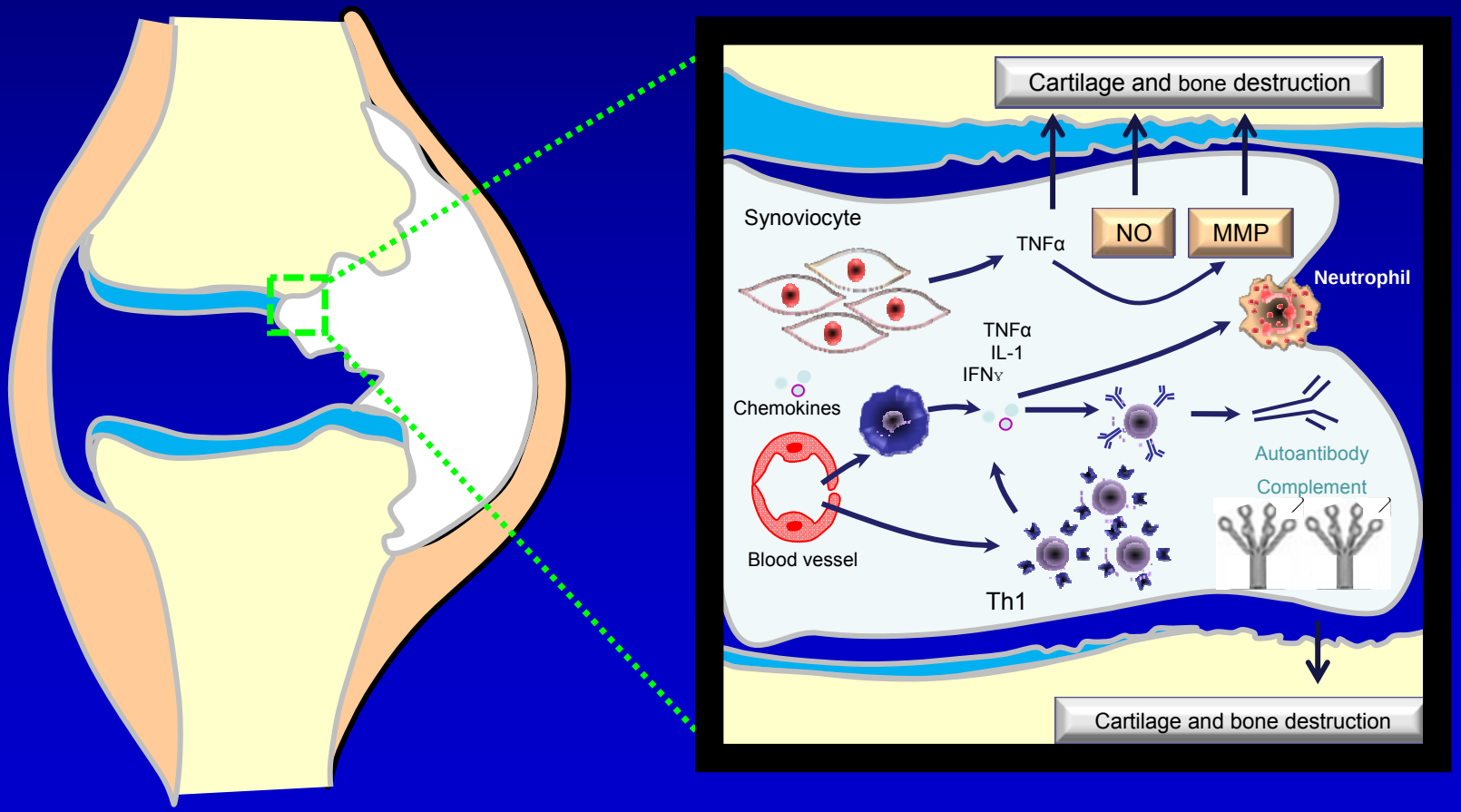
Eyles JL *et al.* (2006) *Nat Clin Pract Rheumatol* 2: 500–510

Neutrophil Numbers

- 2.26 x 10^9/kg in bone marrow (3 x p.b.)
- 0.7 x 10^9/kg in blood compartment
- 75 kg patient has 50 x 10^9 neutrophils in blood compartment
- 5 litre blood volume
- ? Neutrophil count = 10 x 10^9/litre
- But half marginated, $\therefore$ 5 x 10^9/litre

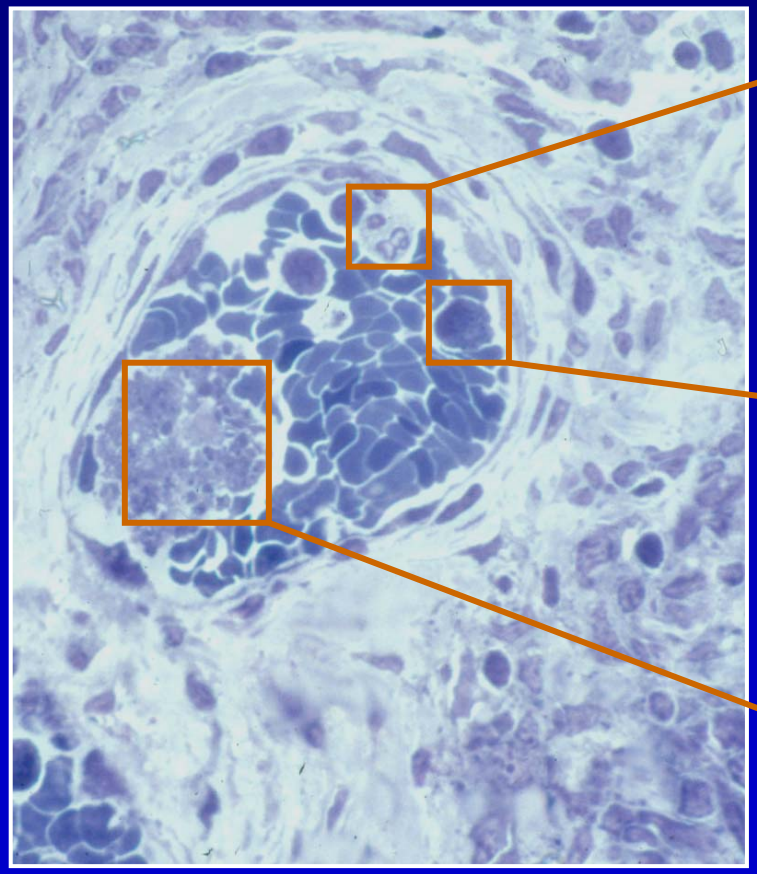
RA inflammation is orchestrated by pro-inflammatory mediators

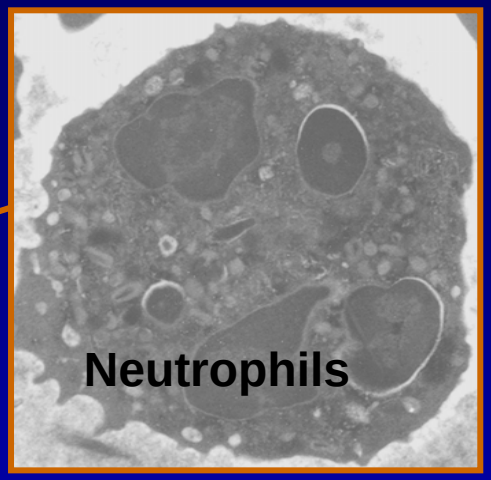
The influx and activation of the various cells are regulated by chemokines, adhesion molecules, and a network of inflammatory cytokines



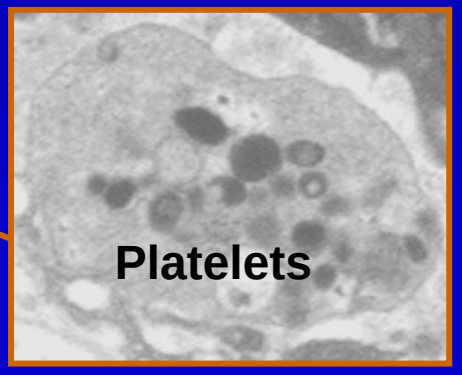
Adapted from Gonzalez-Rey et al. *Nature Reviews Immunology*, 2007; 7, 52-63

Neutrophil margination in RA synovium





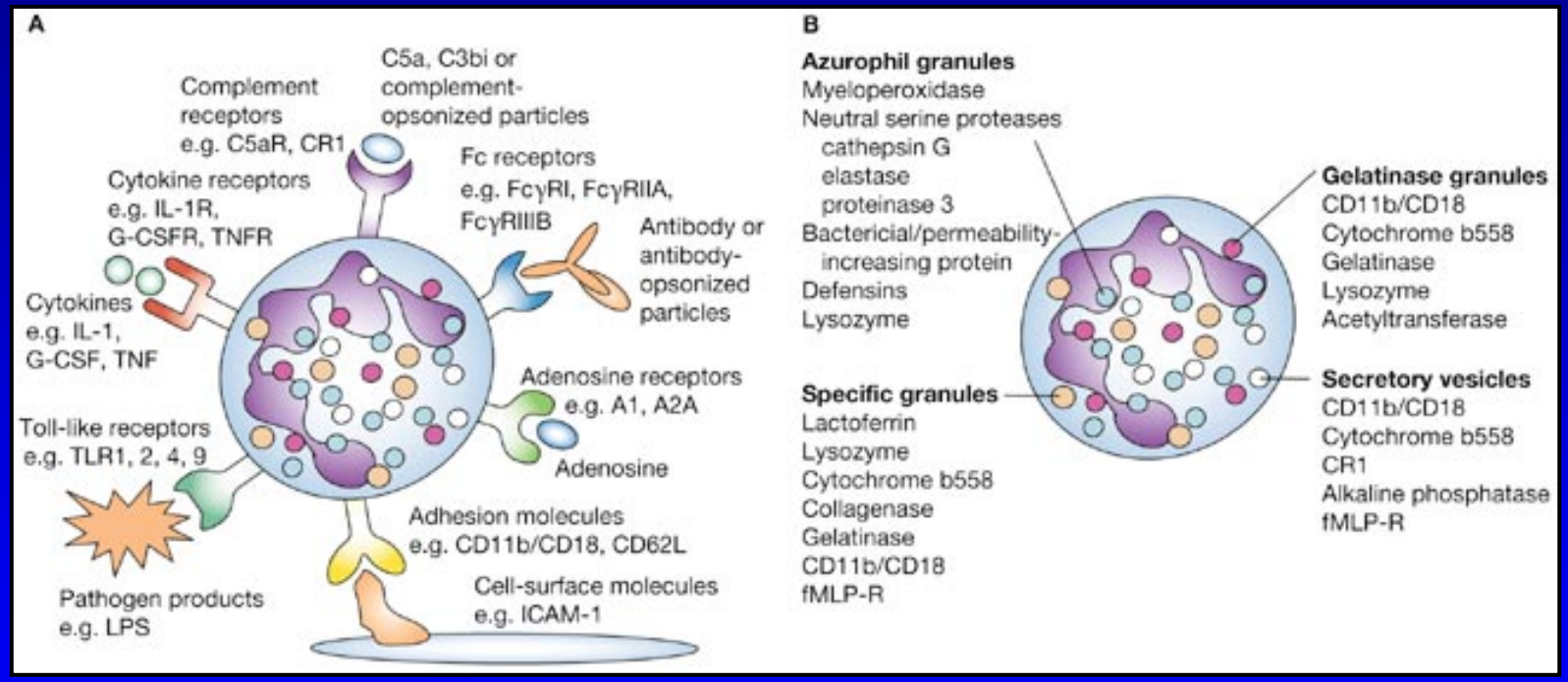
Mononuclear cells



Neutrophil Granules

- Primary
  - “Azurophil”, myeloperoxidase +ve
  - Microbicidal enzymes, hydrolases, etc
- Secondary
  - “specific”, lactoferrin +ve
  - Cytochrome b, lactoferrin, lysozyme, hydrolases, plasminogen activator, B12 binder
- Gelatinase granules + secretory vesicles

Neutrophil Granules

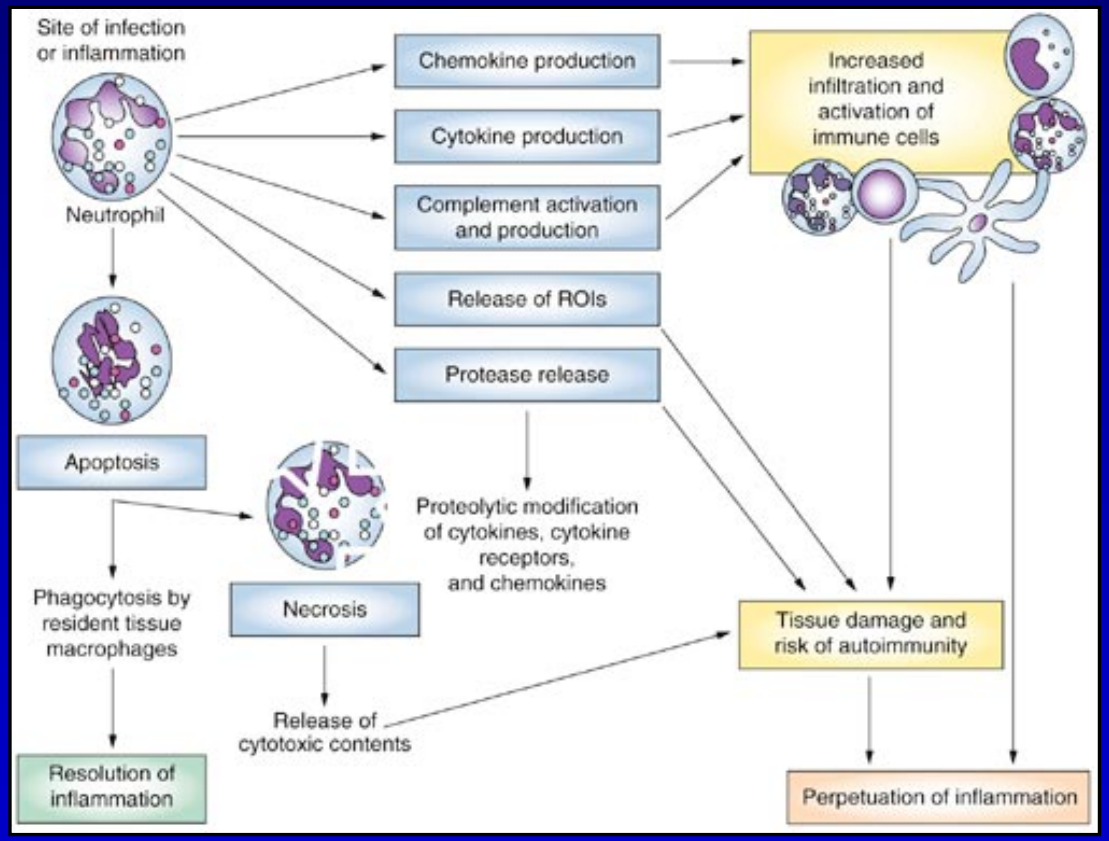


Eyles JL *et al.* (2006) *Nat Clin Pract Rheumatol* 2: 500–510

How Neutrophils Work

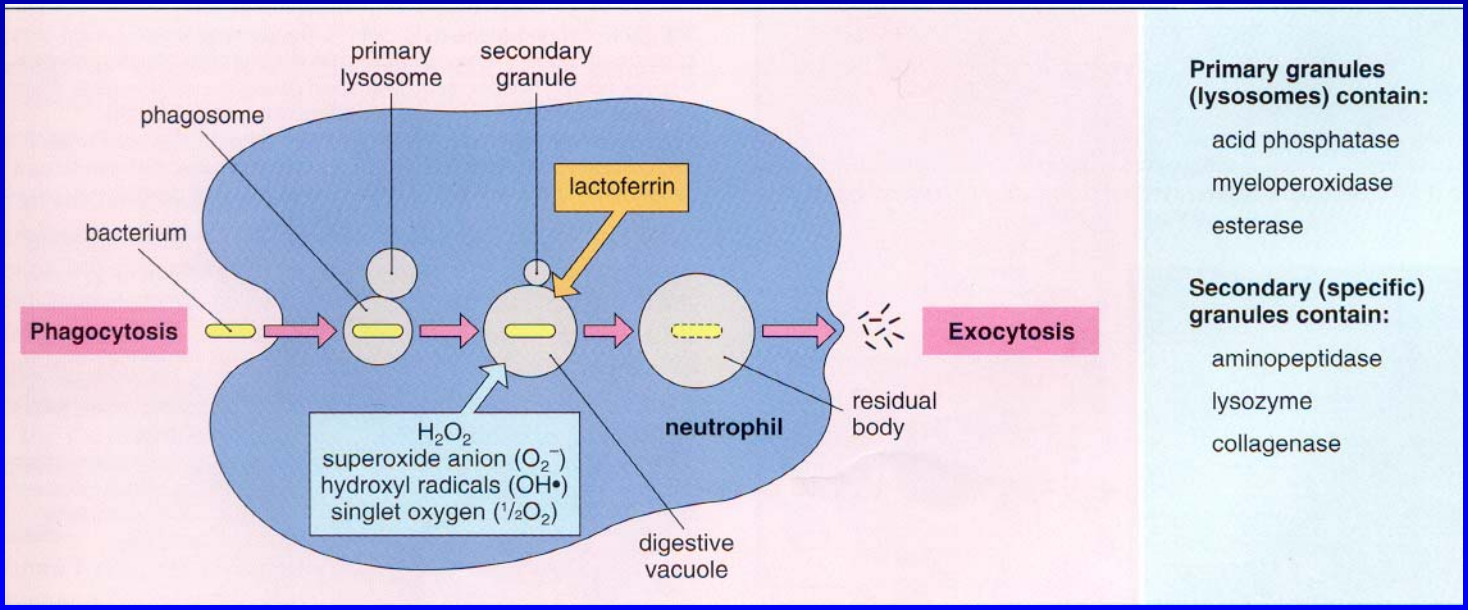
- Circulate at 300-1000 microns/sec
- Tumble along endothelium
- Adhesion and extra-vascular migration
- Chemotaxis
- Phagocytosis
- Respiratory burst
- Nitric oxide generation
- Apoptosis

Potential neutrophil effector functions at sites of infection or inflammation



Eyles JL *et al.* (2006) *Nat Clin Pract Rheumatol* 2: 500–510

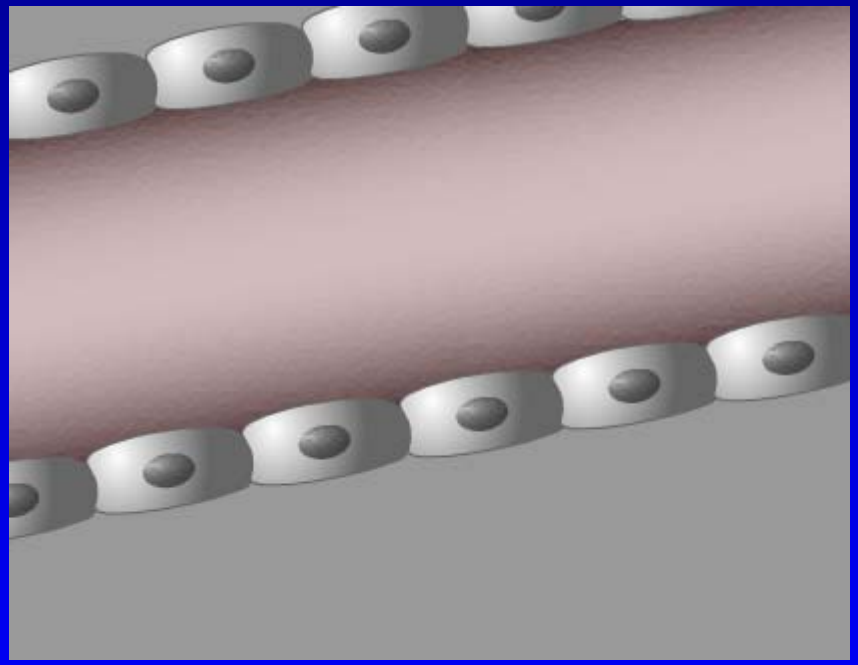
Neutrophil function



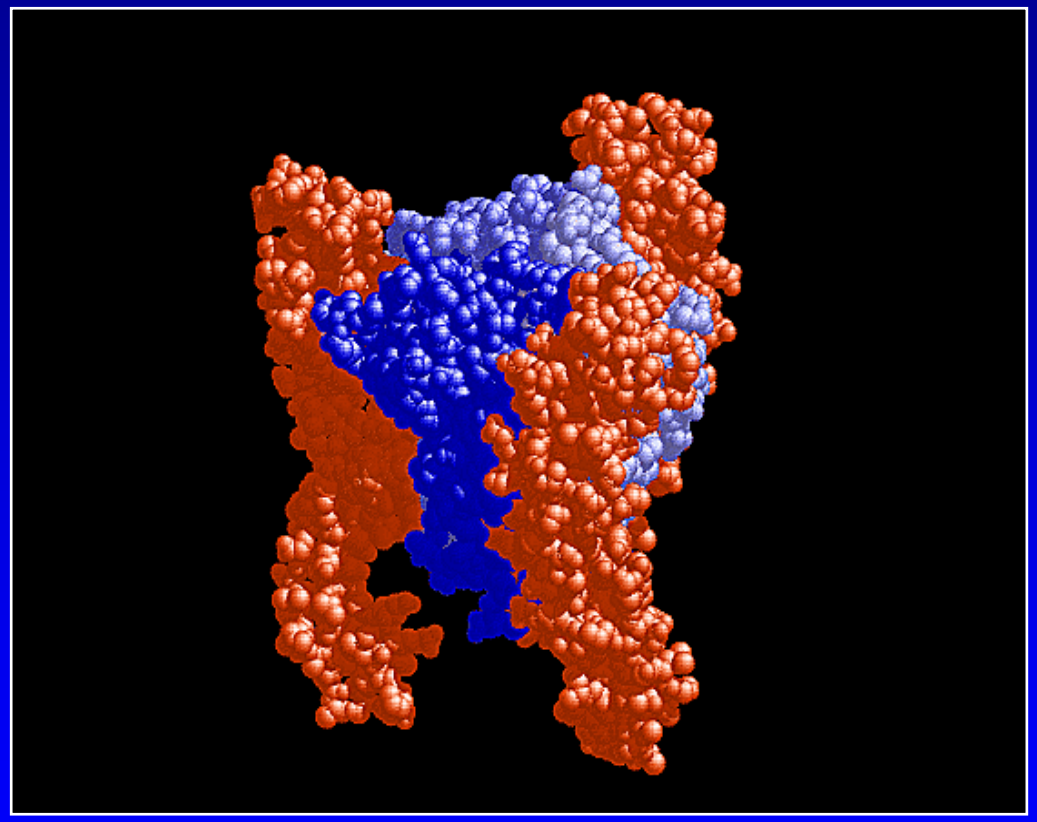
Neutropenia

- Reference range 2 – 7 x 10^9/l
- Ethnic neutropenia 1 x 10^9/l
- Neutropenia = < 1 x 10^9/l
- *Clinical problems uncommon > 0.5 x 10^9/l*
- Severe neutropenia = < 0.1 x 10^9/l

Migration of neutrophils from blood to tissue



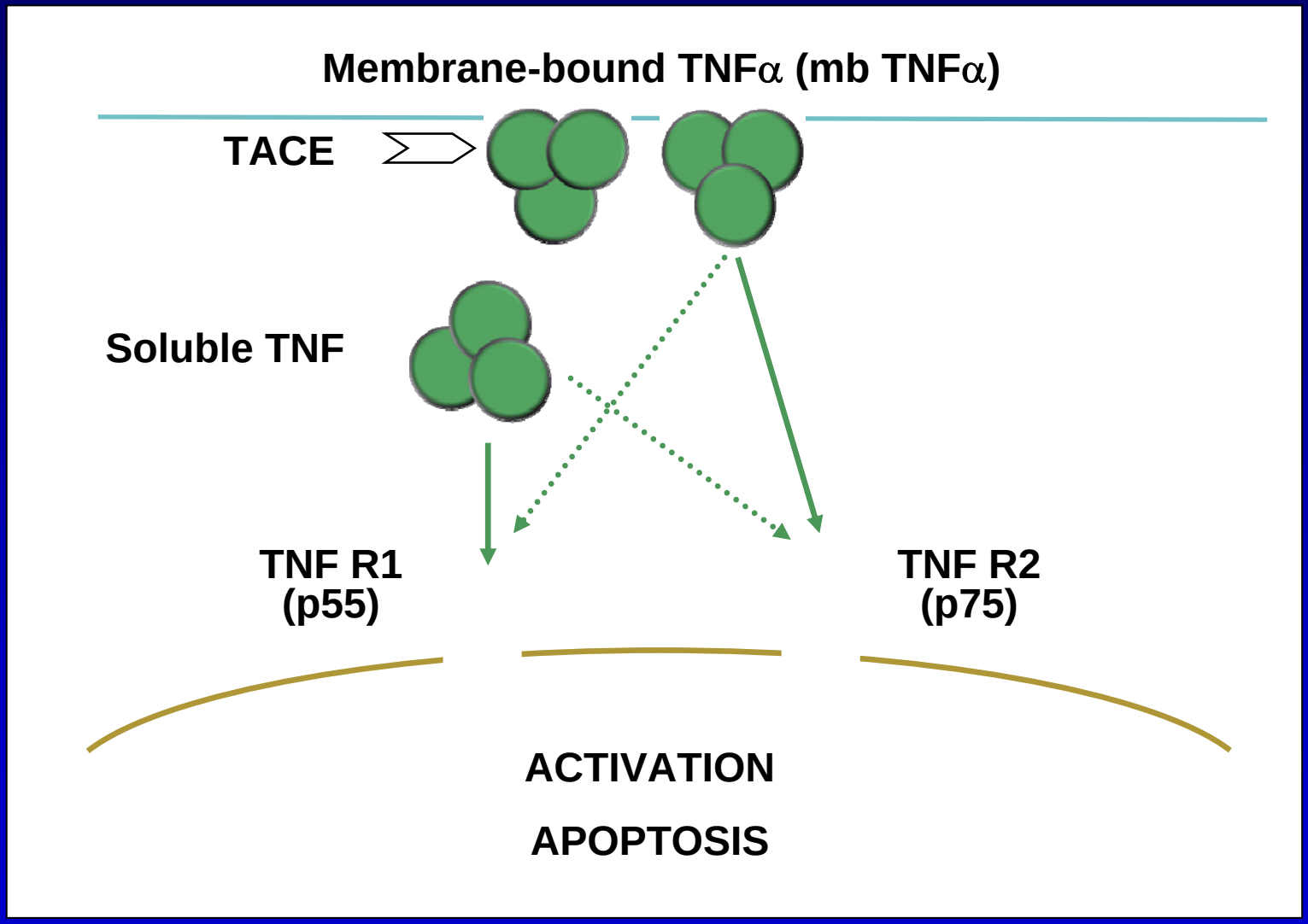
Targeting TNF in rheumatoid arthritis



Receptor-bound tumour necrosis factor alpha

TNF Production, Action or Neutralisation

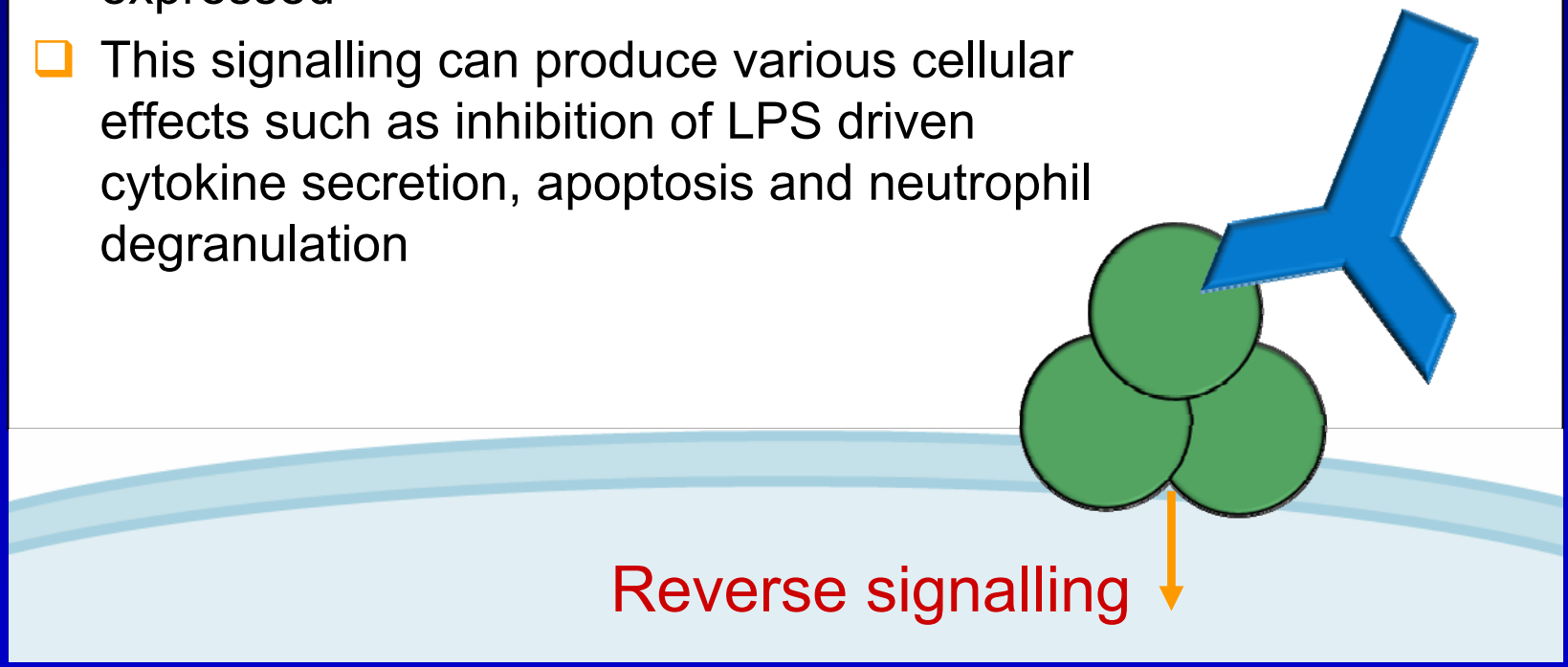
TNF and TNF Receptor "Systems"



Adapted from J. Sibilia with permission

Binding to membrane bound TNF

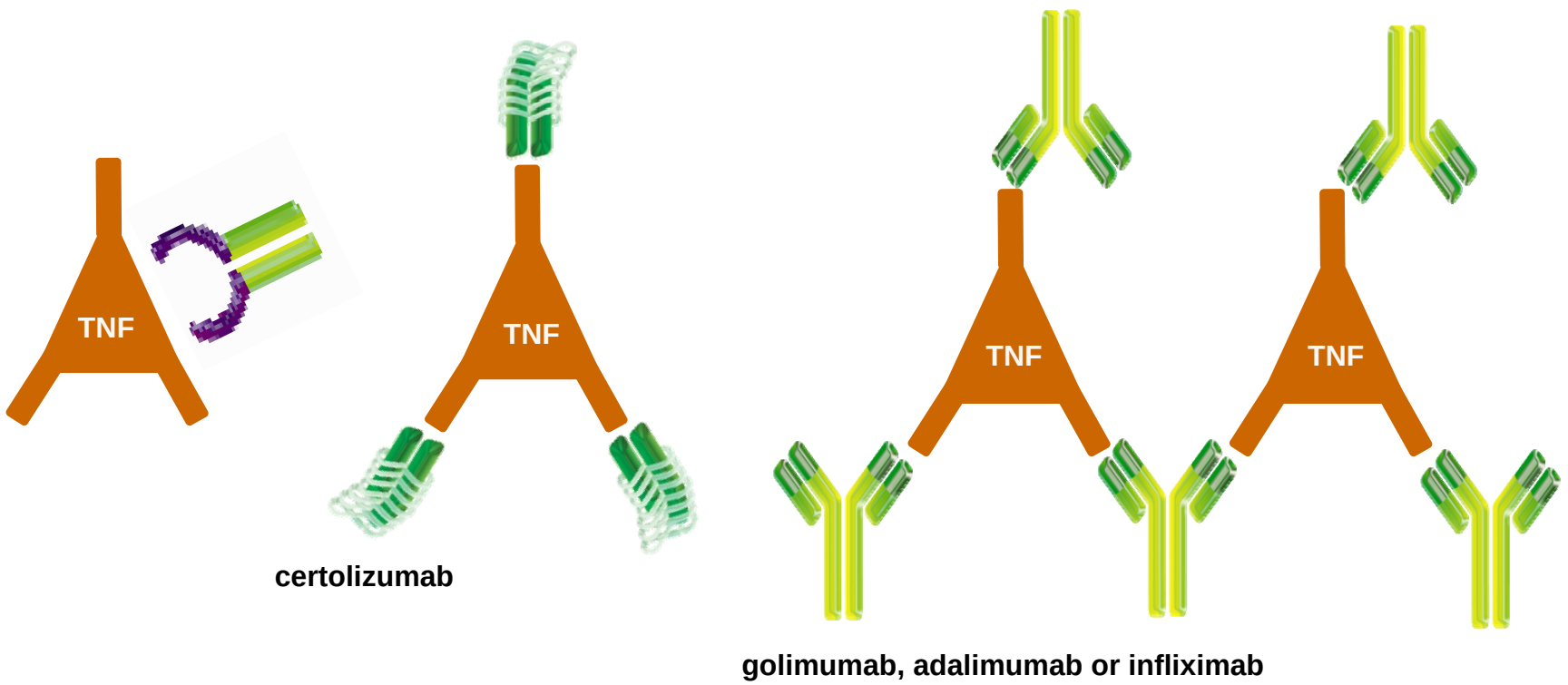
- The anti-TNFs can bind to membrane TNF and reverse signal into the cell on which it is expressed
- This signalling can produce various cellular effects such as inhibition of LPS driven cytokine secretion, apoptosis and neutrophil degranulation



Various intracellular Effects

Mitoma H et al. *Arthritis Rheum* 2008;58:1248-57
Nesbitt A et al. *Inflamm Bowel Dis* 2007;13:1323-1332

TNF inhibitors: biologic-ligand complexes



TNF binding and monomeric complex formation

TNF bivalent binding and polymeric immune complex formation with anti-TNF mAbs

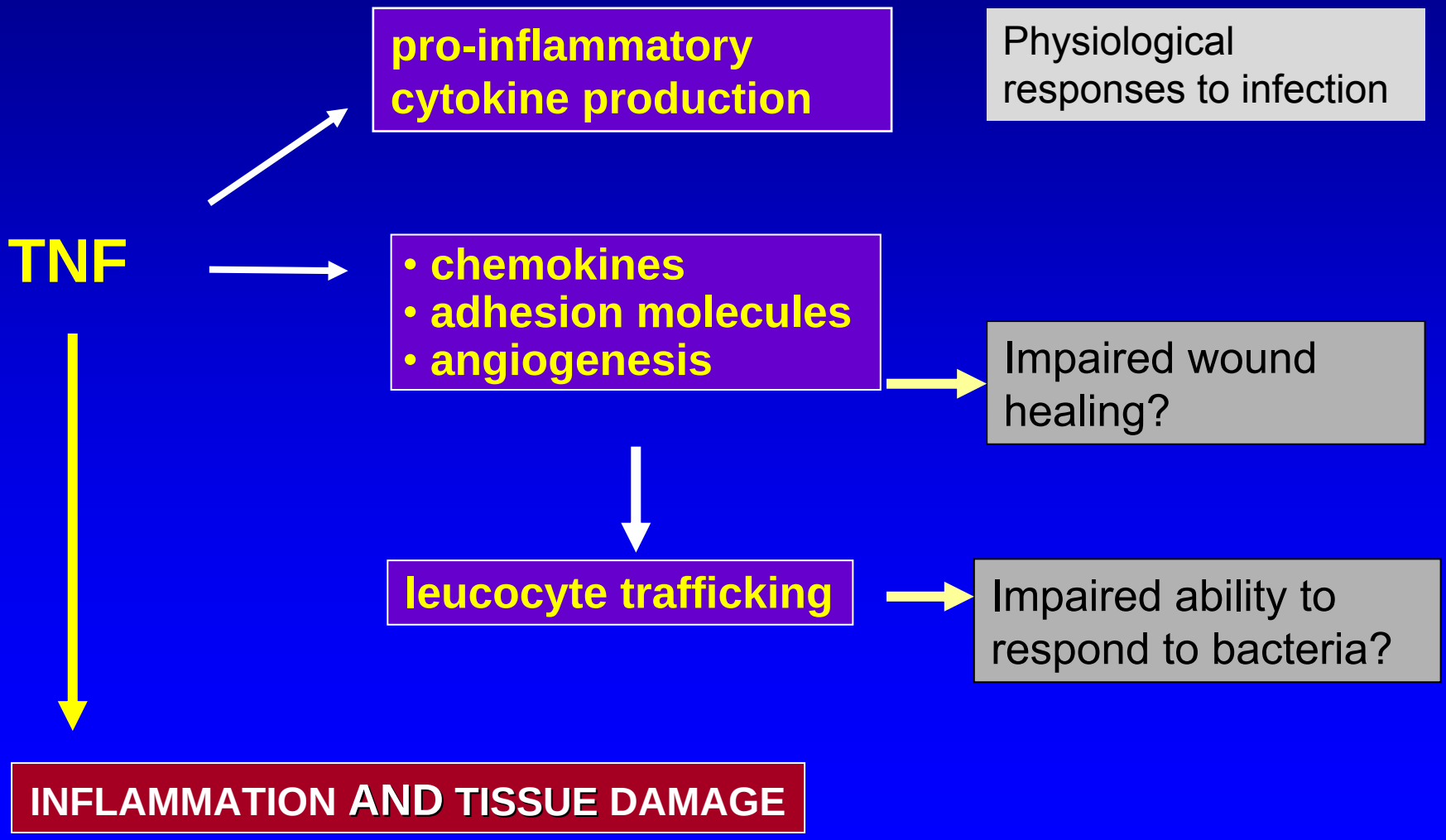
Taylor PC. *Curr Opin Pharmacol* 2010 ;10:308-15.

Anti-TNFs : Differences between Agents

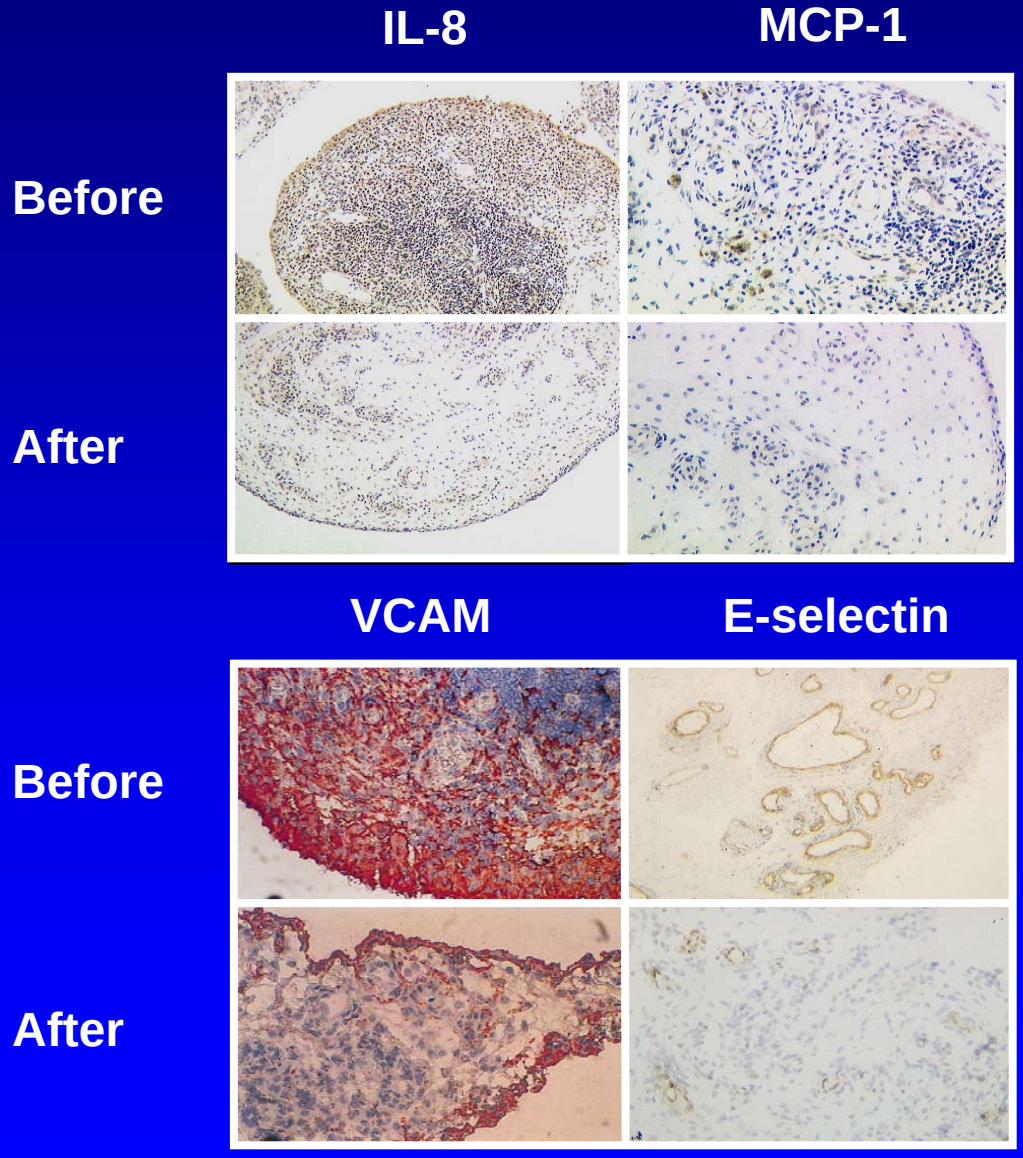
	Neutralisation of sol. TNF	Binding to m/b TNF	Complem. lysis and ADCC	Apoptosis	Complex formation	Neutrophil death	Inhibition of cytokine secretion
Etanercept	+++	++	++	++	-	++	-
Adalimumab	++	+++	+++	+++	+++	+++	++
Infliximab	++	+++	+++	+++	+++	+++	++
Certolizumab	+++	+++	-	-	-	-	+++

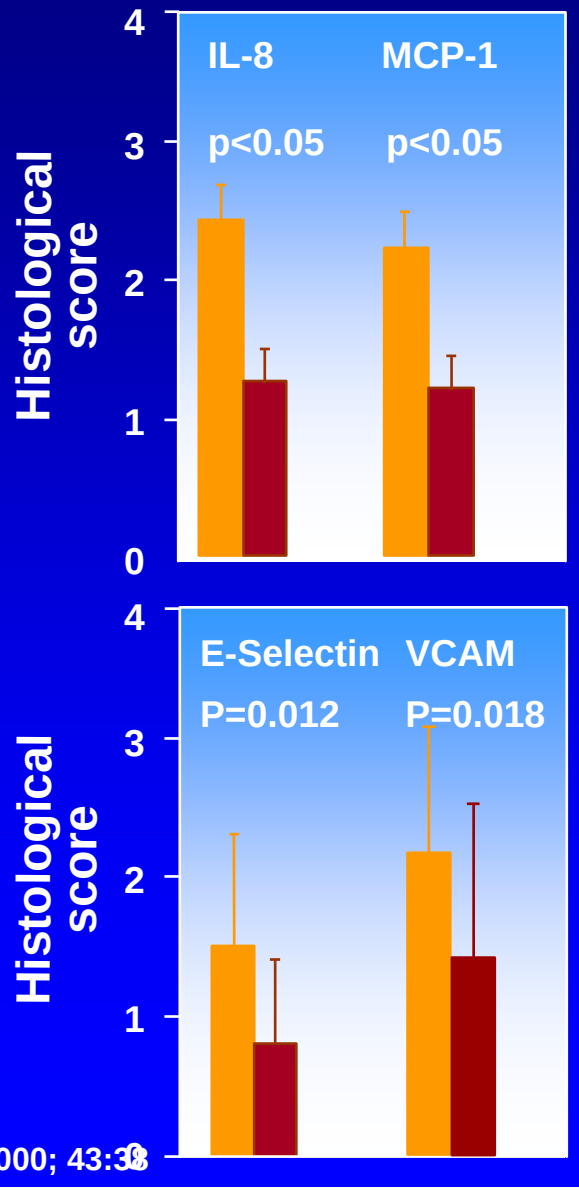
Taylor PC. *Curr Opin Pharmacol* 2010 ;10:308-15.

Biology of TNF in RA



TNF modulates both arms of inflammatory cell recruitment cascade

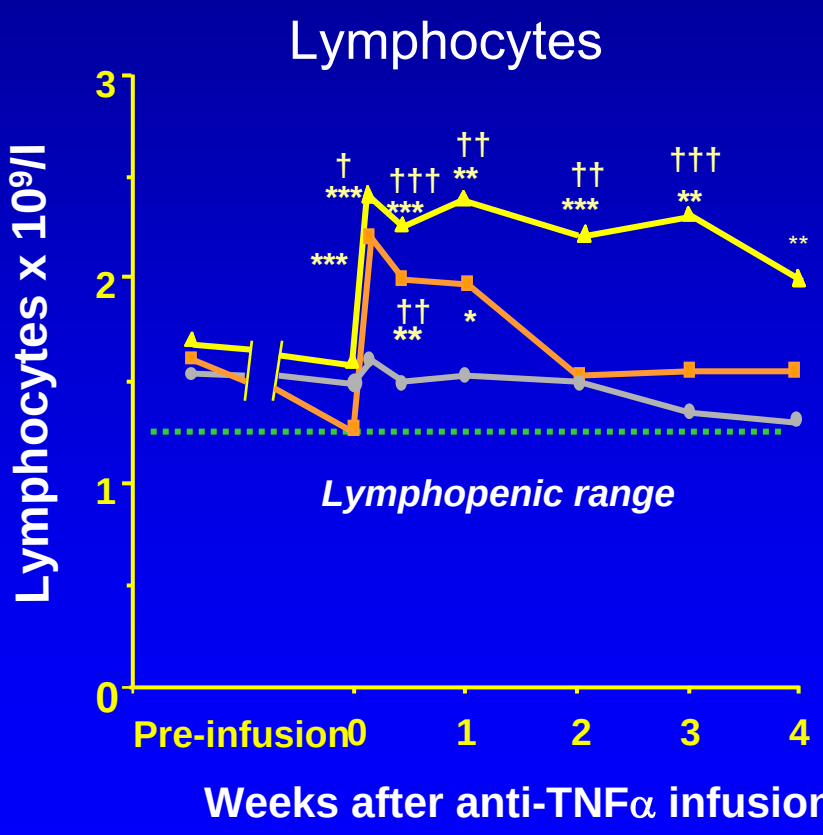


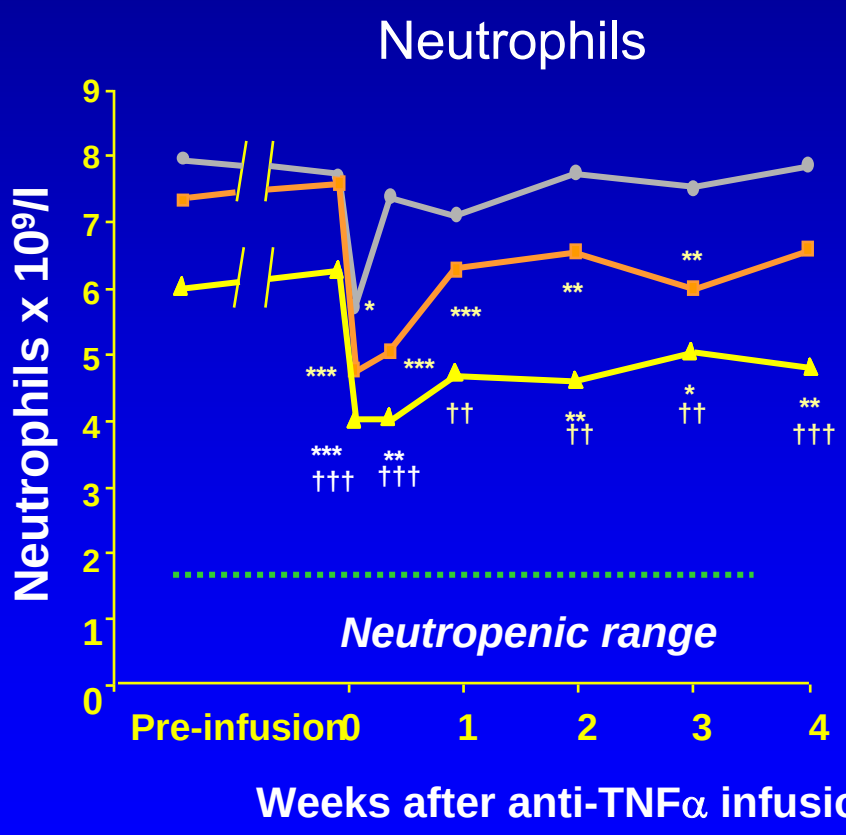


Tak, **Taylor** *et al* Arthritis Rheum 1996, 39; 1077, **Taylor *et al*, Arthritis Rheum 2000; 43:38**

Reciprocal effects of anti-TNFα on lymphocyte and neutrophil counts

Placebo ● 1mg/kg cA2 ■ 10mg/kg cA2 ▲





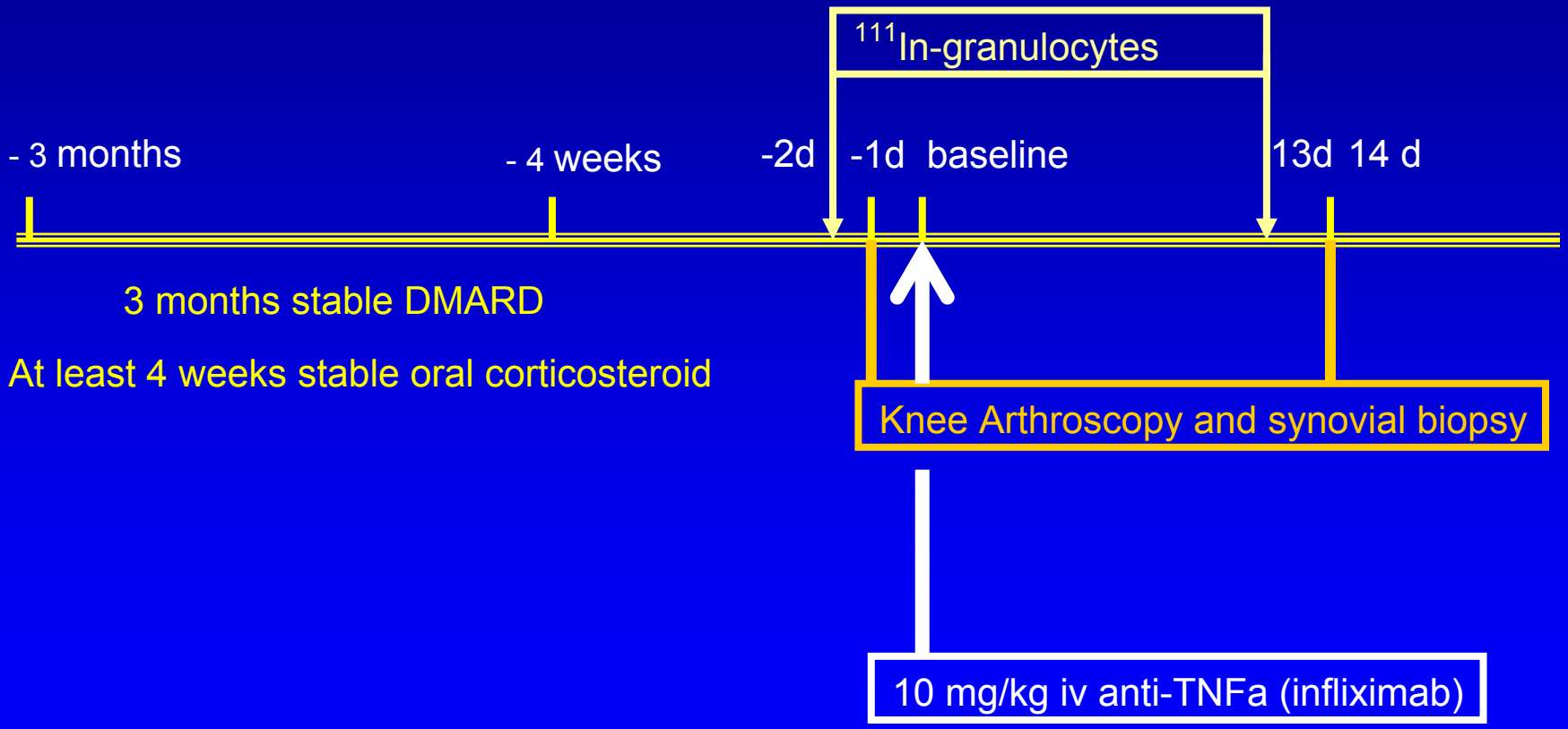
Paleolog *et al*, Arthritis Rheum 1996, 39; 1082

p<0.05, ** p<0.01, *** p<0.001 *versus* pre-infusion

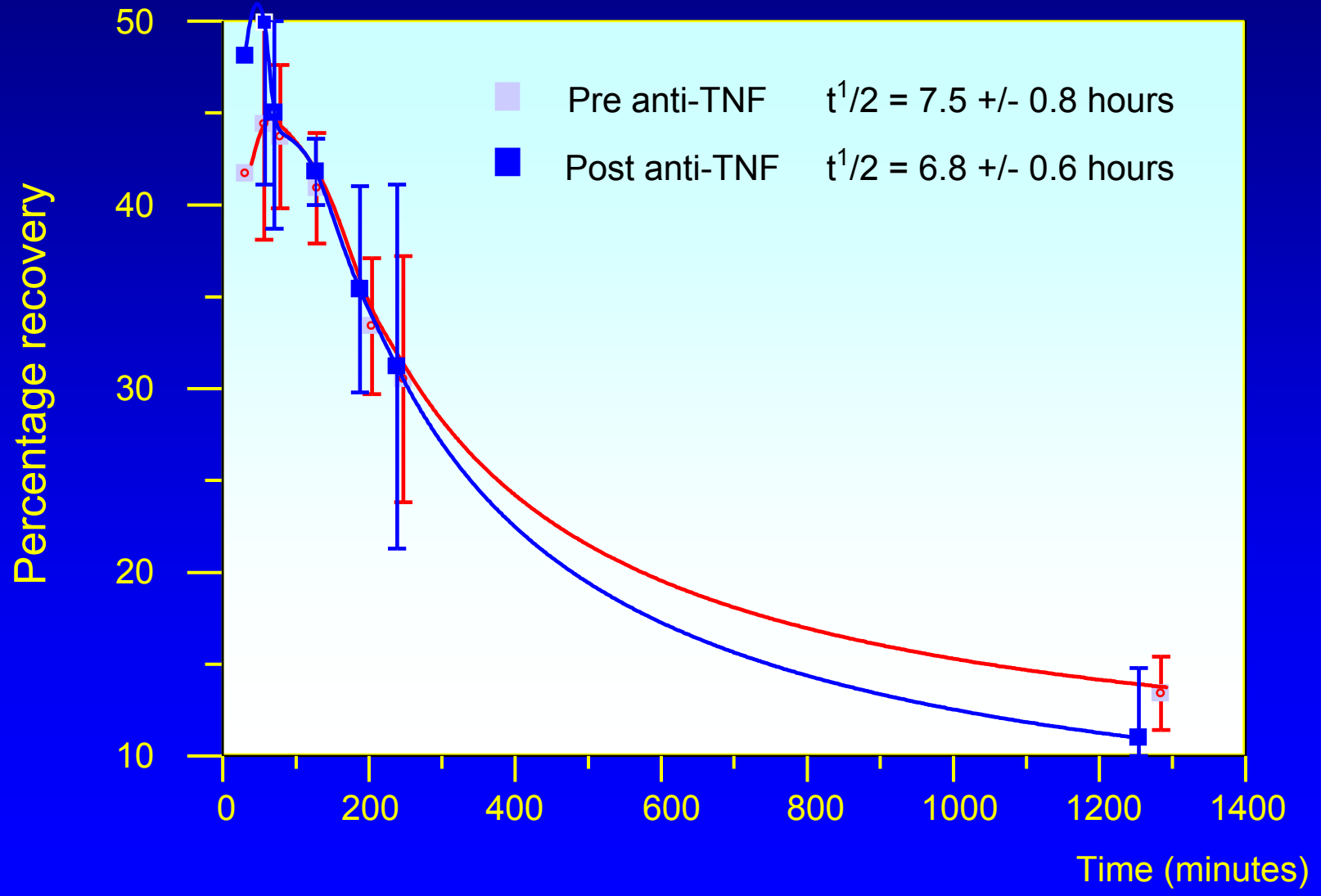
† p<0.05,†† p<0.01,††† p<0.001 *versus* change in placebo

STUDY DESIGN

10 Patients with active RA (including at least one swollen knee)

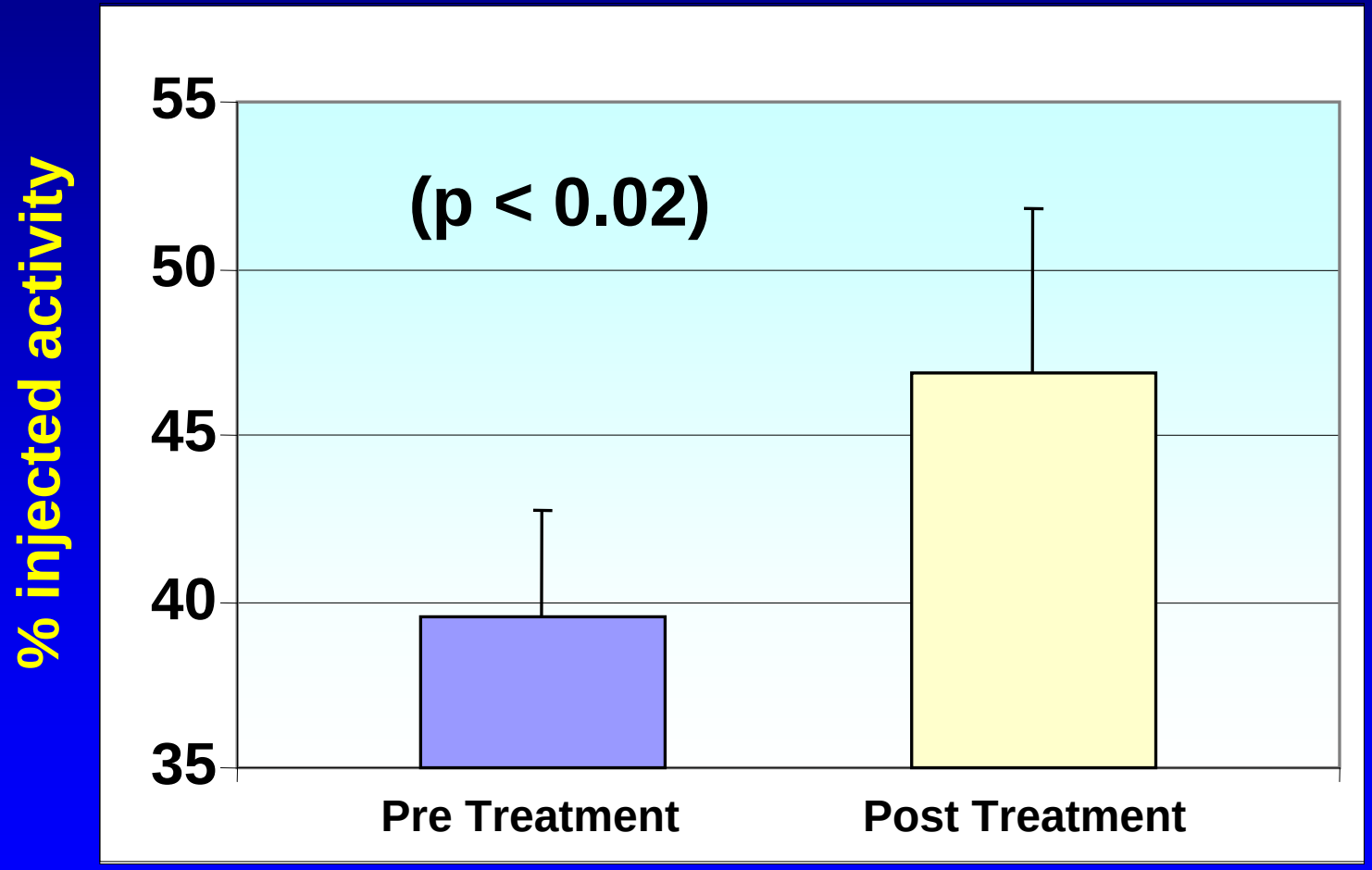


Mean percentage radiolabeled granulocyte recovery



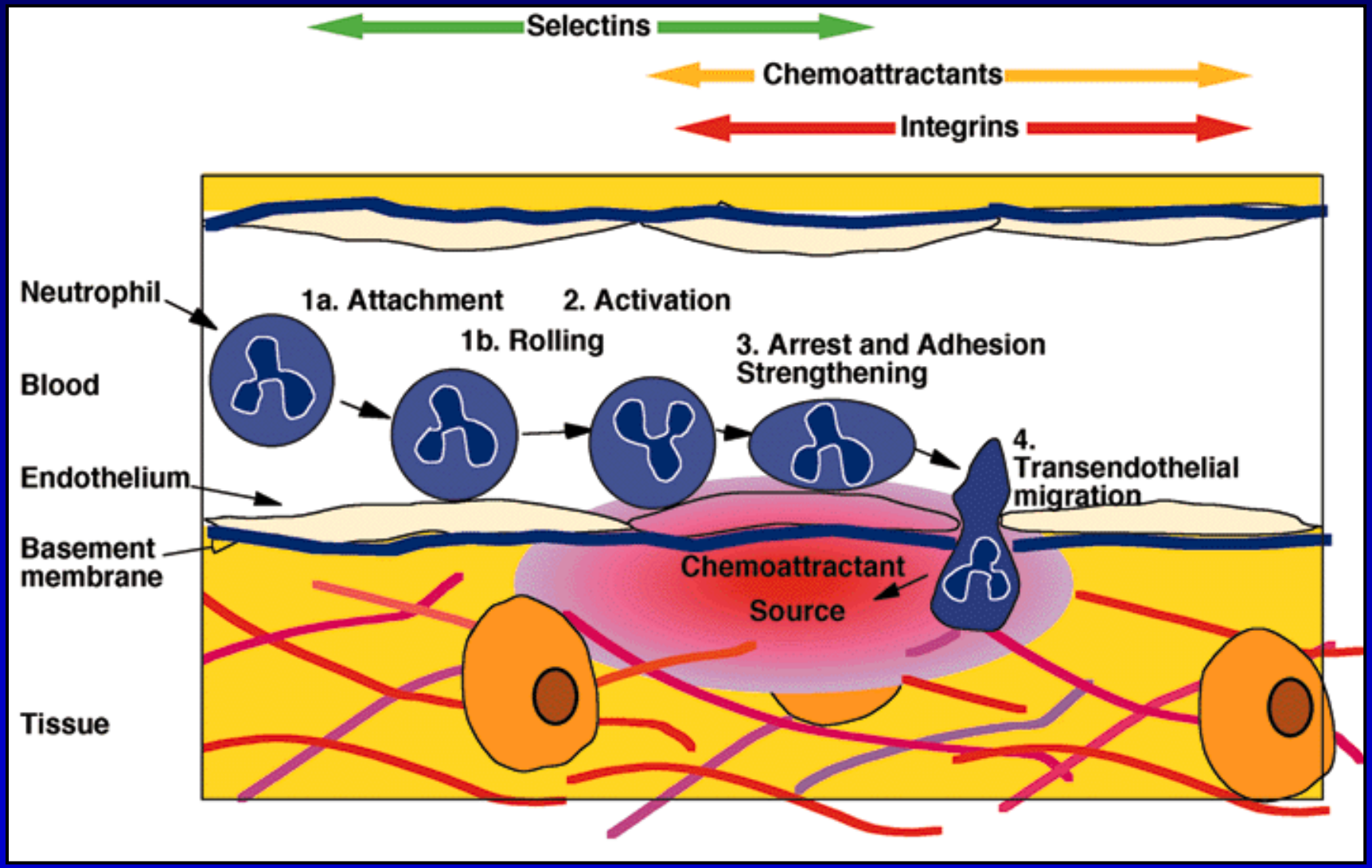
Taylor PC *et al*, Clin Sci 1999, 97: 85

Increased intravascular recovery of ^{111}In-granulocytes after 10 mg/kg infliximab

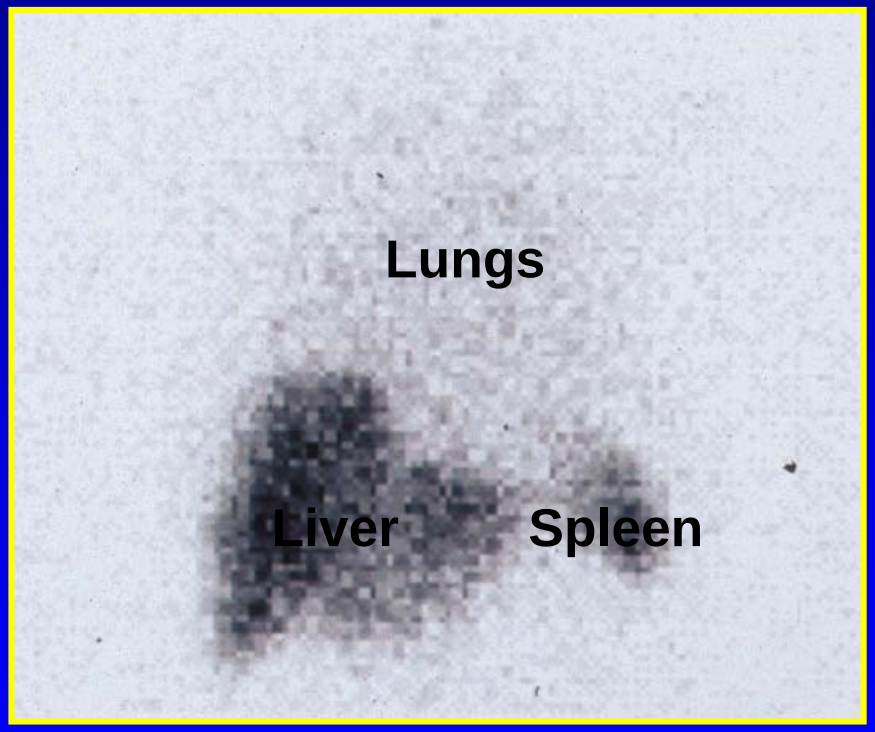


Taylor *et al*, Clin Sci 1999, 97: 85

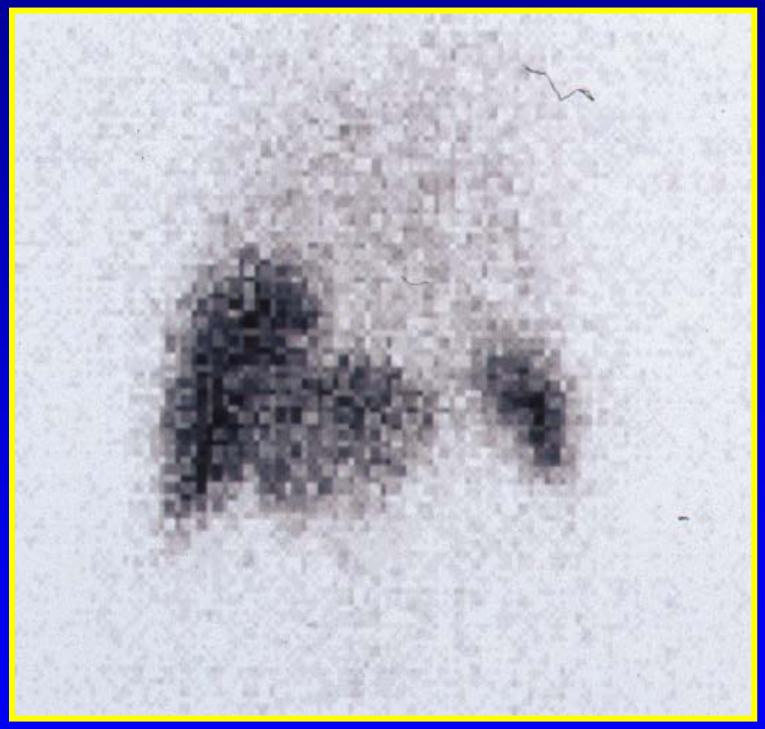
Neutrophil margination



Which sites in the whole body marginating granulocyte pool are affected?

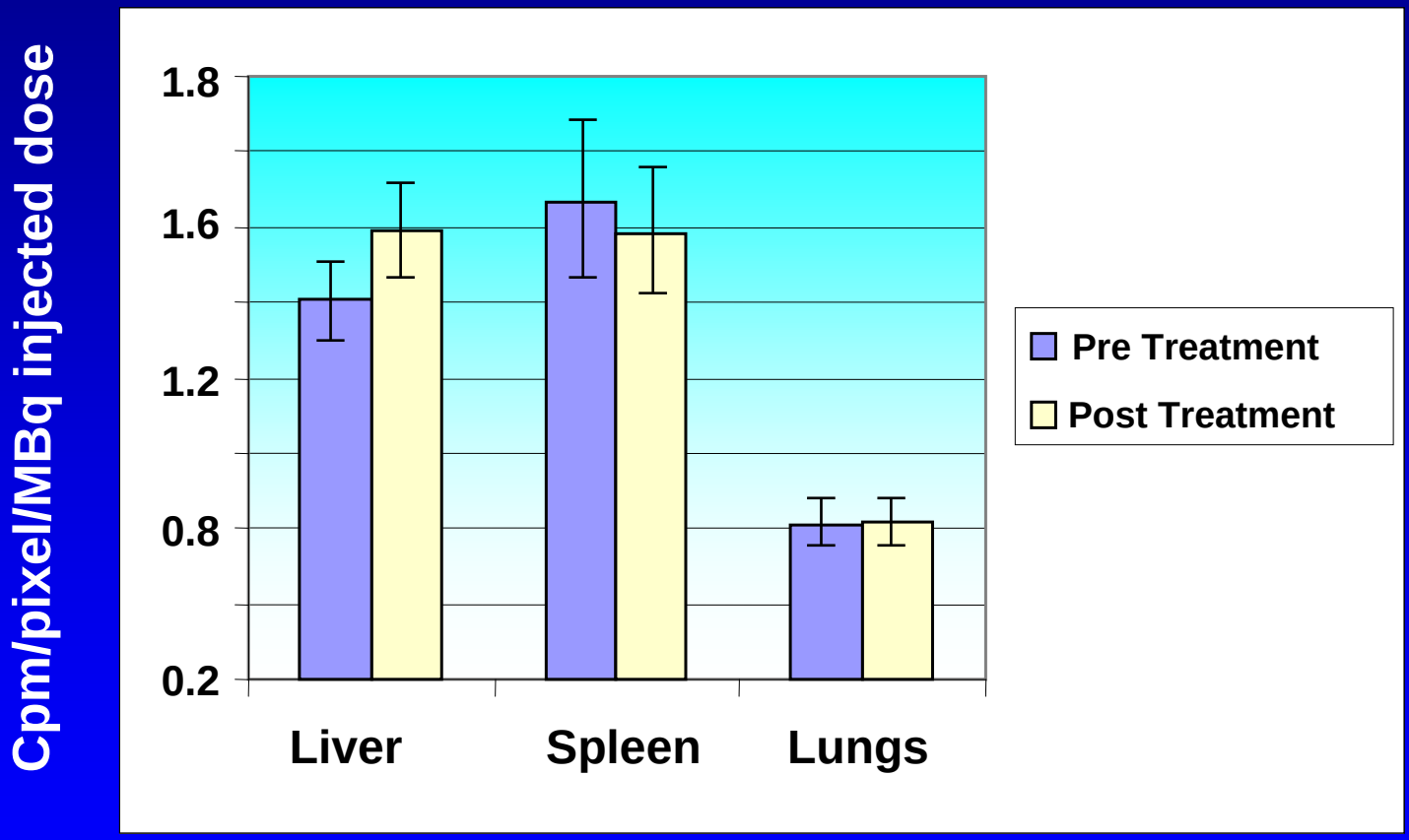


Pre- treatment



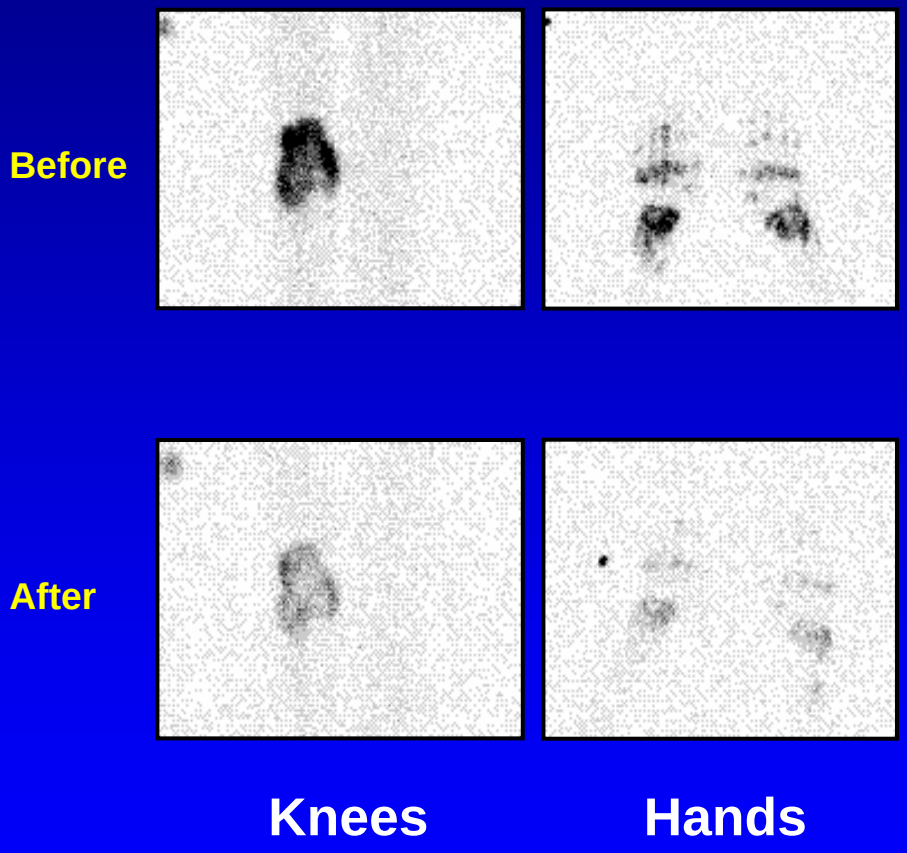
2 weeks post 10mg/kg

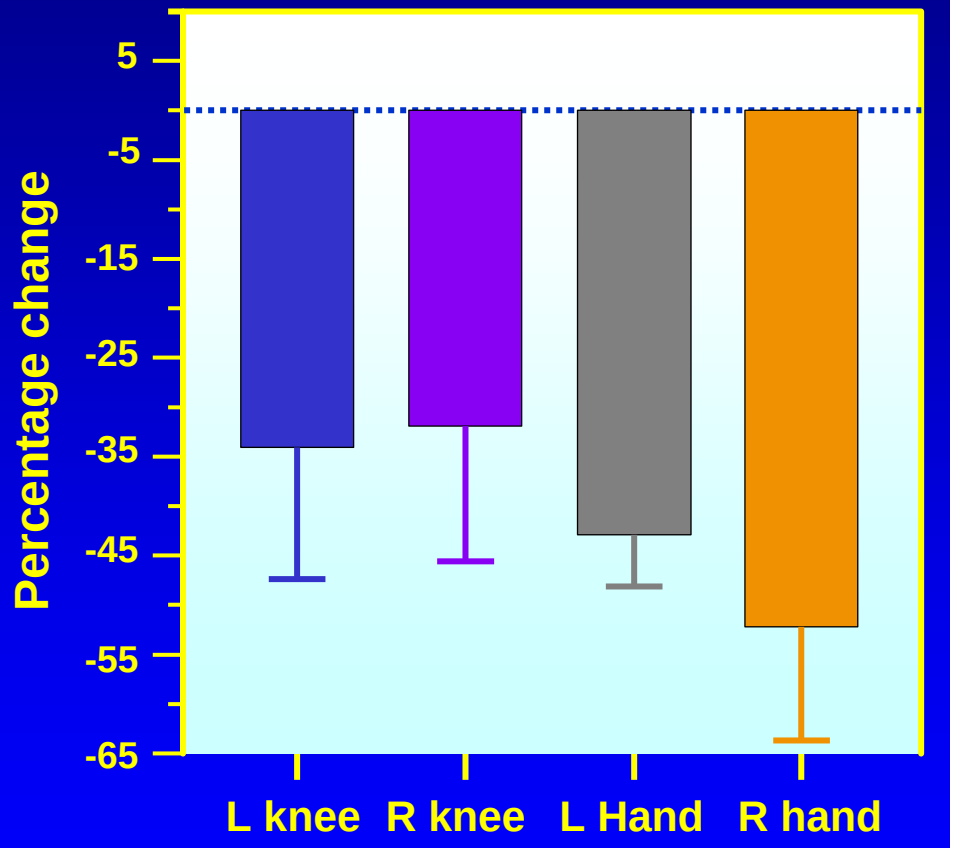
Organ activity is unchanged after injection of ^{111}In-granulocytes infliximab



Taylor *et al*, Clin Sci 1999, 97: 85

Infliximab reduces inflammatory cell recruitment to joints





Taylor *et al*, Arthritis Rheum 2000; 43:38

Review of reported adverse events with etanercept and infliximab

Clinical Category Adverse Event*	Etanercept Pts (Rate/100 Pts)	Infliximab Pts (Rate/100 Pts)
All Fatalities	342 (0.274)	199 (0.097)
Fatal Sepsis	63 (0.050)	28 (0.014)
Anemia NOS†	57 (0.046)	32 (0.016)
Neutropenia/Agranulocytosis	29 (0.023)	15 (0.007)
Leukopenia Excluding Neutropenia	75 (0.060)	11 (0.005)
Thrombocytopenia NOS†	64 (0.051)	21 (0.010)
Pancytopenia	29 (0.023)	16 (0.008)
Lymphoma	41 (0.033)	44 (0.021)
Cardiopulmonary Arrest	43 (0.034)	18 (0.009)
Heart Failure	248 (0.198)	132 (0.064)
Anaphylaxis	0 (0.000)	40 (0.020)
Serum Sickness	1 (0.001)	24 (0.012)
Lupus-like Syndrome	16 (0.013)	59 (0.029)
Histoplasma Infections	0 (0.000)	22 (0.011)
Tuberculosis	15 (0.012)	73 (0.036)
Central Demyelination/NOS†	33 (0.026)	8 (0.004)

All adverse events were coded using MedDRA; †NOS = Not Otherwise Specified.

Callegari P, et al. EULAR; June 9-12, 2004; Berlin. Abstract SAT0456. [Evidence Level B]

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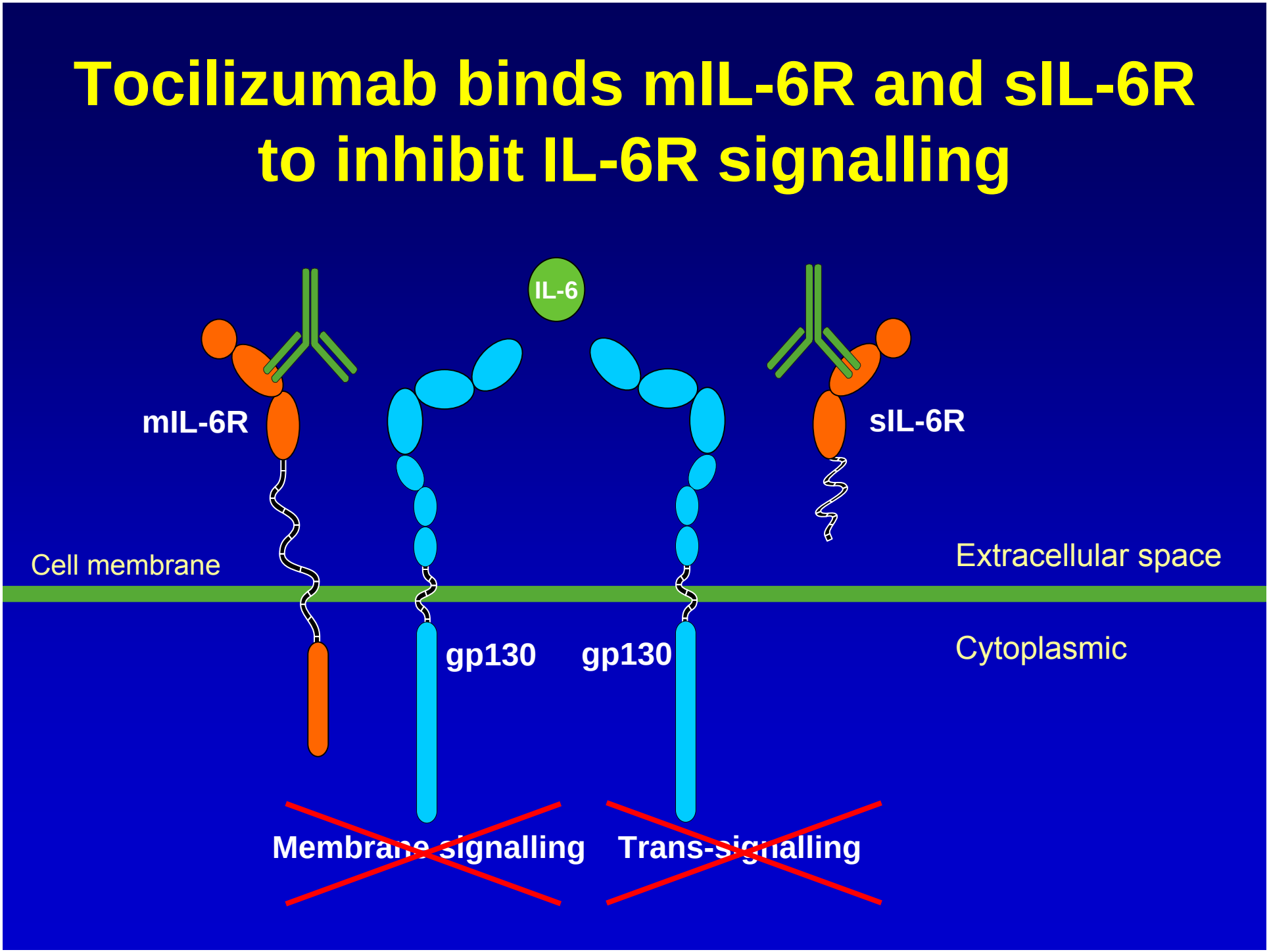
Targeting IL-6 in rheumatoid arthritis

IL-6 membrane receptor

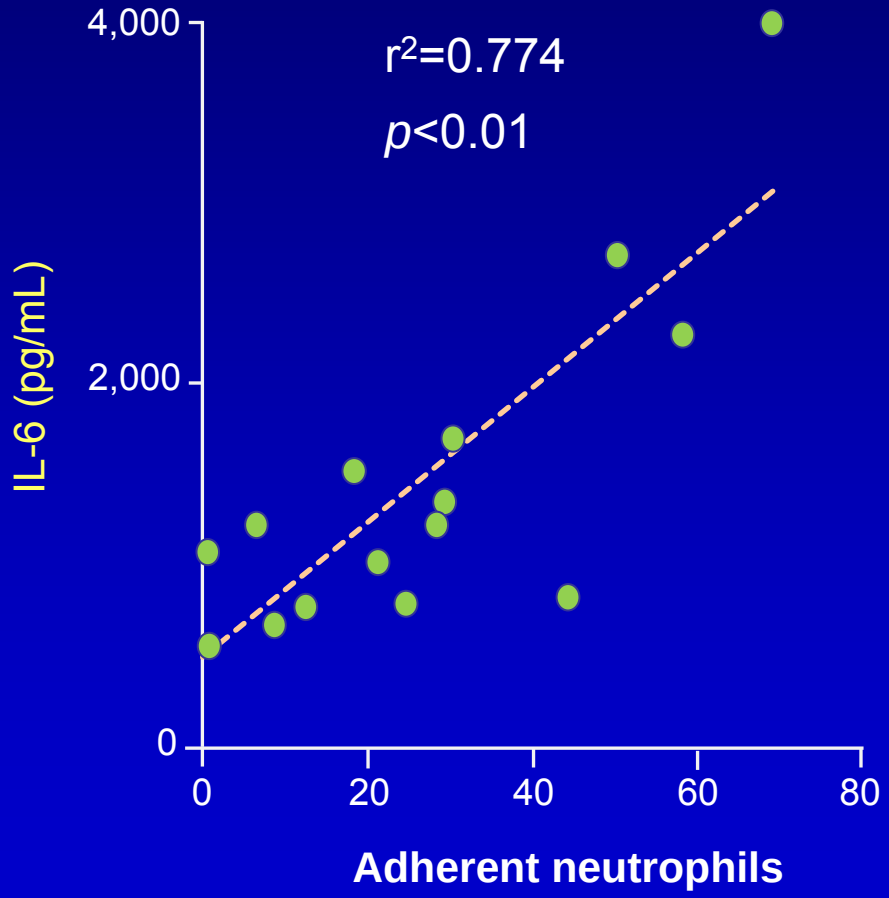
Tocilizumab receptor complex

Tocilizumab binds mIL-6R and sIL-6R to inhibit IL-6R signalling



Immune effects: IL-6/sIL-6R contribute to leukocyte recruitment

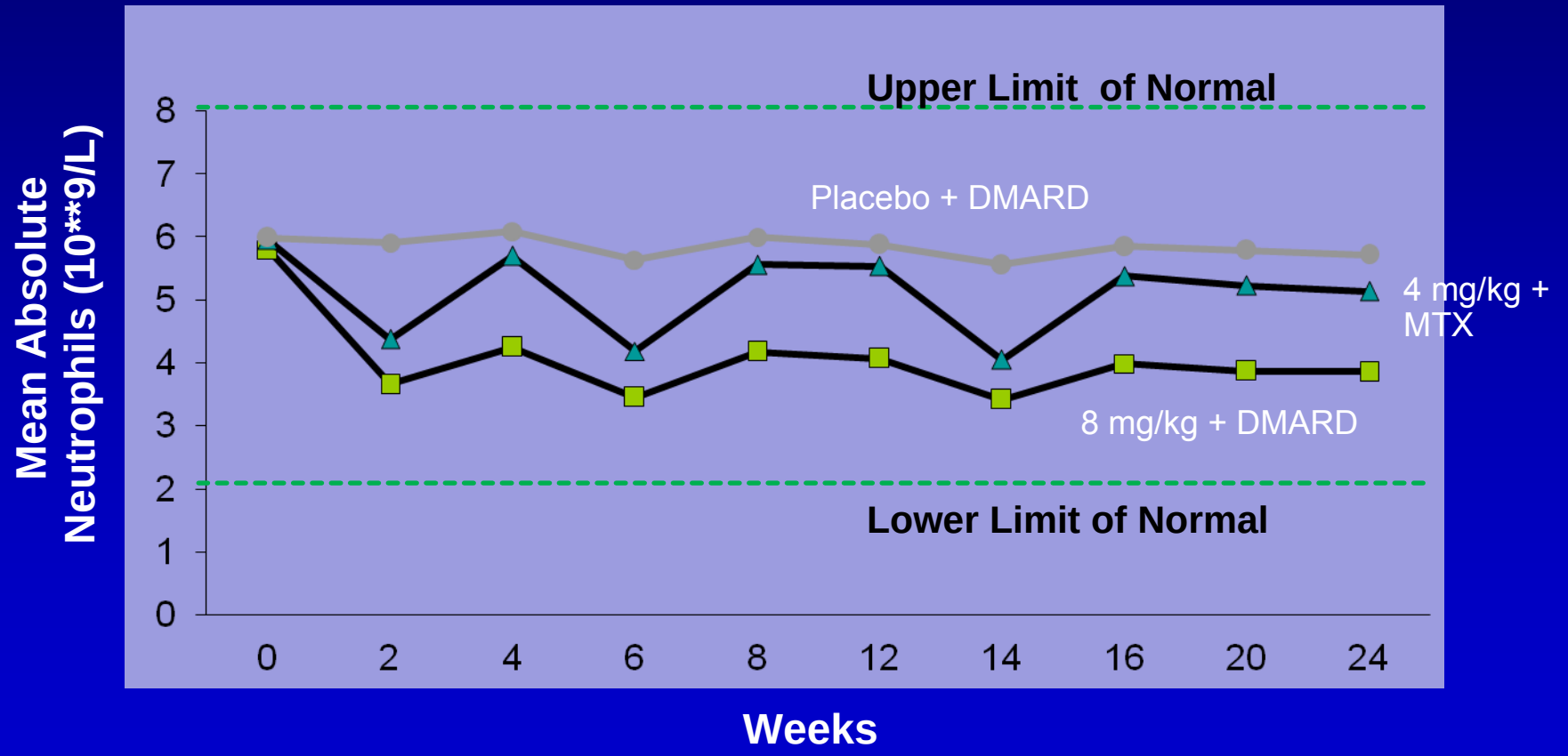
- **IL-6/sIL-6R increases leukocyte recruitment by:**
  - **Activating production of a subset of chemokines by endothelial cells[1]**
  - **Upregulating expression of adhesion molecules[1]**
- **IL-6 supports neutrophil recruitment[2]**



1. Romano M, *et al. Immunity* 1997; **6**:315–325;
2. Lally F, *et al. Arthritis Rheum* 2005; **52**:3460–3469.

IL-6R inhibition and neutrophil counts

Controlled 6-Month Studies (Combination Therapy)



Pooled data from 6 month tocilizumab studies safety population

Overview on Grade 3 and 4 Neutropenia: lowest value reported (CTC Grades)

	TCZ 8 mg (n=288)	MTX (n=284)	TCZ 4 mg + DMARD (n=774)	TCZ 8 mg + DMARD (n=1582)	DMARD (n=1170)
Grade 1 (1500-LLN/mm^3)	51 (18%)	22 (8%)	88 (11%)	298 (19%)	30 (3%)
Grade 2 (1000-1500/mm^3)	30 (10%)	6 (2%)	53 (7%)	179 (11%)	10 (< 1%)
Grade 3 (500-1000/mm^3)	**9 (3%)**	**1 (< 1%)**	**9 (1%)**	**48 (3%)**	–
Grade 4 (< 500/mm^3)	–	–	**3 (< 1%)**	**5 (< 1%)**	–

- **No serious infections observed with grade 3 and 4 neutropenia**
- **5 Non-serious infections seen in grade 3, 4 neutropenia (two bronchitis events, one sinusitis, one pharyngitis, and one conjunctivitis)**

Pooled data from 6 month tocilizumab studies safety population

No link between degree of neutropaenia following TCZ and incidence of infections

6-month pooled data from TCZ trials (safety population)

Patients, n (%)	Placebo + DMARD (n = 1170)	TCZ 4 mg/kg + MTX (n = 774)	TCZ 8 mg/kg + DMARD (n = 1582)
Normal	1122 (95.9)	614 (79.3)	1047 (66.2)
Grade 1 (≥1.5 to <2.0 × 10^9/l)	30 (2.6)	88 (11.4)	298 (18.8)
Grade 2 (≥1.0 to <1.5 × 10^9/l)	10 (<1.0)	53 (6.8)	179 (11.3)
Grade 3 (0.5 to <1.0 × 10^9/l)	–	9 (1.2)	48 (3.0)
Grade 4 (<0.5 × 10^9/l)	1 (<1.0)	5 (<1.0)	6 (<1.0)

No serious infections reported within 30 days of lowest neutrophil count

Pooled trial data to October 2009

Serious infections (TOWARD study)

Patients, % (n)	Placebo + DMARDs (n = 414)	Tocilizumab 8mg/kg + DMARDs (n = 802)
Patients with at least one serious infection	1.9% (8)	2.7% (22)
Total number of events	8	23
Cellulitis	-	0.6% (5)
Pneumonia	0.5% (2)	0.4% (3)
Herpes Zoster	-	0.4% (3)
Bronchitis	0.2% (1)	0.1% (1)
Pyelonephritis	0.2% (1)	0.1% (1)
Abscess limb	0.2% (1)	-
Arthritis bacterial	-	0.1% (1)
Cellulitis staphylococcal	-	0.1% (1)
Endocarditis enterococcal	-	0.1% (1)

Genovese MC *et al*. Arthritis Rheum 2008;58:2968-2980

Changes in neutrophil counts (CTC grade)* TOWARD study

Common Terminology Grade	Placebo + DMARDs n = 414	Tocilizumab 8 mg/kg + DMARDs n = 802
Normal	94.9%	65.6%
Grade 1 (<LLN – 1.5 x 10^9/L)	4.3%	18.8%
Grade 2 (<1.5 – 1.0 x 10^9/L)	<1%	11.6%
Grade 3 (<1.0 – 0.5 x 10^9/L)	-	3.7%
Grade 4 (<0.5 x 10^9/L)	-	-

*Neutrophil count decreases were transient

Genovese MC *et al*. Arthritis Rheum 2008;58:2968-2980

Neutropaenia

- Clinically significant neutropaenia occurred in 3.4% (Grade III*) and 0.3% (Grade IV**) of patients treated with TCZ plus DMARDs
- There was no clear association between decreased neutrophils and the occurrence of serious infections
- If clinically significant neutropaenia follow SPC recommendations for dose modification

*CDC Grade III neutropenia (0.5 to < 1 × 10^9/l)

**CDC Grade IV neutropaenia (<0.5 × 10^9/l)

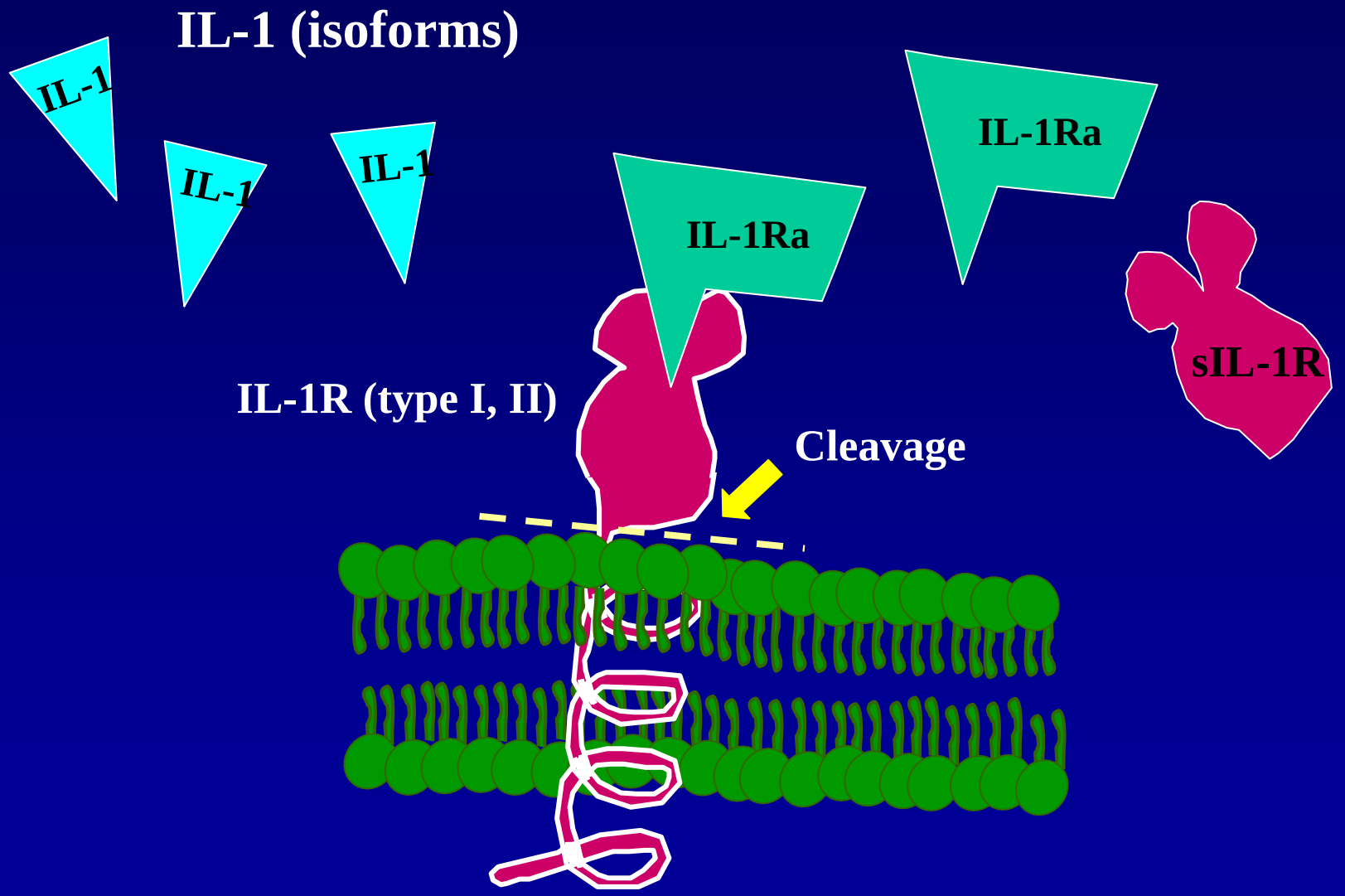
Tocilizumab: Summary of Product Characteristics.

Risk Mitigation: Neutrophils

- TCZ should not be initiated in patients with neutrophils < 2000 cells/mm^3
- Neutrophils should be monitored 4-8 weeks after the first infusion in all patients
  - Repeat labs as clinically indicated

Lab Value (cells/mm^3)	TCZ Modification
ANC ≥ 1000	Maintain dose
ANC 500-1000	Temporarily stop TCZ when ANC > 1000 then resume with 4 mg/kg and return to 8 mg/kg as clinically appropriate
ANC < 500	Discontinue TCZ

Targeting IL-1 in rheumatoid arthritis



From: Moreland *et al*, Arthritis Rheum **40**: 397

IL-1 Receptor Antagonist

In anakinra trials:-

- Neutropenia occurred in 2.4% patients
- Versus 0.4% in placebo treated patients.

(Amgen SPC for Anakinra)

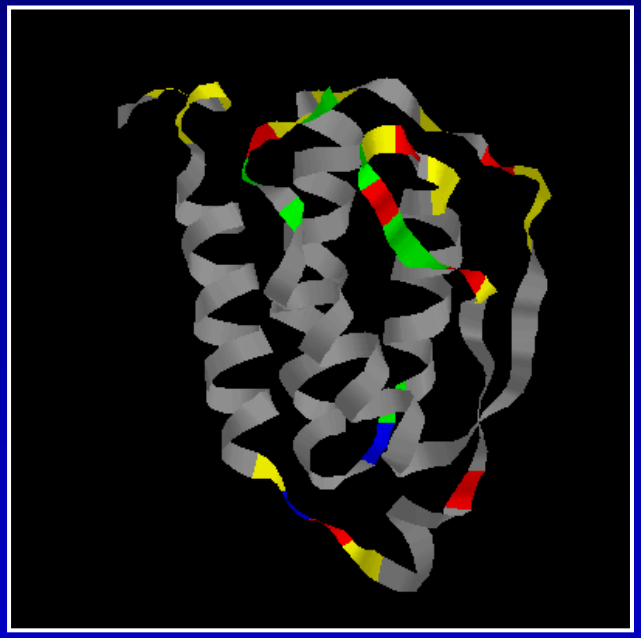
Combination IL-1 and TNF blockade

Event	Etanercept only (n 80)	Half-dosage etanercept + anakinra (n 81)	Full-dosage etanercept + anakinra (n 81)
Any infection	32 (40.0)	30 (37.0)	38 (46.9)
Infection resulting in antibiotic administration or hospitalization	0 (0.0)	3 (3.7)	6 (7.4)
Infection leading to withdrawal	0 (0.0)	2 (2.5)	2 (2.5)
Serious infection	0 (0)	3 (3.7)	6 (7.4)
Serious pneumonia	0 (0)	1 (1.2)	2 (2.5)
Serious cellulitis	0 (0)	1 (1.2)	2 (2.5)

- Two patients in the full-dosage etanercept plus anakinra group experienced neutrophil count < 1.0 10^9/l
- Both patients completed the study, and no clinical events were associated with the neutropenia.

Genovese *et al*, Arthritis Rheum 2004; 50:1412–1419

Targeting GM-CSF in RA



- mAbs against GM-CSF and GM-CSF R in development.
- Will there be effects on neutrophils?
- Clinical trial data awaited.

Management of the severely neutropenic (ANC <500 cells/mm^3) **RA pt**

- Discontinue the causative agent if known; if not known, stop administration of all drugs until the aetiology is established.
- Which category does the patient fall into:-

  Otherwise well patient with neutropenia?

  Immunocompetent patient presenting with neutropenia and compromised, requiring urgent treatment?

  Immunocompromised patients presenting with neutropenia and compromised, requiring urgent treatment?

- Barrier nurse if ANC < 100 cells/mm^3.

Management of the severely neutropenic RA pt: some issues

- Urgent management of septic shock
- Haematology specialist input

- Start specific antibiotic therapy. This often involves the use of third-generation cephalosporins or equivalents.
- Treat areas of stomatitis and skin infections with local cleaning, antisepsis, and dental care.
- Control oral and gingival lesion pain with saline and hydrogen peroxide rinses and local anesthetic gels and gargles.
- If the condition is mild, with only neutropenia without a serious infection, withhold G-CSF.
- However, G-CSF may shorten the period to recovery and the duration of infection.

Summary

- Neutrophils are an important cell type in innate immunity
- Biologics targeting pro-inflammatory cytokines have two effects on neutrophils (best evidence for anti-TNF):
  1. Decreased magination leading to an increase in peripheral blood ANC
  2. Decreased neutrophil production from the bone marrow. This effect predominates in peripheral blood compartment because of the very short half life of neutrophils.
- Relative neutropenia occurs in a minority of patients treated with biologics targeting TNF, IL-1 and IL-6R. But severe neutropenia is rare.
- There is no obvious relationship between drug-induced neutropenia and serious infection in trial data.
- But we must be vigilant for the rare patient with complications!!