

## Biological Response Modifiers: Cytokines & Anti-cytokines

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## Cytokines

- Polypeptides produced in response to microbes and other antigens that mediate and regulate immune and inflammatory reactions

## General properties of cytokines

- Cytokine secretion is brief, self limited
- Autocrine, paracrine
- Pleiotropy, redundancy
- Synergism, antagonism
- Initiate actions by binding to specific membrane receptors on target cells
- Cellular responses: changes in gene expression -> new function, proliferation

## Functional categories of cytokines

- Proinflammatory cytokines: TNF- $\alpha$ , IL-1
- Antiviral cytokines: IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$
- Anti-inflammatory cytokines: IL-10, TGF- $\beta$
- Chemokines
- Hematopoietic growth factors
- Lymphocyte regulatory cytokines
  - Th1 cytokines: IL-2, IFN- $\gamma$ , TNF- $\beta$
  - Th2 cytokines: IL-4, IL-5, IL-10, IL-13

## Cytokine receptors classification

- Based on structural homologies among the extracellular cytokine-binding domains
  - Type I cytokine receptors
  - Type II cytokine receptors
  - TNF receptors
  - Immunoglobulin superfamily receptors
  - Seven transmembrane  $\alpha$ -helical receptors

## US FDA approved cytokines <sup>(1)</sup>

- Immunomodulators
  - 1983 interferon alfa – hairy cell leukemia
  - 1993 interferon beta – multiple sclerosis
  - 1999 interferon gamma – chronic granulomatous disease
  - 1992 aldesleukin (interleukin-2) – metastatic renal cell carcinoma, metastatic melanoma
  - 2001 peginterferon alfa – chronic HCV infection

### US FDA approved cytokines <sup>(2)</sup>

- Hematopoietic growth factors
  - 1989 – Epogen® - erythropoietin  
severe anemia in chronic renal failure
  - 1991 Neupogen® -filgrastim (G-CSF)  
Leukine® - sargramostim (GM-CSF)  
myelosuppression from cancer chemotherapy
  - 1997 Neumega® - oprelvekin (interleukin-11)  
thrombocytopenia
  - 2002 Neulasta® - pegfilgrastim

### Interferons

- Antiviral, antiproliferative, immunomodulatory activities.
- Type I: IFN- $\alpha$ , IFN- $\beta$
- Type II: IFN- $\gamma$
- Binding to specific receptors on cell surface, initiate complex cascade of intracellular events that leads to the expression of numerous gene products

### Interferon alfa: indications

- Neoplasms of lymphatic or hematopoietic system:
  - hairy cell leukemia, multiple myeloma, cutaneous T-cell lymphoma, chronic myelogenous leukemia, thrombocytosis associated with myeloproliferative disease, low-grade non-Hodgkin's lymphoma
- Solid neoplasms:
  - AIDS-related Kaposi's sarcoma, recurrent or metastatic renal cell carcinoma, metastatic-malignant melanoma
- Viral disease:
  - chronic active hepatitis B, chronic hepatitis C, condyloma acuminata

### Interferon alfa

- Oct 4, 1983 Intron® A – IFN alfa-2b
  - 165 amino acids, MW 19,270
- Oct 24, 1984 Roferon® A – IFN alfa-2a
  - 165 amino acids, MW 19,000
- Jan 19, 2001 PEG-Intron®
  - covalent conjugate of IFN alfa-2b with monomethoxy polyethylene glycol (PEG), MW 31,000
- Oct 16, 2002 Pegasys®
  - covalent conjugate of IFN alfa-2a with a single branched bis-monomethoxy PEG chain, MW 40,000

### Pegylation

- Modification of molecules through the attachment of polyethylene glycol (PEG)
- Reduces renal clearance, more sustained absorption after SC injection, restricted distribution
- Results in a more constant and sustained plasma concentrations
- Decrease adverse effects caused by
  - the large variations in peak-to-trough plasma concentrations associated with frequent drug administration
  - the immunogenicity of unmodified proteins

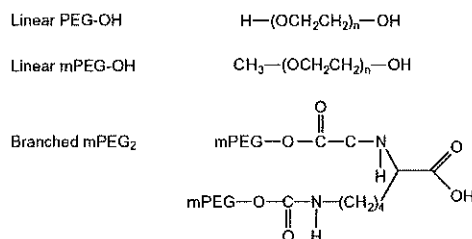
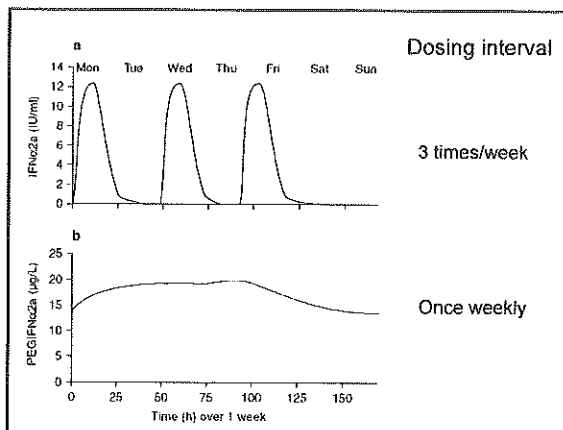


Fig. 1. Structural formulae of poly(ethylene glycol) [PEG] molecules.<sup>(11)</sup> mPEG = monomethoxypoly(ethylene glycol).



### Interferon beta (1)

- 1993 Betaseron® - Interferon beta -1b
  - produced by *Escherichia coli*, 165 amino acids, MW 18,500, no carbohydrate side chains, specific activity 32 million IU/mg
- 1996 Avonex® - Interferon beta -1a
  - produced by Chinese hamster ovary cells, 166 amino acid glycoprotein, MW 22,500, amino acid sequence identical to natural human interferon beta, specific activity 200 million IU/mg
- 2002 Rebif® - Interferon beta -1a

### Interferon beta (2)

- Treatment of relapsing forms of multiple sclerosis to reduce the frequency of clinical exacerbations.
- Betaseron®: 250 mcg SC every other day, Avonex®: 30 mcg IM once a week, Rebif®: 44 mcg SC 3 times weekly.
- Common side effects: flu-like symptoms.
- Potential serious side effects: depression, allergic reaction, injection site necrosis.

### Interferon beta (3)

- Mechanism of action in multiple sclerosis is unknown.
- Induce a number of proteins (neopterin, beta2-microglobulin, MxA protein, IL-10).
- Enhancement of suppressor T cell activity, reduction of pro-inflammatory cytokine production, down regulation of antigen presentation, inhibition of lymphocyte trafficking into CNS.

### Interferon beta (4)

- Clin Neurol Neurosurg 2004 Jun;106(3):255-8
- IFN-beta1a and IFN-beta1b have different patterns of influence on cytokines
- After 1 year of treatment, IFN-gamma (proinflammatory cytokine) concentration was significantly lower in the Betaseron (IFN-beta1b) group
- Concentration of IL-4 and IL-10 (anti-inflammatory cytokines) were significantly higher in the Avonex (IFN-beta1a) group

### Interferon gamma (1)

- Interferon gamma-1b (Actimmune®)
- Single-chain polypeptide, 140 amino acids, produce from *Escherichia coli*
- Non-covalent dimer of 2 identical 16,465 dalton monomers
- Indication: chronic granulomatous disease, severe malignant osteopetrosis.

### Interferon gamma <sup>(2)</sup>

- Chronic granulomatous disease (CGD): an inherited disorder of leukocyte function caused by defects in the enzyme complex responsible for phagocyte superoxide generation.
- IFN- $\gamma$ , 50 mcg/m<sup>2</sup> SC, 3 times weekly reduce the frequency and severity of serious infections

### Interferon gamma <sup>(3)</sup>

- Severe malignant osteopetrosis: an inherited disorder characterized by an osteoclast defect, leading to bone overgrowth, and by deficient phagocyte oxidative metabolism
- IFN- $\gamma$ , 50 mcg/m<sup>2</sup> Sc, 3 times weekly enhance osteoclast function, delay time to disease progression

### Interferon gamma <sup>(4)</sup>

- In both disorders, the exact mechanism(s) by which IFN- $\gamma$  has a treatment effect has not been established. Changes in superoxide levels during therapy do not predict efficacy.
- Most common adverse reaction: "flu-like" (fever, headache, chills, myalgia, fatigue), which may decrease in severity as treatment continues.

### Hematopoietic growth factors <sup>(1)</sup>

- Erythropoietin
  - glycoprotein produced in the kidney, stimulates red blood cell production
- Epogen® (epoetin alfa)
  - 165 amino acid glycoprotein produced by mammalian cells, MW 30,400
  - Indications: anemia of chronic renal failure, zidovudine-treated HIV-infected, cancer-chemotherapy patients. Reduction of allogeneic blood transfusion in surgery patients.

### Hematopoietic growth factors <sup>(2)</sup>

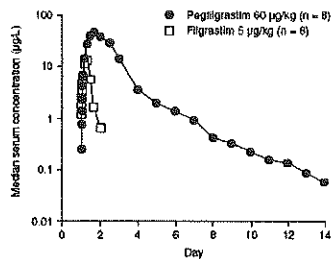
- Granulocyte colony-stimulating factor (G-CSF)
  - Produced by monocytes, fibroblasts, endothelial cells
  - Stimulates neutrophil production
- Neupogen® (Filgrastim)
  - Recombinant methionyl human G-CSF
  - 175 amino acid protein produced by *E coli*, nonglycosylated, MW 18,800
  - Indications: cancer patients receiving myelosuppressive chemotherapy, bone marrow transplant. Severe chronic neutropenia, patients undergoing peripheral blood progenitor cell collection

### Hematopoietic growth factors <sup>(3)</sup>

- IL-11
  - Thrombopoietic growth factor, 178 amino acids
  - Produced by bone marrow stromal cells
- Neumega® (Oprelvekin)
  - Recombinant methionyl human G-CSF
  - 177 amino acids (lacking amino-terminal proline), produced by *E coli*, nonglycosylated, MW 19,000
  - Indications: prevention of severe thrombocytopenia following myelosuppressive chemotherapy

### Pegfilgrastim

- Pegfilgrastim  
6 mg once per cycle  
chemotherapy
- Filgrastim  
5 mcg/kg daily



Drugs 2002; 62 suppl. 1:69-98

Pegfilgrastim serum concentrations after administration to healthy volunteers.

### US FDA approved anti-cytokines

- TNF blockers
  - 1998 Enbrel® - etanercept
  - Remicade® - infliximab
  - 2002 Humira® - adalimumab
- IL-1 antagonist
  - 2001 Kineret® - anakinra

### IL-1 receptor antagonist (IL-1Ra)

- Anakinra (Kineret®)
  - Nonglycosylated form of human IL-1Ra, produced by *E. coli*
  - Addition of a methionine at amino terminus
  - 153 amino acids, MW 17.3 kD
  - Indication: rheumatoid arthritis
  - 100 mg/d Sc

### Tumor Necrosis Factor (TNF)

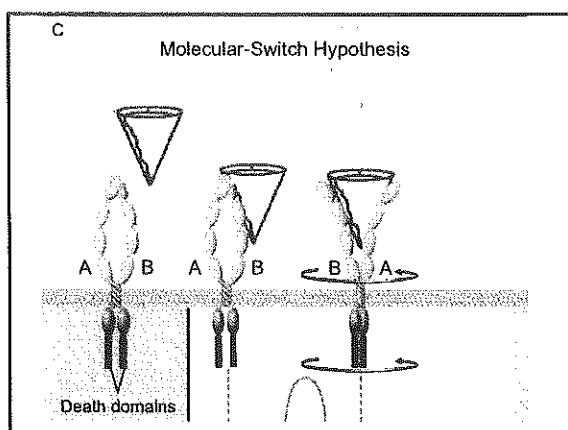
- Principal mediator of the acute inflammatory response to gram-negative bacteria and other infectious microbes
- Major cellular source: activated macrophage
- Originally identified as a substance present in the serum of animals treated with bacterial LPS that caused the necrosis of tumors in vivo
- Also called TNF- $\alpha$   
TNF- $\beta$  = lymphotoxin

### TNF

- Synthesized as homotrimer membrane protein
- Membrane TNF is cleaved by a membrane-bound metalloproteinase, producing the secreted form
- Homotrimer of 51 kD (3x17 kD subunits)
- Triangular pyramidal shape

### TNF Receptors

- Type I TNF receptor (TNF-RI), 55kD, p55 activates caspases -> apoptosis, but also activate transcription factors
- Type II TNF receptor (TNF-RII), 75kD, p75 activates NF- $\kappa$ B, AP-1 -> gene transcription
- Both TNF receptors are present on almost all cell types



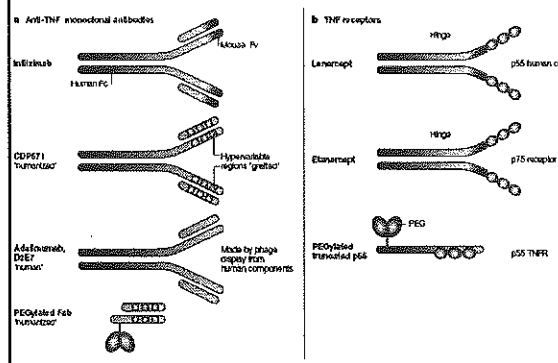
### Biological actions of TNF

- Stimulate the recruitment of neutrophils and macrophages and activate them
  - Endothelial cells express adhesion molecule
  - Endothelial cells & macrophages secrete chemokines
  - Macrophages secrete IL-1 (functions like TNF)
- Pathologic abnormalities in severe infections
  - Fever, increase synthesis of serum amyloid A protein and fibrinogen from hepatocyte
  - Appetite suppression, reduced lipoprotein lipase → cachexia

### Septic Shock

- Vascular collapse, disseminated intravascular coagulation, metabolic disturbances
- Is due to LPS-induced production of TNF and other cytokines (IL-12, IFN- $\gamma$ , IL-1)
- Serum TNF concentration may predict outcome
- Can be reproduced in experimental animals by administration of LPS or TNF
- TNF antagonists can prevent mortality in experimental models

### Anti-TNF

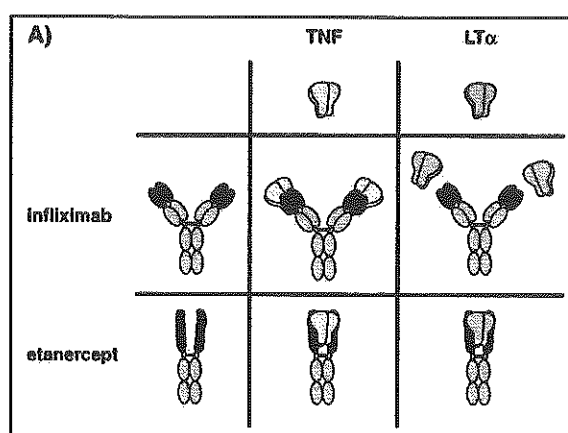


### Etanercept

- Dimeric fusion protein:
  - extracellular ligand binding portion of p75 TNFR
  - Fc portion of human IgG1 (CH2, CH3, hinge region, not CH1 domain)
  - 934 amino acids, MW 150 kD, produced by CHO cell
- Indication:
  - Rheumatoid arthritis
  - Polyarticular-course juvenile rheumatoid arthritis
  - Ankylosing spondylitis
  - Psoriatic arthritis, plaque psoriasis

### Anti-TNF antibodies

- Infliximab
  - Chimeric IgG1 $\kappa$ , MW 149 kD
  - Indication: Crohn's disease, rheumatoid arthritis, ankylosing spondylitis
- Adalimumab
  - human IgG1 $\kappa$ , MW 148 kD, 1330 amino acids
  - Indication: rheumatoid arthritis



### Development of anti-TNF therapy for chronic inflammatory diseases (1)

- 1975 TNF characterized, named by Carswell *et al.*
- 1982-1988 Analysis of synovial tissue in RA shows high levels of inflammatory cytokines (TNF, IL-1, IL-6)
- 1984 TNF cloned
- 1989 Anti-TNF antibody abrogates IL-1 production in synovial-cell culture
- 1989-1992 humanized mouse anti-TNF antibody developed initially intended for treatment of sepsis
- 1990-1992 Anti-TNF antibody efficacy shown in mouse models of RA

### Development of anti-TNF therapy for chronic inflammatory diseases (2)

- 1991 First TNFR-Ig fusion protein developed
- 1992 Small Phase I/II clinical trial of anti-TNF antibody in RA - proof of principle and safety
- 1993 Phase II clinical trial of anti-TNF antibody in RA - a formal proof of clinical efficacy
- 1993-1998 Clinical trials of etanercept in RA result in approval in USA (November 1998)
- 1993-1996 Retreatment and long-term Phase II clinical trials show that chronic treatment is feasible and that methotrexate improves efficacy of anti-TNF antibody

### Development of anti-TNF therapy for chronic inflammatory diseases (3)

- 1993-1996 Phase I and II clinical trials of anti-TNF antibody in Crohn's disease
- 1997-1999 Phase III clinical trial of anti-TNF antibody plus methotrexate combination therapy
- Anti-TNF antibody approved in USA (1998) and Europe (1999) for the treatment of severe Crohn's disease
- 1999-2000 Anti-TNF antibody (infliximab) approved for use in USA (1999) and Europe (2000)

### Development of anti-TNF therapy for chronic inflammatory diseases (4)

- 2002 Anti-TNF therapeutics (infliximab and etanercept) recommended as effective treatment for severe arthritis by the UK's National Institute of Clinical Excellence (NICE)
- More than 250,000 RA patients treated with anti-TNF antibody (infliximab) or TNFR-Ig (etanercept)
- 2004-2005 Approval of other anti-TNF biologicals
- (2005-2010) Orally available anti-TNF antibody drugs?

### TNF $\alpha$ and Congestive Heart Failure

- TNF $\alpha$  over-expressed in myocardial tissue in heart failure
- Increasing serum TNF $\alpha$  level is correlated with a worse New York Heart Association (NYHA) functional class for congestive heart failure (CHF), an increase in hospitalisations due to CHF, and an increase in mortality
- Initial data from preclinical and pilot studies were encouraging, showing some efficacy of TNF $\alpha$  antagonist therapy in the treatment of CHF

### Etanercept - phase III trial in CHF

- RENAISSANCE  
(Randomized Etanercept North American Strategy to Study Antagonism of Cytokines)
- RECOVER  
(Research into Etanercept Cytokine Antagonism in Ventricular Dysfunction)  
Europe, Israel and Australasia
- The two studies were of a similar design, combined and presented as the Randomized Etanercept Worldwide Evaluation (RENEWAL)

### Etanercept Label

#### Precaution: Patients with Heart Failure

Two large clinical trials evaluating the use of ENBREL in the treatment of heart failure were terminated early due to lack of efficacy. Although the studies did not demonstrate harm, there was a suggestion of worse heart failure outcomes with ENBREL treatment in one of the two trials. There have been post-marketing reports of worsening of congestive heart failure (CHF), with and without identifiable precipitating factors, in patients taking ENBREL. Physicians should exercise caution when using ENBREL in patients who also have heart failure.

### Infliximab – pilot study in CHF

- ATTACH (Anti-TNF alpha Therapy Against Chronic Heart Failure)
- 150 patients were recruited.
- Strong trends towards an increase in the percentage of patients with worsening clinical status with increasing infliximab dose
- Mortality rate for the high doses of infliximab (10 mg/kg) was statistically greater than that observed in the placebo arm.

### Adverse events – Anti-TNF drugs

- As a class, anti-TNF alpha drugs have demonstrated both efficacy and a number of serious adverse events including infections, congestive heart failure, neurologic events including demyelination, lymphomas and autoimmunity including lupus like syndromes
- Decision to use should consider both their efficacy and potential side effects

### Clinical trials of other anti-cytokines

- TNFR55-IgG1 fusion protein (lenercept)
- TNF binding protein pegylated dimer
- Anti-TNF antibody F(ab')<sub>2</sub> fragment (afelimomab)
- Anti-IL-6 receptor antibody
- Anti-IL-12 Antibody

### Lenercept

- TNFR55-IgG1 fusion protein
- Critical Care Medicine 2001 Mar;29(3):503-10
  - Phase III study, 1342 severe sepsis and early septic shock patients
  - Lenercept had no significant effect on mortality
- J Rheumatol 2003 Apr;30(4):680-90
  - RA patients improved after one day of first IV infusion
  - Treatment benefit maximized by 2 weeks but diminished thereafter
  - The third dose clearance rate was more than double compared with the first due to development of antibody



### Afelimomab

- Anti-TNF antibody F(ab')<sub>2</sub> fragment
- Murine antibody fragment against human TNF
- Reduce immunogenicity, improve tissue penetration, minimized interaction with Fc receptors
- Crit Care Med 2004 Nov;32(11):2173-82
  - Phase III trial
  - Afelimomab reduces mortality, attenuates the severity of organ dysfunction in patients with elevated IL-6 levels

### TNFbp pegylated dimer

- TNF binding protein pegylated dimer
- J Rheumatol 2000 Mar;27(3):601-9
  - IV TNFbp pegylated dimer in RA patients
  - Significant anti-TNFbp antibody response affected the half-life and clearance of drug

### Anti-interleukin-6 <sup>(1)</sup>

- Atlizumab (Actemra, MRA) - humanized anti-Interleukin-6 receptor monoclonal antibody
- Chugai Pharmaceutical + Roche
- Blood 2000 Jan;95(1):56-61
  - Improvement in Castleman's disease by 50-100 mg atlizumab once or twice weekly
- April 2003, regulatory filing submitted in Japan for use in giant lymph node hyperplasia (Castleman's disease)

### Anti-interleukin-6 <sup>(2)</sup>

- Atlizumab in rheumatoid arthritis
  - Arthritis Rheum 2002 Dec;46(12):3143-50
    - Phase I/II randomized, double-blind, placebo-controlled trial, suggests that IL-6 blockade may be beneficial
    - Levels of the acute-phase reactants ESR and CRP normalized after a single dose of 5 or 10 mg/kg and the effects lasted for 4 weeks
  - Arthritis Rheum 2004 Jun;50(6):1761-9
    - multicenter, double-blind, placebo-controlled trial
    - Atlizumab was generally well tolerated and significantly reduced disease activity

### Anti-interleukin-6 <sup>(3)</sup>

- Atlizumab in Crohn's disease
  - Gastroenterology 2004 Apr;126(4):989-96
    - A pilot randomized, placebo-controlled trial,
    - A biweekly 8 mg/kg infusion was well tolerated, normalized the acute phase responses and suggested a clinical effect in active Crohn's disease

### Anti-interleukin-12

- Anti-IL-12 antibody for active Crohn's disease
- N Engl J Med 351;20:2069-79. Nov 11, 2004
  - Multicenter, randomized, placebo-controlled, double-blind, phase 2 clinical trial
  - One injection/wk (3 mg/kg anti-IL-12) for 7 weeks resulted in higher response than placebo
  - Decrease in secretion of IL-12, IFN- $\gamma$ , TNF by mononuclear cells of colonic lamina propria