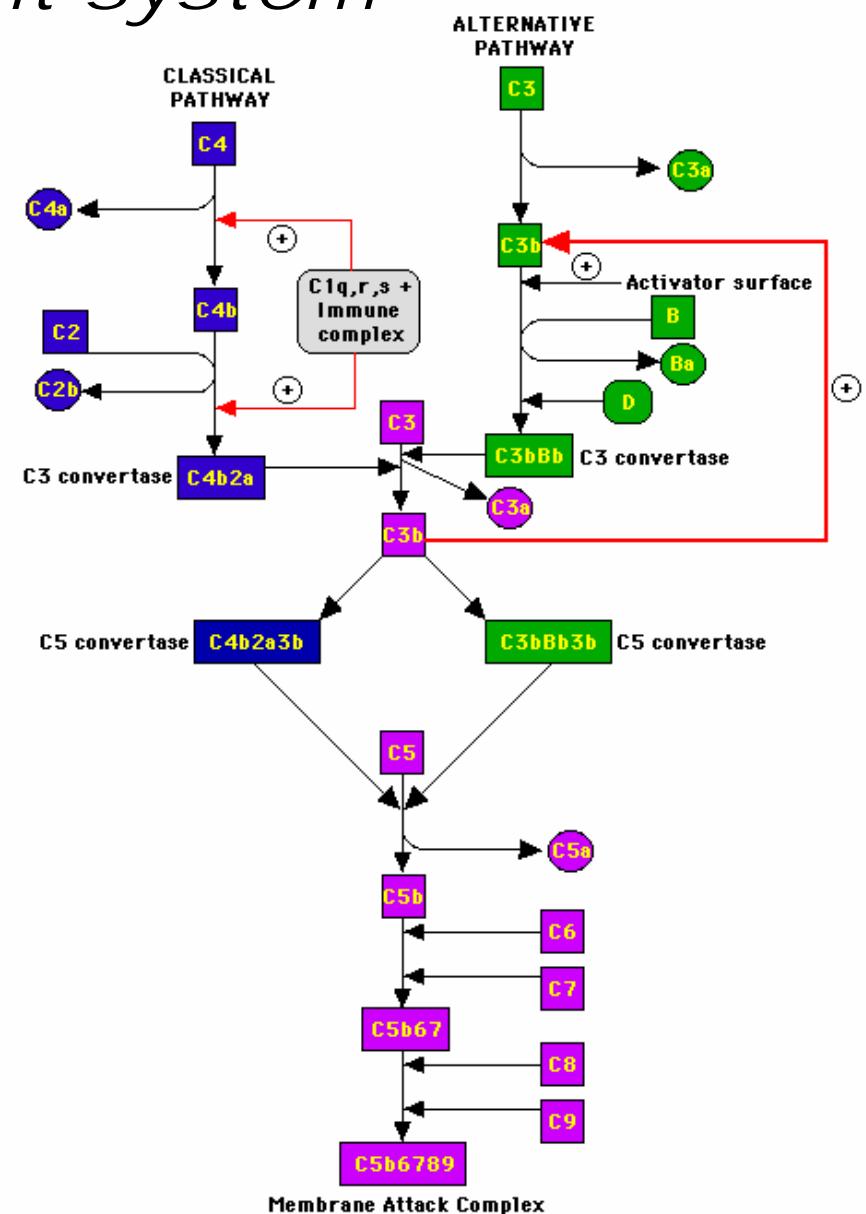


# Complement system

- Is a system of more than 30 distinct plasma proteins (produced by hepatocytes and monocytes) involving 3 separate pathways:
  - classical (activated by Ig-Ag complexes) and
  - alternative (activated by surfaces)
  - Lectin (activated by certain complex carbohydrate structures)
- The Complement directly and indirectly contributes to the acute inflammatory reaction (essential part of the innate IS) and the immune response.
- Principle function: recognition and elimination of foreign elements from the body by coating with C-factors which allows phagocytosis by granulocytes.
- The alternative pathway can be activated by various biomaterial surfaces.
- The Lectin pathway is activated by mannose-binding lectin (MBL) which binds specific sugar structures occurring on the surface of foreign organisms only

# Complement system

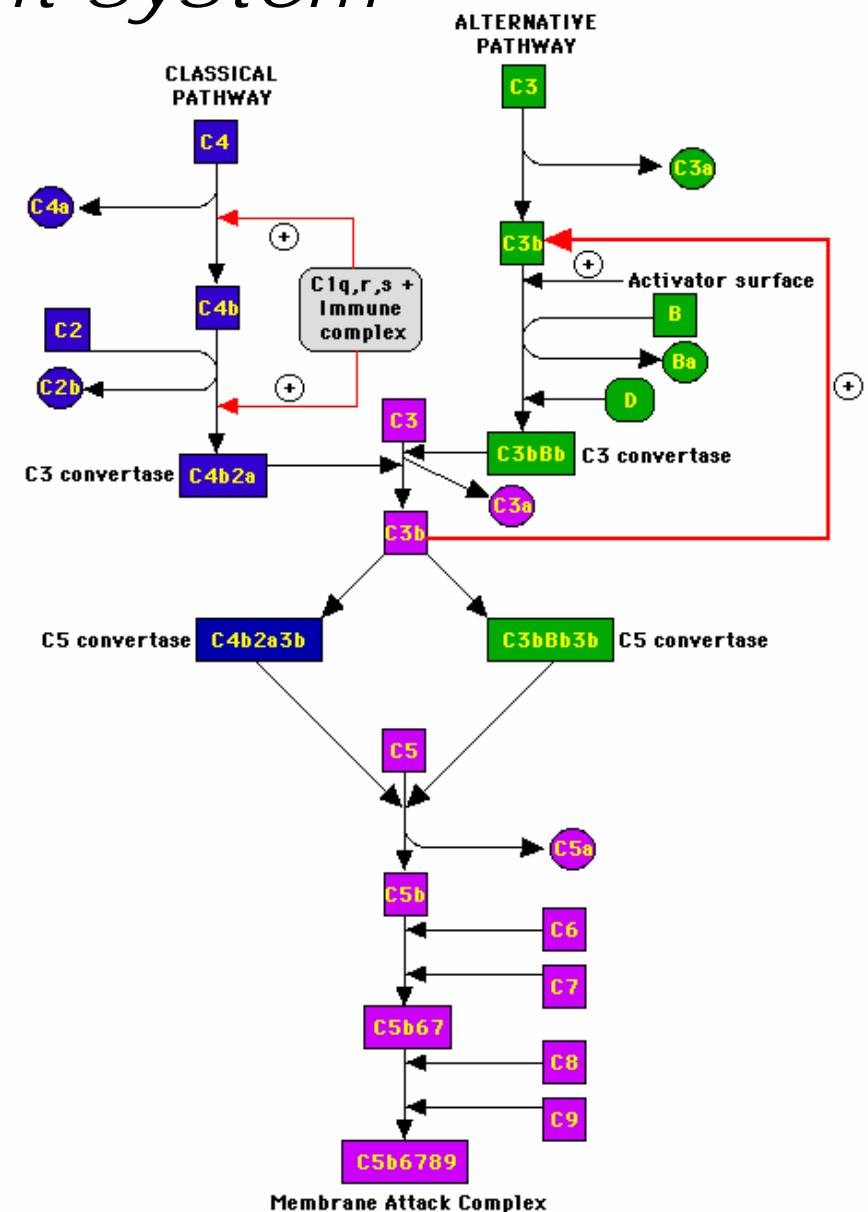
- Classical pathway (CP):
  - Activated by immune complexes (Ig-Ag).
  - C1, 2, 4, C1 inhibitor and C4 binding protein.
  - Enzyme cascade in which each step involves enzymatic reactions that result in signal amplification, finally leading to precipitation and activation of the membrane-attack (lysis) component on the activating foreign organism.
  - Signal amplification is achieved because several of the steps involve activation of enzymes, which in turn can activate several copies of downstream factors
  - The Lectin pathway activates the same cascade as the CP



# Complement system

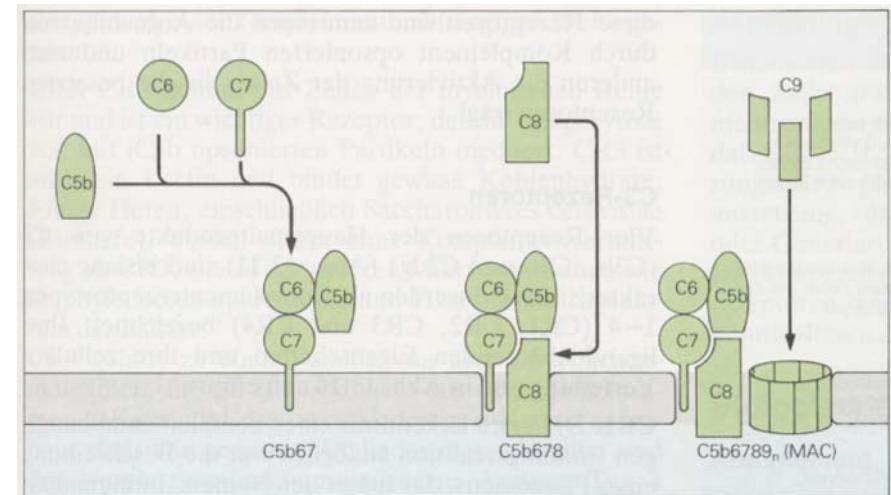
- Alternative pathway (AP):

- Activated by bacterial LPS, foreign surfaces (incl biomaterials)
- C3, B, D, inhibitory factors H, I
- Enzyme cascade with initiation, amplification, and regulation steps.
- Initiation always active at low level. When high concentration of C3b is reached the C5 convertase is active and the strong amplified effect is executed

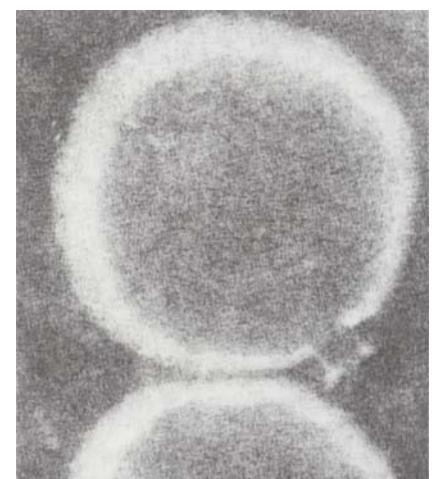


# Complement system

- Complement activation (CP and AP) result in formation of the membrane attack complex (MAC).



- C6 and C7 bind to membranes
- C5b binding induces recruitment of C8 and multiple C9 proteins forming pores, leading to loss of membrane integrity and cell death.



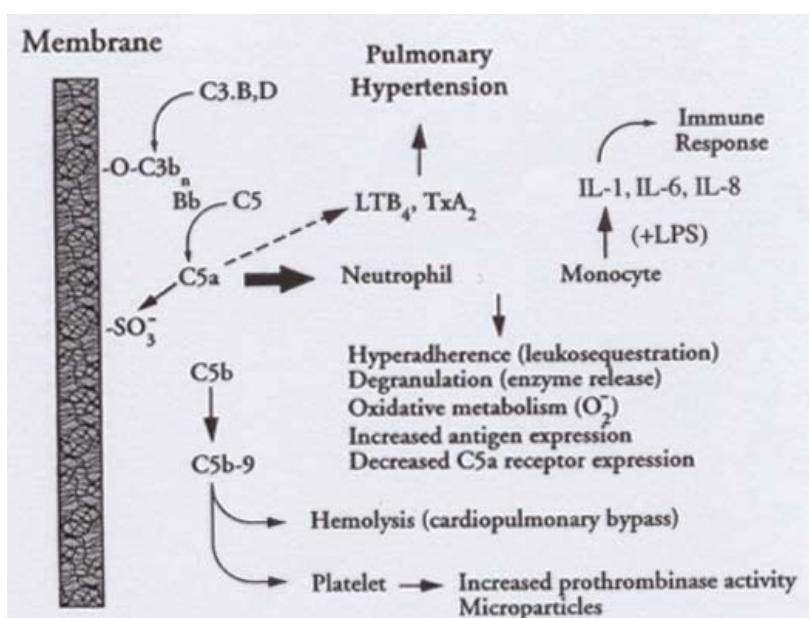
C5a is a potent inflammatory mediator (activating neutrophils and macrophages)

# Complement system

- The CS can become clinically relevant in situations where it either fails to function or where it is activated inappropriately:
  - Lack of activity (genetic deficiency)
    - Increased incidence of recurrent infections
    - glomerulonephritis
  - Innappropriate activation
    - In various autoimmune diseases
    - During extracorporeal therapies:
      - Hemodialysis
      - Cardiopulmonary bypass

# Complement system

- Activation of CS by the various biomaterials is found in different medical devices:
  - E.g. Cuprophan hemodialysis membranes (cellulosic)
  - Leads to MAC hitting „innocent bystander cells“



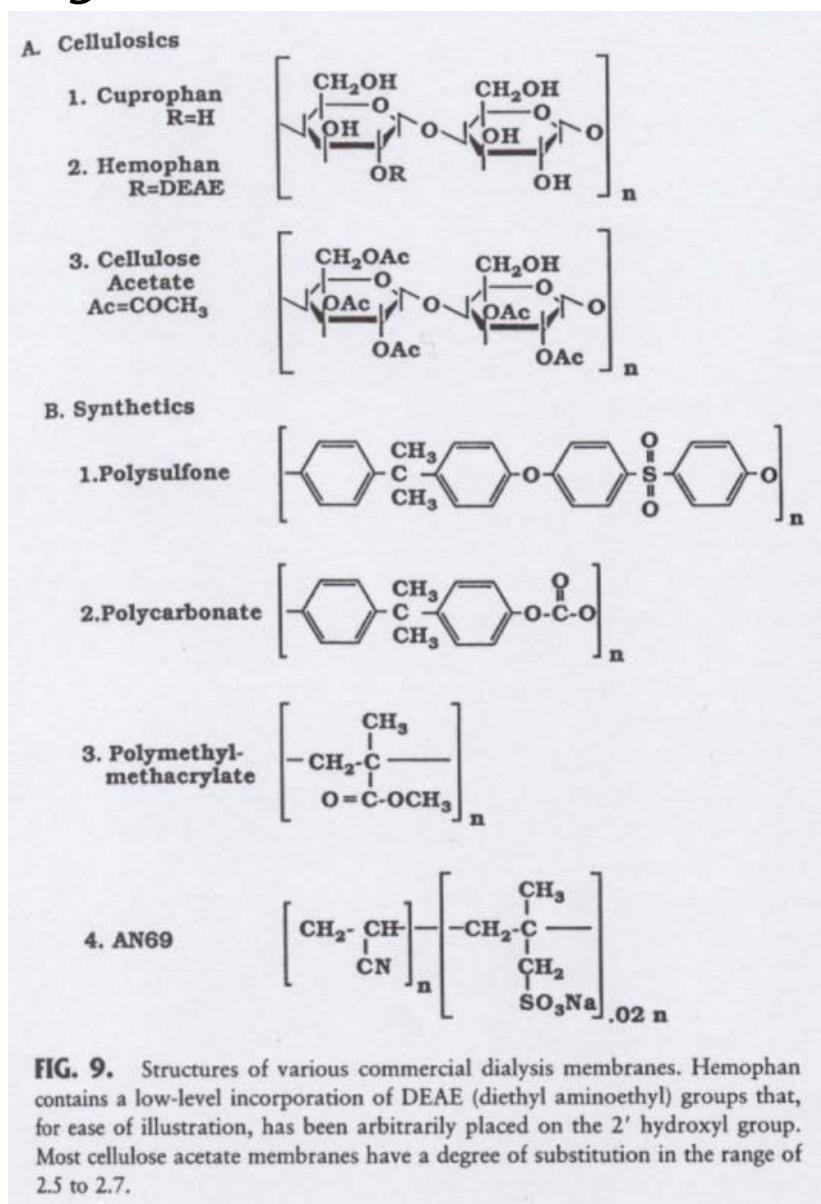
**TABLE 5** Clinical Symptoms Associated with Cuprophan-Induced Biocompatibility Reactions

Cardiopulmonary:	Pulmonary hypertension Hypoxemia Respiratory distress (Dyspnea) Neutropenia (pulmonary leukosequestration) Tachycardia Angina pectoris Cardiac arrest
Other:	Nausea, vomiting, diarrhea Fever, chills, malaise Urticaria, pruritus Headache

# Complement system

- New membranes were developed:

- Moderately activating modified cellulosis
- Low activating synthetics



# Cytokines

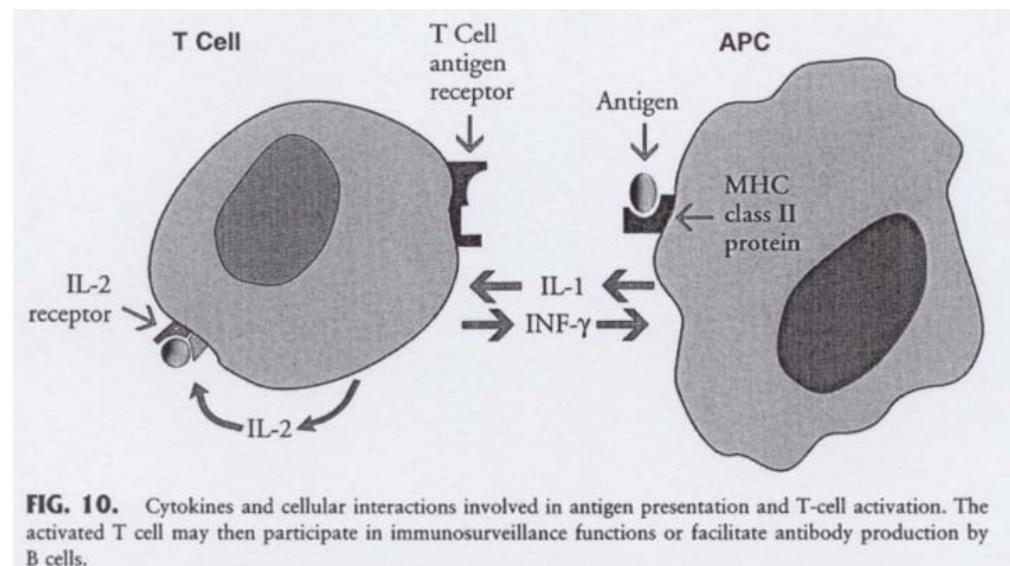
- Group of proteins involved in the cellular response of the immune system.
  - Communication between leucocytes (e.g.: Interleukine 1-20)
- Some retained their original names (based on a particular aspect of their function):
  - IFNs (interferons; antiviral activity)
  - TNF (tumor necrosis factor)
- Cytokines are extremely potent (active in  $10^{-12}M$  range)
- Many cytokines have overlapping functions:
  - Growth factors for immune cells
  - Regulation of the immune response
  - Mediators of inflammation

# Cytokines

Mediator	Source	Induced by	Actions/activities
IL-1 (13–17 kDa) <sup>a</sup>	Macrophages Endothelium NK cells Glial cells Keratinocytes Smooth muscle	Endotoxin, C5a, TNF/IL1	Hepatic acute phase response, leukocyte adherence to EC; production of PGI <sub>2</sub> , PGE <sub>2</sub> , PAF, and TF activity from EC; fibroblast collagen synthesis; neutrophilia; T, B and NK cell activation; PMNL TxA <sub>2</sub> release; fever (PGE <sub>2</sub> ); ACTH production
IL-2 (15.5 kDa)	T cells	IL-1 + Antigen	Proliferation and differentiation of T, B, and LAK cells; activation of NK cells
IL-3 (28 kDa)	T cells, NK cells		Hemopoietic growth factor for myeloid, erythroid, and megakaryocyte lineages
IL-4 (20 kDa)	T cells	Mitogens (Con A)	Proliferation and differentiation of B cells, isotype switching (IgE), mast and T cell proliferation, antagonistic with IFN- $\gamma$
IL-5 (45 kDa)	T cells	Mitogens (Con A)	Proliferation and differentiation of B cells and eosinophils, increased secretion of IgM and IgG from activated B cells
IL-6 (23–30 kDa)	T cells Monocytes Fibroblasts Endothelium	IL-1	Acute phase response (hepatic), proliferation and Ig secretion by activated B cells, T-cell activating factor
IL-7 (25 kDa)	Bone marrow stroma cells		Proliferation of large B progenitors, thymic maturation of T cells
IL-8 (14 kDa)	T cells, PMN Monocytes	Antigen Mitogen IL-1 TNF- $\alpha$	T-cell and neutrophil chemotactic factor

# Cytokines

- Many cytokines are involved in regulating the immune response through their action on T cells, B cells and APCs (antigen presenting cells: macrophages, dendritic cells, B cells).
- A number of cytokines are known to contribute to inflammation:
  - IFN $\gamma$ , GM-CSF, TNF- $\alpha$ , IL-6, IL-8, IL-1

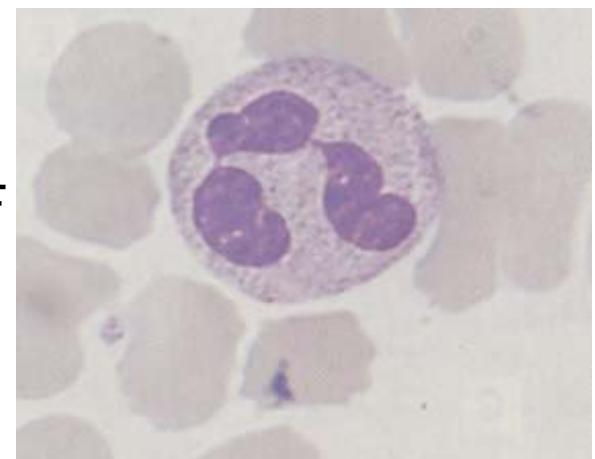


**FIG. 10.** Cytokines and cellular interactions involved in antigen presentation and T-cell activation. The activated T cell may then participate in immunosurveillance functions or facilitate antibody production by B cells.

# *Cellular components of the IS*

- Neutrophil (polymorphnuclear leucocyte)

- 60-70% of white blood cells
- 1-2 days live span after leaving the bone marrow
- Receptors for C5a, C3b, F<sub>cy</sub>, IL-1 and TNF
- Attach to vascular endothelium and migrate into the site of inflammation upon stimulation (e.g. C5a)
- Contain granules filled with:
  - Proteases (elastase, cathepsin, collagenase)
  - Bactericidal proteins (lysozyme, myeloperoxidase)
- Produce (when stimulated): H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub><sup>-</sup>, HO<sup>·</sup>

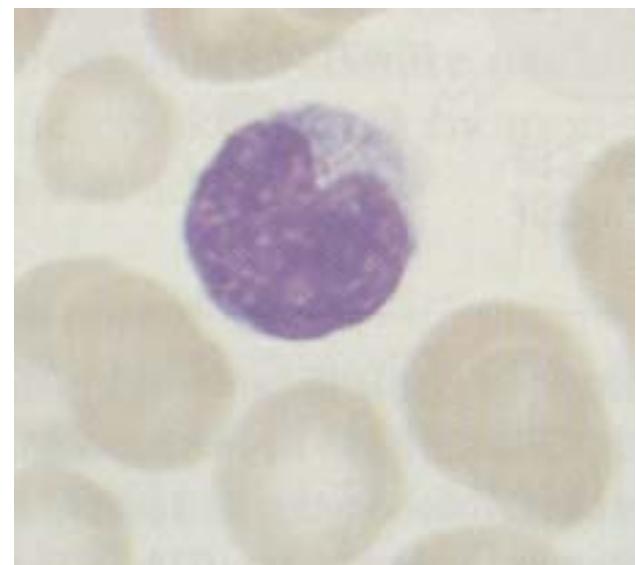


= first line of defense against infection

# *Cellular components of the IS*

## ● Monocyte

- Originate in the bone marrow
- Constitute ca. 5% of peripheral white blood cells
- Transit time in the blood: about 24h
- Migrate into the tissues and differentiate into macrophages
- Produce: C1q, C2, C4, factor H and I, lysozyme constitutively and factor B, C3, collagenase, IFNs, TNF, IL-1 upon stimulation.
- Share many characteristics with neutrophils:
  - Contain Receptors for C5a, C3b,  $F_c$
  - Activated in the same manner
  - Recruited by chemotaxis
  - phagocytotic



# *Host reaction to biomaterials*

- The immune response to a biomaterial involves humoral and cellular components:
  - Activation of the complement cascade by either classical or alternative pathway.
  - Activation of granulocytes:
    - Production of degranulative enzymes and
    - Destructive oxygen metabolites.



# *Systemic toxicity and hypersensitivity*

*Biocompatibility is influenced by:*

*the various classes and designs of biomaterials, modes of application, duration of contact, and general and specific biological reactions.*

*Therefore, no general statements can be made on biomaterials, rather current knowledge is mainly derived from collective classifications based on clinical reports.*

- Systemic effects of biomaterials may be due to:
  - Direct chemical toxicity
  - Accumulation of products from wear, corrosion or degradation
  - Excess inflammatory response
  - Generation of vasoactive products in the activation of the complement system
  - Reactions of the immune system

# Systemic toxicity and hypersensitivity

- Non-immune systemic toxicity:

- Toxicity at some distance from the site of initial insult.
- The mechanisms are varied and complex.
- Typically caused by the accumulation, processing and subsequent reaction of the host to degradation products.
- The manifestations of toxic reactions vary depending on the site at which the response occurs.
- Only few substances are classified as certified toxicants. These mainly include metal ions and salts such as nickel, mercury, and chromium. –even then, released systemic concentrations have to be found above a critical threshold for a biomaterial containing those toxicants to be discarded (withdrawn from the market).

**TABLE 1** Target Organs and Signs and Symptoms in Local and Systemic Toxicity

Systemic responses	
Lungs	Alteration in air exchange and breathing patterns
Kidney	Alterations in urine excretion, pain
Joints	Pain, swelling, loss of function
Liver	Alterations in blood chemistry
Lymphoid	Swelling, alteration of blood count
GI tract	Diarrhea or constipation
The following usually give local responses but may also be involved in systemic responses.	
Skin	Rashes, swelling, discoloration
Eyes	Swelling, itching, watery
Nose	Itching, running, sneezing
The following usually do not give observable signs and symptoms until damage is extreme.	
Brain, skeletal system, muscles	

# *Systemic toxicity and hypersensitivity*

- Non-immune systemic toxicity (cont.):
  - Biomaterials must be carefully evaluated for toxicity *in vitro* (and *in vivo*) before being implanted.
    - Intact material
    - Degradation and wear products
    - Long-term effects can only be estimated.... –and are often the cause for withdrawal of approved materials
  - Non-immune systemic toxicity caused by a biomaterial is generally:
    - Dose related, with higher doses giving a more severe response.
    - There is usually a threshold below which the material shows no toxicity.
    - Repeated exposure to the same substance give responses similar to those of the first exposure.

# *Systemic toxicity due to immune response*

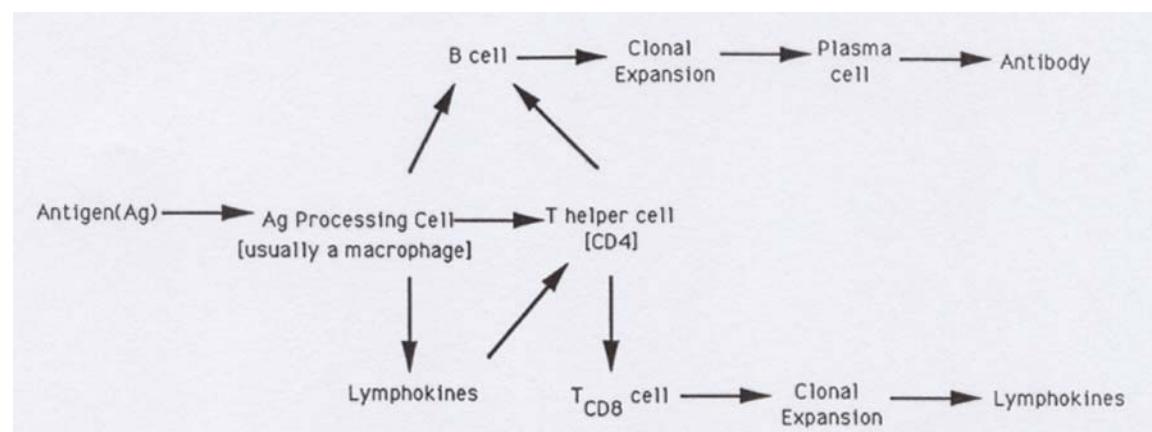
- Systemic reactions caused by an immune response:
  - often have low threshold levels.
  - The response may not be worse with increasing doses
  - Repeated exposure (even at low dose) can induce greater toxicity
- To be antigenic, a substance must be
  - Foreign and
  - Large ( $>5\text{kd}$ )
  - Small molecules (hapten) may become antigenic by binding to a host cell or protein, thereby providing the foreign antigenic site.

e.g. Drugs that bind to host cells or metal salts that bind to host components

# Systemic toxicity due to immune response

Review from previous lesson

- The immune response recognizes that a substance is antigenic but can not distinguish bad foreign substances (e.g. Bacteria) from good foreign substances (implanted biomaterial)



- The specific Immune system has two effector arms:
  - The humoral response is mediated by B cells with production of antibody by plasma cells and
  - The cell mediated response mediated by T cells
- Most antigens are first encountered and processed by a macrophage and subsequently „presented to B and T cells.
- Presentation and recognition of antigens stimulate the clonal expansion of B and T cells.
- The specific immune response recognizes a single molecule entity.

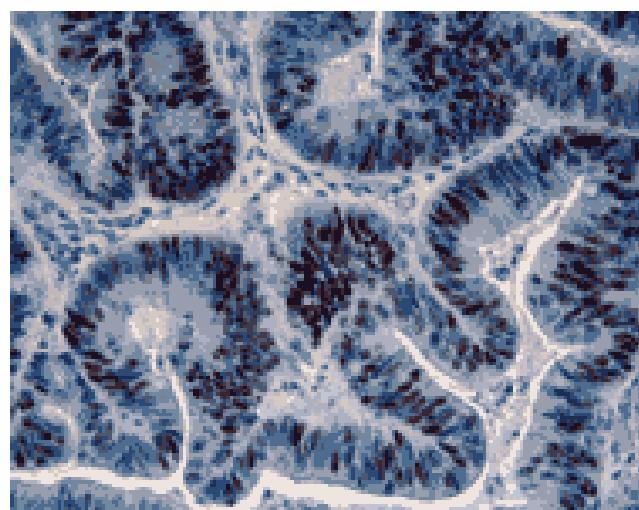
## Excursus: antibody

- The specific recognition of antigens can be used as a sensitive and specific tool for detection and quantification of substances:

Antibodies can be generated against known antigens and used for Clinical test for antigen levels :

- IHC (Immunohistochemistry)
- FACS (fluorescence assisted cell sorting)
- ELISA (Enzyme-Linked ImmunoSorbent Assay )

Each of these assays applies specific antibodies linked to a label (fluorescent dye, or color-generating enzyme)  
The intensity of the dye is then a measure for the Ig-Ag concentration



**IHC for the oncogene protein p53 in colon cancer**

## Excursus: antibody

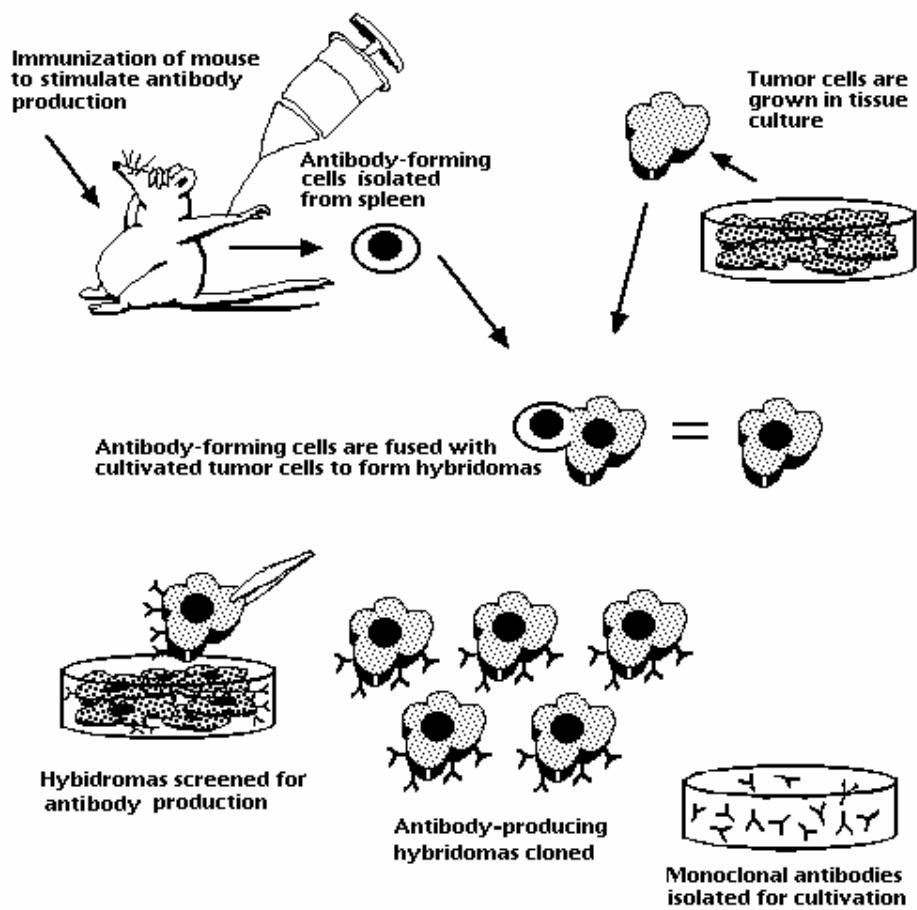
- Monoclonal antibodies recognize a specific part (epitope) of an antigen.

- can be easily produced

- are a valuable tool

- are commercially available

### Monoclonal Antibody Production

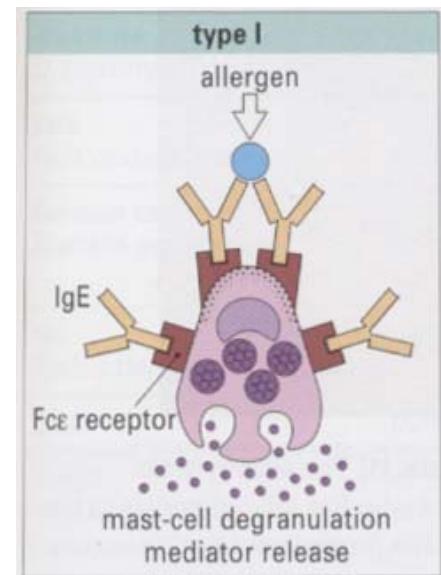


# Hypersensitivity

- Hyper-sensitivity reactions that result from unusual, excessive, or uncontrolled immune reactions are commonly called **allergies**
- Damage results from the release of chemicals normally confined to the internal of cells, or by overstimulation of the inflammatory response.
- It is not possible to predict systemic toxicity caused by immune reactions against a biomaterial or its degradation and wear products:
  - The immune response will depend on the genetics of the individual and
  - the nature,
  - dose and
  - location of release products
- Immune-based Hypersensitivity reactions have been divided into 4 types of allergies:

# Hypersensitivity

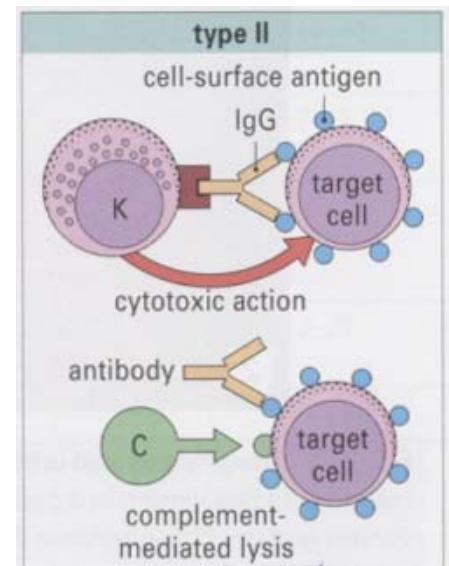
- Type I:
  - Involve interaction of antigen with IgE, which attaches to host cells in the skin and other tissues (mast cells, basophils, platelets and eosinophils).
  - Result in release of cell content (histamine, heparin, serotonin and other vasoactive substances).
  - Produces local or systemic symptoms within minutes to a few hours following Ag-IgE interaction (e.g. hay fever).
  - Caused by biomaterials: rare reports



# Hypersensitivity

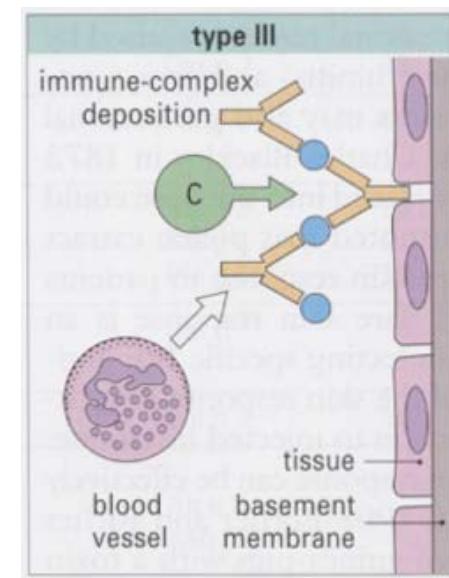
- Type II:

- IgG or IgM recognize antigens formed on cell surfaces
- Production of antibodies and/or complement activation.  
e.g. Drug-platelet combination stimulate the IS to produce antibodies against the drug.
- Type II reactions against biomaterials are rare



- Type III:

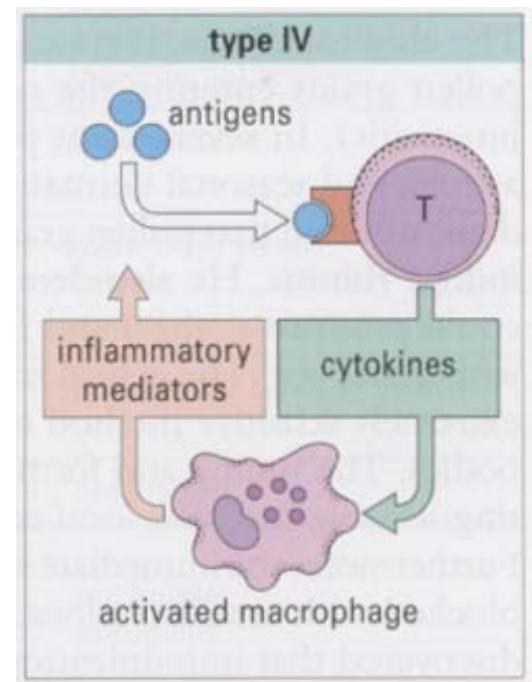
- Immune complexes (antigen-antibody) stimulate the inflammatory response.
- Days to weeks after the Ag-AK interaction.
- Immune complexes can lodge in the walls of blood vessels; activation of the complement system.
- Unlikely for most biomaterial applications, except slowly releasing drug delivery and biodegradation systems.



# Hypersensitivity

- Type IV:

- Involves the production of T cells that react with an macrophage-resistant antigen, or a hapten-based antigen formed on specific cells situated in the lymph nodes.
- Involves complex interaction of T cells, macrophages and soluble mediators
- Prolonged challenge result in granuloma formation (encapsulation of antigen)
- Generally requires previous exposure to the antigen (some biomaterials contain substances that have counterparts met with in every-day life)
- Most frequent response: Eczema (Contact dermatitis); systemic reactions are possible such as swelling, fever or local tissue damage („deep tissue reactions“)
  - Metal objects (jewelry, buttons)
  - Biomaterials:
    - metals (*are recognized as potential allergens*)
    - silicones, acrylics (PMMA) (*might be allergenic factors. Has been a matter of ongoing discussion/research for more than 10 years...*)



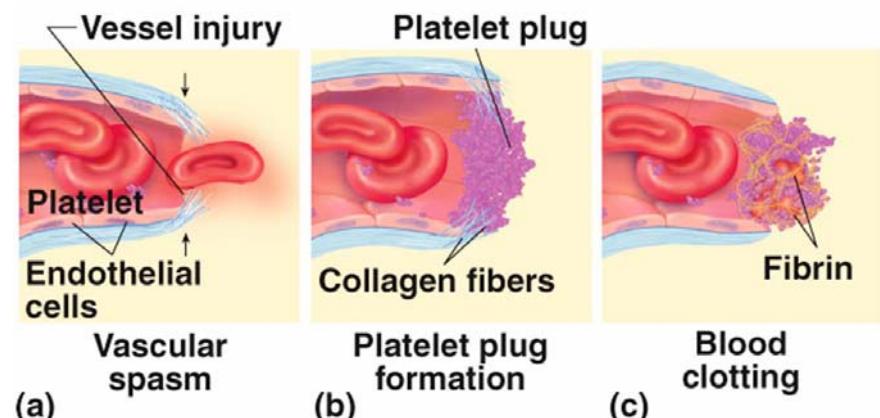
# Hypersensitivity

- Type IV reactions are most common
  - Drug delivery systems or other degradative systems that slowly and continually release potentially antigenic substances provide a model for the production of Type IV responses.
- Type I, II and IV responses are possible but difficult to document.
- The FDA omits the type II and III reactions for reasons of being „relatively rare and less likely to occur with medical devices/materials“ leaving the types I and IV as relevant in the context of biomaterials testing for marketing approval
- Long-term exposure may additionally cause impaired IS (immunosuppression or autoimmune responses) through adjuvant effects (*adjuvant= a substance that enhances interaction between two other substances, eg. „Self“ and antibody*)
- Testing prior to release of a biomaterial (premarketing testing) is extensive; therefore:
  - Relatively few reported clinical reactions of systemic toxicity, immune or non-immune mediated to biomedical applications.
  - But, with an increasing number of synthetic materials on the market it is difficult to predict single or synergistic toxic effects of leachable components in the future...

# Blood coagulation and Blood-Material interactions

- The hemostatic mechanism:

- designed to arrest bleeding from injured blood vessels.
  - May produce adverse consequences when artificial surfaces are placed in contact with blood and involves sets of interdependent reactions between:
    - the biomaterial surface,
    - platelets and
    - coagulation proteins.
  - Results in the formation of a clot or thrombus which may subsequently undergo removal by fibrinolysis.



# Blood coagulation

## ● Platelets:

- „little plates“ are non-nucleated, disc-shaped cells.
- Produced in the bone marrow
- Circulate in the blood ( $5 \times 10^6/\mu\text{l}$ )
- Lifespan: ca. 10 days

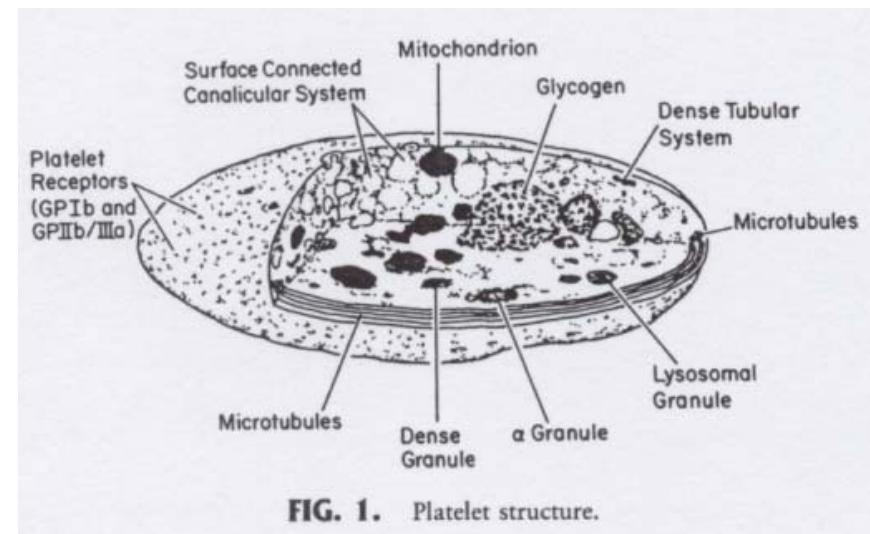
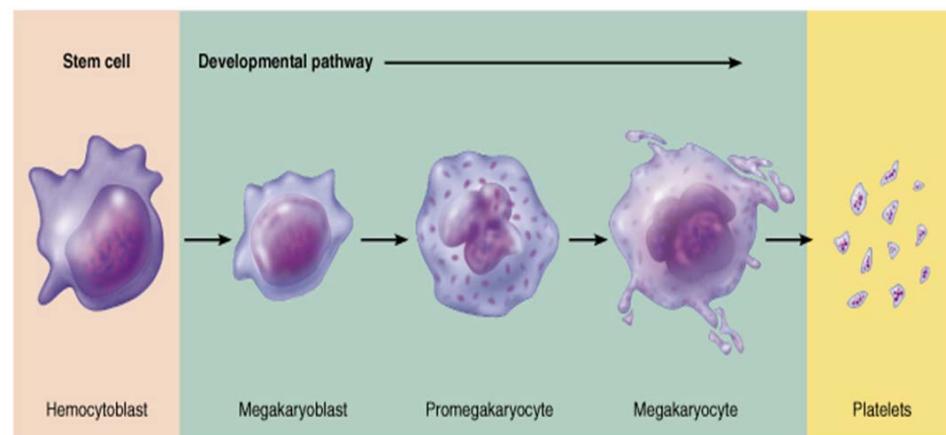


FIG. 1. Platelet structure.

## – Functions:

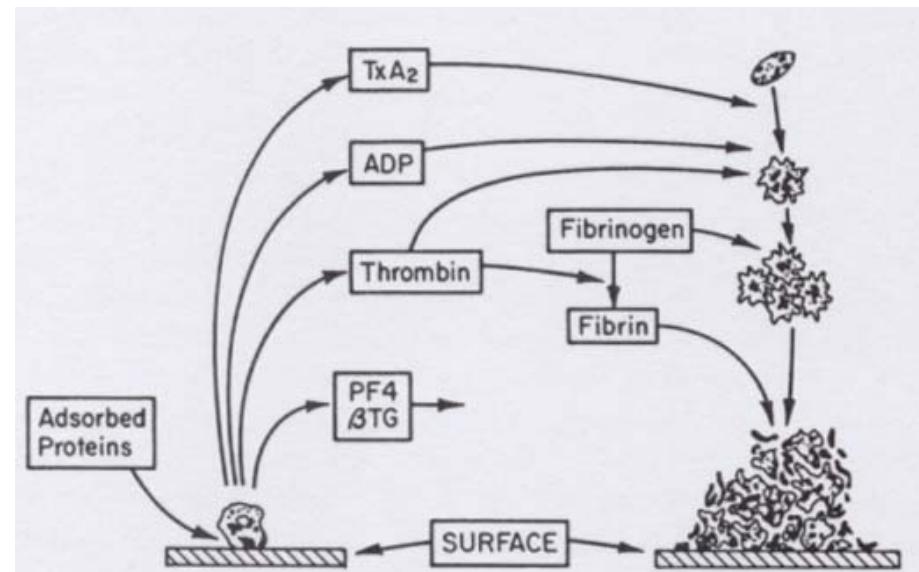
- Arrest bleeding through formation of platelet plugs.
- stabilizing platelet plugs by coagulation reactions, leading to the formation of fibrin.



# Blood coagulation

- Platelets are extremely sensitive cells that may respond to minimal stimulation.

- Activation causes platelets to become sticky and change in shape to irregular spheres with spiny pseudopods.
- Secretion of platelets products lead to:
  - Stimulation of other platelets
  - Irreversible platelet aggregation
  - Formation of a fused platelet thrombus

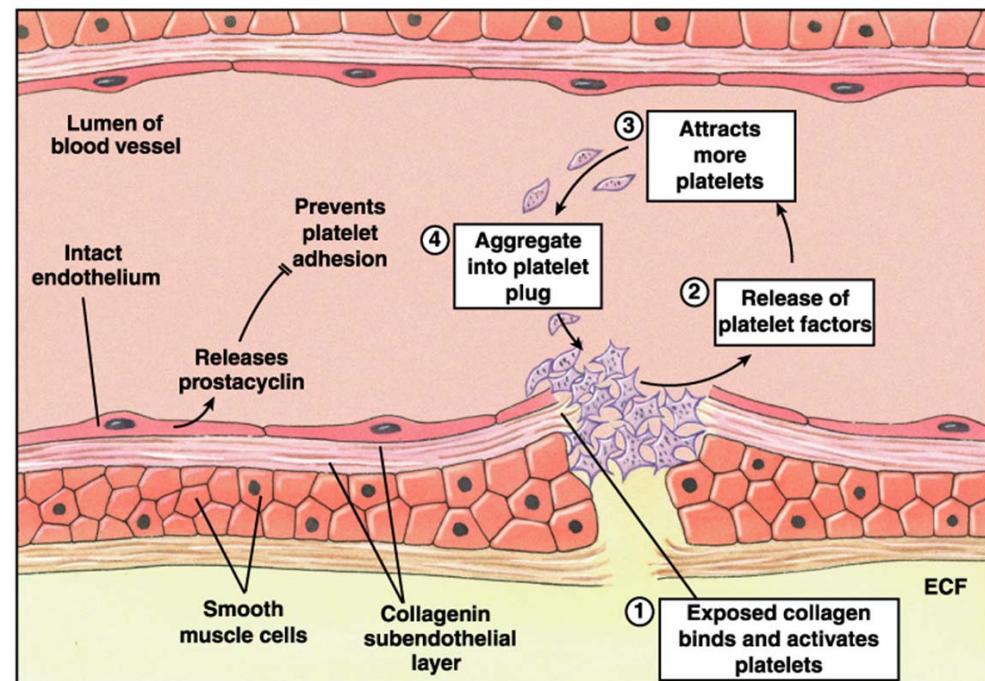


**FIG. 2.** Platelet reactions to artificial surfaces. Following protein adsorption to surfaces, platelets adhere and release  $\alpha$ -granule contents, including platelet factor 4 (PF4) and  $\beta$ -thromboglobulin ( $\beta$ TG), and dense granule contents, including ADP. Thrombin is generated locally through factor XIIa and platelet procoagulant activity. Thromboxane A<sub>2</sub> (Tx A<sub>2</sub>) is synthesized. ADP, Tx A<sub>2</sub>, and thrombin recruit additional circulating platelets into an enlarging platelet aggregate. Thrombin-generated fibrin stabilizes the platelet mass.

# Blood coagulation

- Platelets (activated) adhere to artificial surfaces and injured blood vessels.
- Adhesion to injured vessels involves interaction of:
  - plasma protein:  
von Willebrand factor  
(vWF; VIIIa)
  - Platelet membrane protein:  
vWF receptor (gpIb),
  - connective tissue elements (e.g. collagen)

- Enhanced adhesiveness of platelets (unactivated) towards biomaterial surfaces pre-adsorbed with fibrinogen.
- Adhesion to artificial surfaces may also be mediated through:
  - platelet glycoprotein IIb/IIIa (receptor for adhesive proteins supporting cell attachment):
    - fibrinogen,
    - vWF,
    - fibronectin,
    - vitronectin



# Blood coagulation

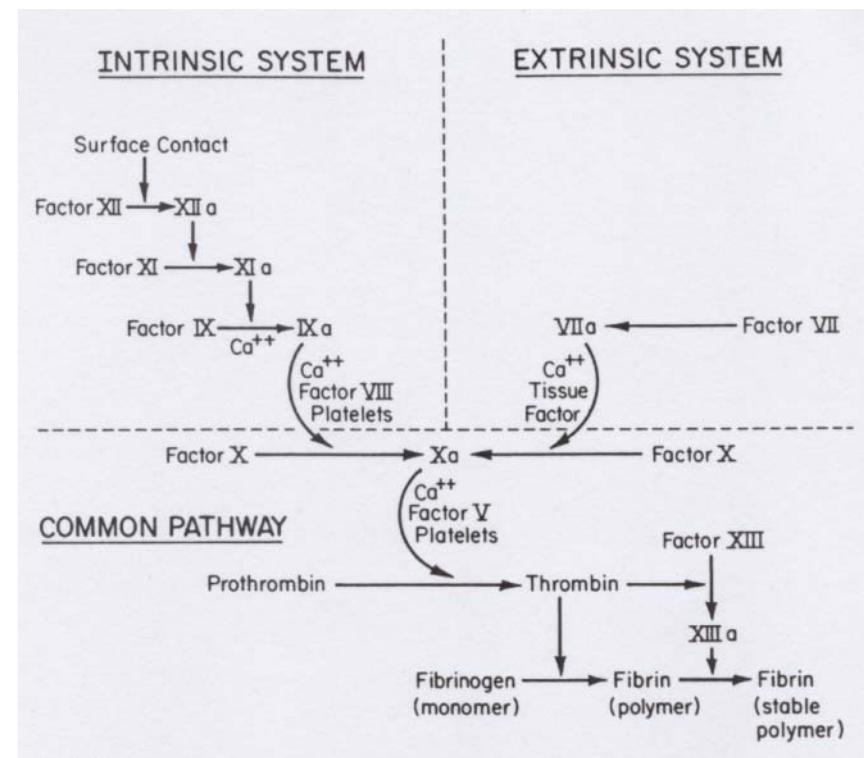
- At least 12 plasma proteins interact in a series of reaction leading to blood clotting.

- Roman numerals in order of discovery
- Initiation of clotting occurs either:
  - Intrinsically (by surface (neg charged) mediated reaction) or
  - Extrinsically (through „tissue factor“ derived from tissues or platelets)

Coagulation factors and their properties			
Factor	Synonyms	Molecular weight	Plasma concentration (mg/dL)
I	Fibrinogen	340 000	200–400
II	Prothrombin	70 000	10
III	Tissue factor (thromboplastin)	44 000	0
IV	Calcium ion	40	9–10
V	Proaccelerin, labile factor	330 000	1
VII	Serum prothrombin conversion accelerator (SPCA), stable factor	48 000	0.05
VIII (vWF)	Antihemophilic factor (AHF)	330 000 (250 000)n	0.01 1
IX	Christmas factor	55 000	0.3
X	Stuart–Prower factor	59 000	1
XI	Plasma thromboplastin antecedent (PTA)	160 000	0.5
XII	Hageman factor	80 000	3
XIII	Fibrin-stabilizing factor (FSF)	320 000	1–2
	Prekallikrein	85 000	5
	High-molecular-weight kininogen (HMWK)	120 000	6
	Fletcher factor		
	Fitzgerald, Flaujeac Williams factor, contact activation cofactor		

# Blood coagulation

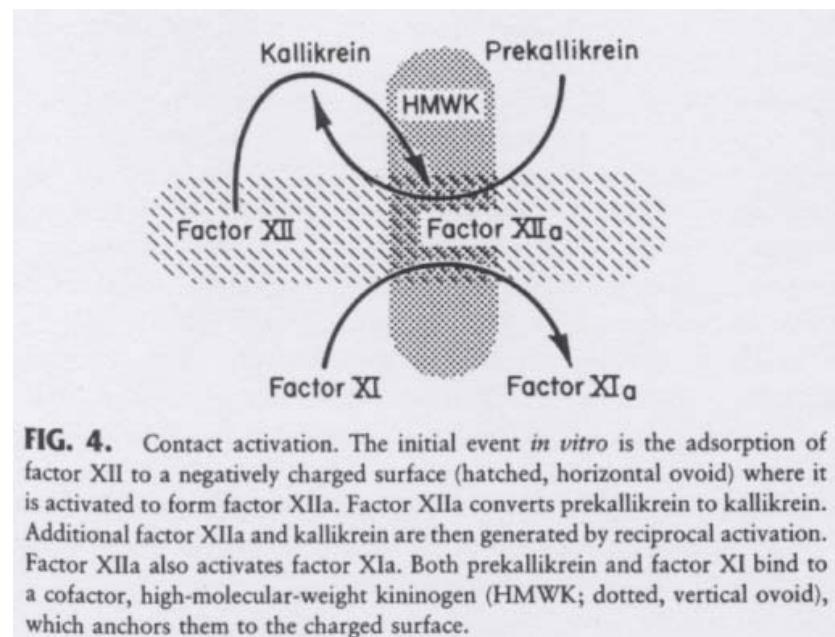
- The two systems converge upon a final common path which leads to the formation of an insoluble fibrin gel when thrombin acts on fibrinogen.
- Coagulation proceeds through a „cascade“ of reactions.
- The final steps require a phospholipid surface
- One activated enzyme can activate many substrate molecules:
  - Quick amplification
  - Significant amount of thrombin is produced
  - Platelets are activated
  - Fibrin is formed and
  - Bleeding is arrested



**FIG. 3.** Mechanisms of clotting factor interactions. Clotting is initiated by either an intrinsic or extrinsic pathway with subsequent factor interactions which converge upon a final, common path.

# Blood coagulation

- Except for the contact phase  $\text{Ca}^{2+}$  is required for most reactions:
  - Chelators of calcium (citrate, EDTA) are effective anticoagulants.
- In the intrinsic system, contact activation refers to reactions following adsorption of contact factors onto a negatively charged surface.
- Involved are the factors:
  - XII, XI
  - Prekallikrein and
  - HMWK (high molecular weight kininogen)



**FIG. 4.** Contact activation. The initial event *in vitro* is the adsorption of factor XII to a negatively charged surface (hatched, horizontal ovoid) where it is activated to form factor XIIa. Factor XIIa converts prekallikrein to kallikrein. Additional factor XIIa and kallikrein are then generated by reciprocal activation. Factor XIIa also activates factor XIa. Both prekallikrein and factor XI bind to a cofactor, high-molecular-weight kininogen (HMWK; dotted, vertical ovoid), which anchors them to the charged surface.

# Blood coagulation

## Control mechanisms:

Promote localized hemostasis while preventing generalized thrombosis

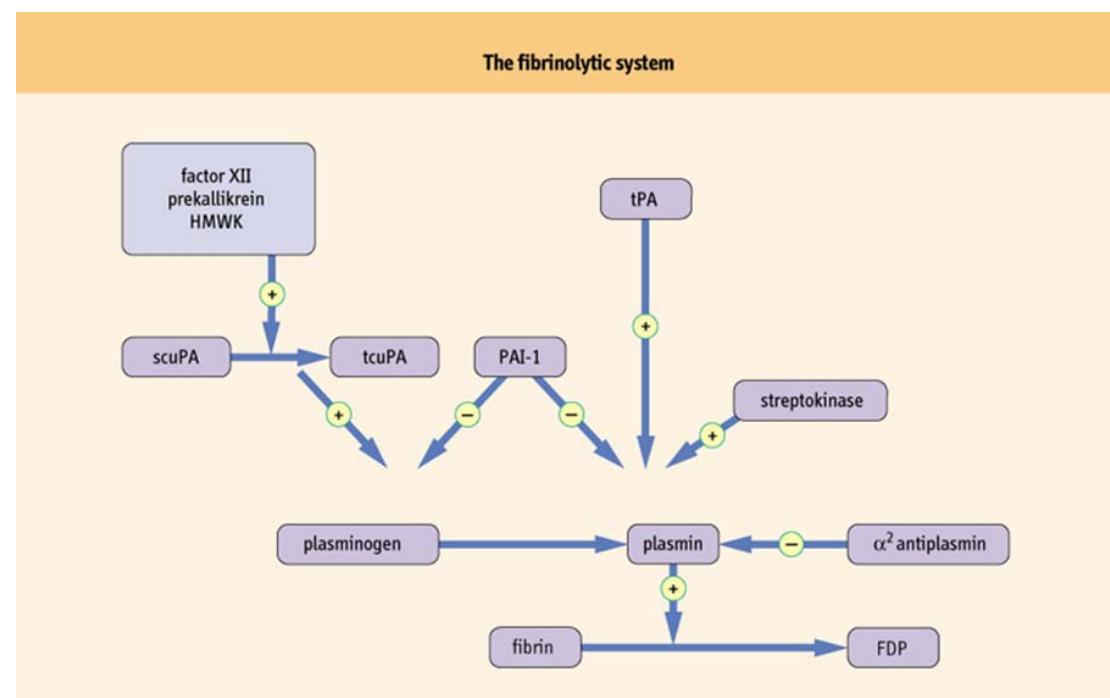
- Blood flow may reduce the localized concentration of precursors/activated proteins.
- The requirement of a phospholipid surface ensures that clotting is localized to around the aggregated platelets
- There are natural inhibitors (a.g. Antithrombin III: inhibits thrombin)
- During coagulation also degrading factors are generated:
  - Plasmin: degrades fibrin, inactivates V and VIII
  - Thrombomodulin: bindes/removes thrombin

Properties of coagulation inhibitors		
Inhibitor (synonym)	Molecular weight	Plasma concentration (mg/dL)
Antithrombin (antithrombin III)	65 000	18–30
Protein C	56 000	0.4
Protein S	69 000	2.5
Tissue factor pathway inhibitor, TFPI (lipoprotein-associated coagulation inhibitor, LACI)	32 000	0.1

# Fibrinolysis

- The fibrinolytic system removes unwanted fibrin deposits to:
  - improve blood flow following thrombus formation, and to
  - facilitate the healing process after injury and inflammation.

- It is a multicomponent system composed of:
  - precursors,
  - activators,
  - cofactors and
  - inhibitors.
- It also interacts with the coagulation system at the level of contact activation



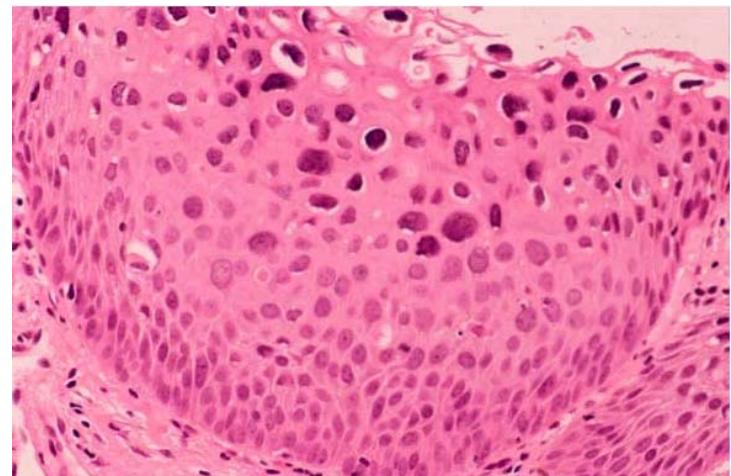
# *Blood coagulation and Blood-Material interactions*

- There are a number of interactions between the complement, coagulation and fibrinolytic systems.
- When artificial surfaces are exposed, an imbalance between activation and inhibition of these systems can lead to excessive thrombus formation and increased inflammatory response.
  - Devices having large surface areas (e.g. Hemodialyzers) may cause:
    - reciprocal activation reaction between complement and white cells
    - complement activation may mediate both white cell and platelet adhesion to artificial surfaces
    - stimulated monocytes express tissue factor (activator of extrinsic coagulation)
    - neutrophils may contribute by releasing potent fibrinolytic enzymes (neutrophil elastase)

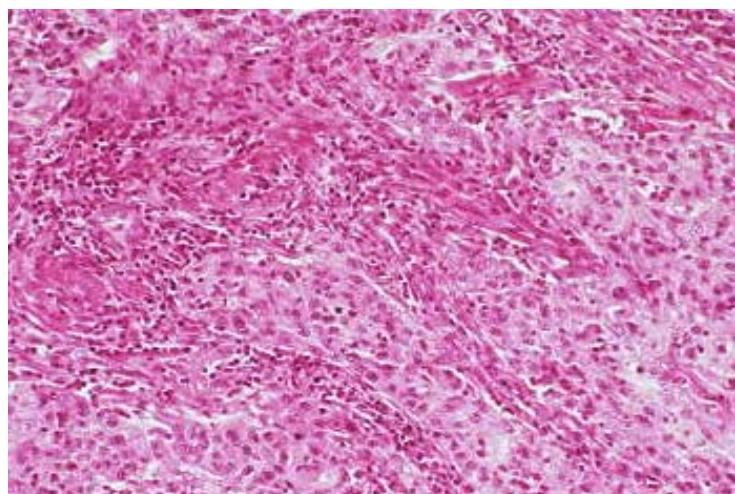
Case study: the first Dacron<sup>TM</sup> vascular graft implants were extensively collagen coated (~1980). As a result patient recovery was often complicated by extensive blood clotting and prolonged inflammation

# Tumorigenesis and Biomaterials

- **Neoplasia** („new growth“) is the process of excessive and uncontrolled cell proliferation (neoplasma or tumor).
  - Benign tumors
  - Malignant tumors
- **Benign tumors:**
  - do not penetrate (invade) adjacent tissues, nor do they
  - metastasize (spread to distant sites).
  - Suffix: -oma (e.g. Papilloma)
- **Malignant tumors** (or cancer)
  - tend to invade adjacent tissues, and
  - metastasize via blood and lymph vessels.
    - Carcinoma (derived from epithelia)
    - Sarcoma (derived from mesenchym)
    - Leukemia (derived from hematopoietic system)
    - Lymphoma (solid tumors of lymphoid tissues)



Human papilloma virus in cervix



Squamous cell carcinoma

# Tumorigenesis and Biomaterials

- Neoplastic growth abnormal proliferation unrelated to the physiological requirements of the tissue, and is unaffected by the stimulus which initially caused it.
- In contrast:
  - Normal proliferation during fetal or postnatal growth
  - Normal wound healing
  - Hyperplastic adaption to physiological needs.

**TABLE 1** Characteristics of Benign and Malignant Tumors

Characteristics	Benign	Malignant
Differentiation	Well defined; structure may be typical of tissue of origin	Less differentiated with bizarre (anaplastic) cells; often atypical structure
Rate of growth	Usually progressive and slow; may come to a standstill or regress; cells in mitosis are rare	Erratic, and may be slow to rapid; mitoses may be absent to numerous and abnormal
Local invasion	Usually cohesive, expansive, well-demarcated masses that neither invade nor infiltrate the surrounding normal tissues	Locally invasive, infiltrating adjacent normal tissues
Metastasis	Absent	Frequently present; larger and more undifferentiated primary tumors are more likely to metastasize

# *Tumorigenesis and Biomaterials*

- Cases of human and veterinary implant-related tumors have been reported.
- Neoplasms occurring at the site of implanted medical devices are unusual.
- The vast majority are sarcomas:
  - Fibrosarcoma
  - Osteosarcoma
  - Chondrosarcoma
  - Angiosarcoma
- Formation of a tumor occurring adjacent to an implant does not necessarily prove that the implant caused the tumor!
- Implant-related tumors have been reported both short and long term following implantation.
- The period of latency is usually relatively long
  - 25% of tumors associated with foreign bodies have developed within 15 years, and
  - Over 50% within 25 years.
- The pathogenesis of implant-induced tumors is not well understood.
- Most data indicate that physical effects rather than chemical characteristics of the foreign body primarily determine tumorigenicity. Solid materials with high surface area being the most tumorigenic.

# Tumorigenesis and Biomaterials

- Tumors are induced experimentally by materials of any kind, including those considered to be non-reactive:
  - glasses,
  - gold or platinum
  - relatively pure metals
  - polymers
- Tumorigenicity corresponds
  - directly to the extent of tissue encapsulation of a foreign body
  - inversely with the degree of active cellular inflammation.
  - directly with the shape of the material
- An active, persistent inflammatory response inhibits tumor formation.
- Direct correlation between biomaterial and tumor formation is often hard to establish, because of multifactorial etiology.

example: one clinical and experimental study suggested a decrease of breast carcinomas in women with breast implants (Su et al., 1995).

**TABLE 3** Steps in Implant-Associated Tumorigenesis: A Hypothesis<sup>a</sup>

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1. Cellular foreign body reaction
2. Fibrous capsule formation
3. Preneoplastic cells contact implant surface during quiescent tissue reaction
4. Preneoplastic cell maturation and proliferation
5. Tumor growth

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<sup>a</sup>Following K. G. Brand and colleagues.

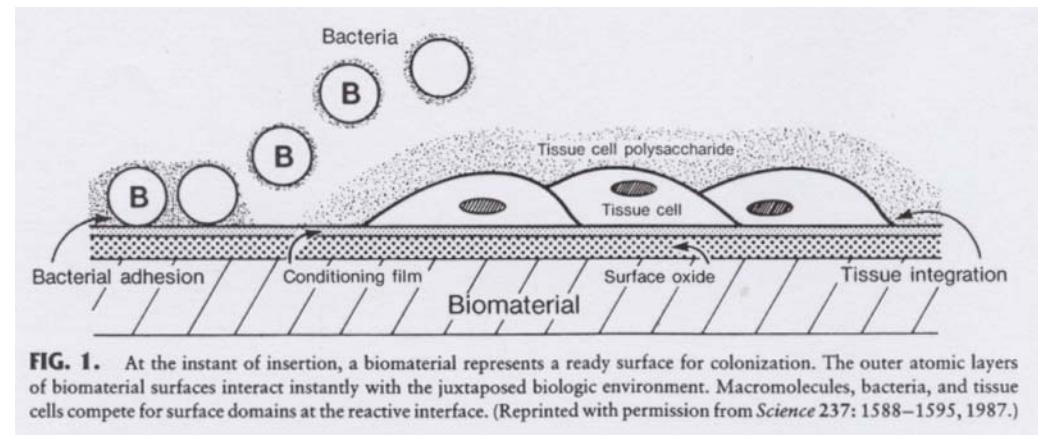
# *Implant associated Infection*

- Infections involving artificial organs, synthetic vessels, joint replacements or internal devices usually require re-operation.
  - Infected cardiac, abdominal or extremity vascular prostheses result in amputation or death in 25-50% of cases.
  - Intravenous catheters, peritoneal dialysis and urologic devices used for more than a few days frequently become infected or raise secondary tissue-sited infections.
  - The rate of infections for the total artificial heart approaches 100% when the heart is implanted for more than 90 days.

# Implant associated Infection

- Infections around biomaterials and damaged tissue is caused by bacteria that have colonized the surfaces.

- If bacterial colonies established on biomaterial surfaces grow uninhibited, they destroy the neighbouring tissue.



- In natural environment bacteria are surface creatures (99% of their biomass exists on surfaces).
- The colonization potential of most synthetic surfaces for bacteria is high compared with tissue cells since the surfaces resemble sub-strata in nature.
- „Wild-type“ bacteria colonizing a surface exhibit extreme changes in gene-expression leading to a firmly settled algine-slime producing phenotype.
- The „slime“ forms a thick protecting **bio-film** which shields the bacteria from the environment

# Implant associated Infection

- The features of implant-associated sepsis include:
  - A biomaterial or damaged tissue substratum
  - Adhesive bacterial colonization of substratum
  - Antibiotic therapy cures the sepsis. But sepsis reoccurs immediately after the antibiotic pressure is terminated.
  - Persistence of infection until removal of substratum
  - Absence of tissue-integration at the biomaterial-tissue interface
  - Presence of tissue cell damage and necrosis
- Analysis of hundreds of implanted devices revealed that they were all coated in bacterial biofilm, although only few of the hosts (patients) had developed symptoms of infection
- Further analysis revealed:
  - Biofilm protects the bacteria from the host immune response and from antibiotics
  - Bacteria settled in biofilm do not interfere with the host (no release of toxic substances)
  - When the bio-film coated bacterial colony reaches a mature size (within 1-2 weeks) planktonic clusters of bacteria are released from the biofilm. The host immune system immediately kills these floating bacteria.
  - The mature biofilm size remains unchanged as a result of equilibrium between growth and host immune defense.
  - ....Unless the host immune defense weakens and fails.....

# Implant associated Infection

- Few bacterial species dominate biomaterial-centered infections:
  - *Staphylococcus epidermidis*, *S. aureus*
  - *Escherichia coli*
  - *Pseudomonas aeruginosa*
- *S. epidermidis* (human skin bacteria) is a primary cause of infection of implanted polymeric biomaterials
  - Artificial hearts, total joints, vascular grafts, catheter, shunts
- *S. aureus* is the major pathogen isolated from metallic-related bone, joint and soft tissue infections.
- *P. aeruginosa* is the primary pathogen of special sites:
  - Contact lenses, total artificial hearts
- Implant associated infectious bacteria (the above) are all naturally occurring in normal human micro-environment → normal healthy humans have a functional immune defense against those bacteria (!)

# *Implant associated Infection*

- Initial attachment of bacteria (reversible nonspecific adhesion) depends on:
  - the general physical characteristics of the bacterium,
  - the fluid interface and
  - the substratum.
- Subsequent specific irreversible adhesion may occur as a time dependent chemical process involving:
  - Chemical binding
  - hydrophobic interactions,
  - specific receptor-ligand interactions as well as
  - nonspecific exopolysaccharide to surface interactions

# *Implant associated Infection*

- Implanted biomaterials are rapidly coated by constituents of the serum and surrounding matrix:
  - Fibronectin
  - Collagen
  - Osteonectin
  - Vitronectin
  - Albumin,....
- Bacterial and tissue cells may then adhere to constituents of this film:
  - Certain strains of *S.aureus*, *S.epidermidis* and *E.coli* have receptors for fibronectin or collagen.

# Implant associated Infection

- Patients with immune system depression or aberration are predictably at risk:
  - Rheumatoid patients manifest a spontaneous and somewhat cryptic sepsis in joints
  - Diabetes,
  - infants, children,
  - the aged,
  - patient with vascular disease,
  - drug abusers and
  - HIV patients are at increased risk from specific organisms.
- Infections involving biomaterials are generally considered resistant to antibiotic therapy and require the removal of implant to resolve the infection.
- Researchers search for means of controlling bacterial settlement on material implant surfaces. Several approaches are made:
  - „non-fouling“ surfaces are sought. Substances like PEG (polyethyleneglycol) are almost resistant to settlement
  - Application of ultrasonic radiation or DC potentials to the surfaces seem to antagonize settlements
  - Marine organisms such as algae have biofilm coats of constant, controlled size. These organisms control the biofilm by secreting controlling substances
  - Constant delivery of antibiotics to the materials surface (eg. By gradual release from surface coating) would kill the planktonic bacteria before they can settle into biofilm
  - Re-sterilization immediately upon insertion of the implant, before colonization occurs