B Cell Receptor for Antigen (BCR)

The B cell receptor for antigen (BCR) consists of membrane-bound antibodies of different classes.

Idiotype Network

B Cell Maturation and Immunological Memory

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Synonyms
B lymphocyte, antigen dependent B cell development, affinity maturation of the immune response, germinal center reaction, B memory cell, B cell receptor modification.

Definition
Efficient protection from pathogens and toxins requires high affinity antibodies. These antibodies are generated by a process referred to as affinity maturation which takes place in the germinal centers. Within the micro-environment of the germinal center activated B cells differentiate into plasma and memory cells. Plasma cells secrete large quantities of high affinity antibodies whereas memory B cells give long-term protection. On subsequent contact with the same antigen, memory cells can rapidly differentiate into plasma cells secreting high affinity protective antibodies.

Characteristics
B Cell Maturation
B cells are generated in the bone marrow from hematopoietic stem cells. By stepwise rearrangement (Fig. 1) of the Ig V-region genes (V, (D), J) these cells become competent to express a B cell receptor (BCR). The naive B cells express a broad repertoire of antigen-specific receptors, but for a given antigen there are only a few B cells available. In addition, the overall affinity of these B cells will be low.

The process of B cell affinity maturation is antigen dependent. It takes place in the peripheral lymphoid organs where the naive B cell is activated by antigen binding to its receptor. In order to induce a germinal center reaction the B cell needs help from T lymphocytes (T cell-dependent immune response) and if this is available then clonal expansion of the antigen specific B cell and differentiation into plasma and memory cells occurs (1).

Germinal Center Reaction
In order to induce a germinal center reaction, antigen-activated T and B cells—as well as antigen-presenting dendritic cells—have to come together in close vicinity. When this happens a micro-environment is set up which permits rapid proliferation of B cells so that within a few days a single antigen-activated B cell can form a large clone consisting of several thousand cells.

It takes about 1 week for the mature germinal center structure to be formed (2). Such a germinal center is composed of a dark and a light zone. In the dark zone B cells proliferate, whereas in the light zone they differentiate into plasma and memory cells (Fig. 2). During proliferation in the germinal center a process of hypermutation is activated (1). This highly specific mechanism introduces single nucleotide exchanges into the genes coding for the V region of the H-chain and L-chain of the B cell receptor. In this way B cells with variant receptors are generated and these variants may have different affinities for the antigen. Only those B cells with high affinity receptors are selected to differentiate into plasma and memory B cells.

This selection process takes place in the light zone of the germinal center where the B cells are embedded in...
a network of follicular dendritic cells (1). These are specialised cells which do not process antigen, but rather present intact antigen in form of antigen-antibody complexes bound to their complement and Fc receptors. Here, the B cell variants compete for binding to the antigen. Those cells which—due to low affinity receptors—fail to interact with antigen die by apoptosis while those with high affinity receptors survive and differentiate. Thus the interplay of hypermutation and selection ensures that only B cells with high affinity receptors develop into plasma and memory cells.

Ig Class Switch and Hypermutation

The V region of the B cell receptor defines its antigen specificity. However, functions such as complement binding, secretion, opsonization depend on the constant region of the H-chain which defines the so-called Ig class of the B cell receptor (see Fig. 3). During B cell differentiation an Ig class switch may take place, so that the same V region gene may be combined with a different constant region gene. The naïve B cell expresses Ig receptors of the IgM and the IgD class. A switch to IgG may take place in antigen-acti-
vated B cells during the germinal center reaction and it requires the activity of the enzyme AID (activated cytidine deaminase) which is also essential for hypermutation (3). Nevertheless, these two processes of B cell receptor modification are independently controlled. Somatic mutations are found in V region genes of memory B cells, independent of expression of IgM or IgG.

**Immunological Memory**

The process of affinity maturation is surprisingly efficient. However it is dependent on clonal expansion and clonal selection, both of which are time consuming processes. For immediate protection, for example against bacterial toxins, one needs a pool of memory B cells which have developed in a germinal center reaction and hence already have high affinity receptors for the antigen. This is the reason for vaccination. In contrast to the naive B cell, the memory cell responds immediately to an antigenic stimulus and rapidly differentiates into plasma cells secreting high affinity antibodies.

Plasma cells generated in a germinal center reaction have the capacity to migrate back into the bone marrow where they may survive for many months. In this way the level of antigen-specific antibodies stays elevated for a long time after antigenic contact and these antibodies provide protection from further infections. However, even long-lived plasma cells need finally to be replenished.

Little is known about the mechanisms controlling the long-term maintenance of immunological memory. It is still not clear whether it is the memory cell itself which is long-lived or whether it is the memory B cell clone that survives over the years. Low doses of antigen stored in the network of follicular dendritic cells may be sufficient to continuously stimulate memory B cell proliferation. There is also evidence that signals provided by the innate immune system (innate immunity) might be involved in the homeostatic control (4). Since memory B cells—in striking contrast to naive B cells—express Toll-like receptors they may respond to non-specific environmental stimuli. Since the size of the memory B cell pool is limited, there must be strong competition for space and indeed the proliferative activity of these cells in the absence of antigen is low.

**Preclinical Relevance**

The germinal center reaction is a useful marker for the antigen-dependent activation of the immune system. In preclinical studies immunohistology is used to assess whether reagents have stimulating or immune suppressive activity. Specific staining of tissue section allows the visualisation of germinal centre formation. Germinal center evaluation belongs to the key parts of histopathological examinations in immunotoxicologic screening studies.

**Relevance to Humans**

The importance of B cell memory and affinity maturation is clearly shown in patients with immune deficiencies (5), such as in X-linked hyper-IgM syndrome. A genetic analysis revealed that these patients have a defect in the gene for the CD40 ligand, which prevents efficient communication between antigen-activated T and B cells. Without adequate T cell help no germinal center will develop and no memory B cells will be generated. In these patients B cells do differentiate into plasma cells, but there is no Ig class switch and the secreted antibodies are IgMs of low affinity. As a result patients suffer from recurrent infections from early in life.

**References**


**B Cell Receptor Complex**

The B cell receptor complex (BCR complex) is expressed on the surface of a B cell and is composed of six chains. There are two immunoglobulin (Ig) heavy chains and two Ig light chains that form the portion of the complex that is involved in antigen specificity. Associated with heavy and light immunoglobulin chains are two other chains, Igα and Igβ. These chains have long cytoplasmic regions and are involved in the signaling process.

**Signal Transduction During Lymphocyte Activation**

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B Lymphocytes

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Synonyms
B cell, plasma cell, antibody-forming cell

Definition
B lymphocytes (1–2) are a subset of white blood cells, also termed leukocytes, which are characterized by their ability to synthesize and secrete antibodies. B lymphocytes also express antibody molecules on their surface, which serve as antigen receptors allowing for the recognition of non-self. When terminally differentiated into antibody producing cells, B lymphocytes are termed plasma cells or antibody-forming cells. The primary function of B cells is to detect and tag foreign or non-self molecules (►antigens) through the secretion of antibodies that will specifically bind to the foreign antigen, allowing removal by other cells of the immune system, or activation of the complement cascade.

Characteristics
B lymphocytes develop from the pluripotent hematopoietic stem cells located in the bone marrow in mice, humans, and other mammals, or in the bursa of fabricius in birds, hence the denotation ‘B’. Stem cell commitment to the lymphoid lineage, either B or T cell, is controlled by the Ikaros gene. The Ikaros gene codes for a DNA-binding protein which, in combination with other transcription factors, regulates the expression of genes that yield the phenotypic characteristics of lymphocytes including the immunoglobulin heavy chain, the light chain, the essential components of gene rearrangement machinery and members of the CD3 complex of the T cell antigen receptor. Once the stem cells have committed to the lymphoid lineage, the specific mechanism responsible for commitment to B lymphocytes is poorly understood but is thought to involve a progressive loss in the ability to differentiate into other lineages. However, B cell commitment has an essential requirement for an interaction with stromal cells. Stromal cells signal the progenitor cells to continue the B cell development program through cell-to-cell interactions that are mediated by adhesion proteins and through the release of soluble growth factors. Once stem cell commitment to the B cell lineage has been made, the B cell undergoes four stages of development. The first stage of development, termed the pro-B cell stage, occurs in the bone marrow and is identified by the expression of cell surface proteins associated with early B cell development and the capability of ► VDJ gene rearrangement and joining. The second stage is the pre-B cell stage, which is marked by the expression of small amounts of µ (immunoglobulin (Ig)M heavy chain) on the cell surface. It is notable that the expressed µ also possesses a surrogate light chain making up the pre-B cell receptor. In the third stage of B cell development, light-chain gene assembly takes place, which can be either the λ or κ form, resulting in the expression of surface IgM and is the hallmark of the immature B cell. B cell development up to the immature B cell stage takes place in the bone marrow. Immature B cells then undergo positive and negative selection in order to eliminate those cells that react to self antigens. B cells that survive the selection process and are present in the periphery are termed mature B cells. These mature B cells express IgM and IgD on their surface and are present in the circulation and in secondary lymphoid organs such as the spleen and lymph nodes. A single mature B cell can recognize a specific antigen through its B cell receptor (BCR) which is a membrane-bound Ig molecule. Binding of the antigen to the BCR with the help of secondary cellular and soluble mediators can result in activation of the B cell and proliferation or ►clonal expansion. Following clonal expansion, the B cells, all with specificity for the activating antigen, differentiate into antibody-forming cells (AFC; also termed plasma cells), or into ►memory cells. AFCs secrete antibodies, a soluble form of Ig, which coat a specific antigen and facilitate antigen clearance or activation of the complement cascade.

The basic Ig molecule is composed of two identical heavy chains and two identical light chains, and has two antigen binding sites that are held together by disulfide bonds. There are two classes of light chains, κ and λ, adding to the diversity of the antigenic repertoire, and five heavy chain classes, IgM, IgG, IgA, IgE and IgD, that are encoded, respectively, by µ, γ, α, ε, and δ heavy chain genes. Each class of Ig appears to have unique biological properties. In addition, certain classes of antibodies can be joined together by additional disulfide bonds and by a polypeptide chain termed the J chain, which is also synthesized in B cells. For example, IgM is pentameric or hexameric resulting in high antigen valence (10 or 12 antigen-binding sites). IgM also has relatively low affinity for antigen, and is the major Ig involved in a ►primary antibody response. IgG is monomeric, has high affinity for antigen, can cross the placental barrier and is the hallmark of a ►secondary antibody response. IgE is also monomeric and is involved in allergic and an-
tiparasitic responses. IgA is monomeric or dimeric, is very efficient at bacterial lysis and is the main secretory antibody (found in saliva, mucus, sweat, gastric fluid, and tears). IgD is monomeric, is a major surface component on many B cells and has unknown biological properties. Regulation of Ig expression and isotype class switching is governed through a complex interaction of several regulatory elements whose activity is B cell specific and dependent on the state of B cell maturation. The most 5' regulatory element in the Ig heavy chain gene is the variable heavy chain (VH) promoter, which lies immediately upstream of each variable region and contributes to B cell-specific activity of the Ig heavy chain. Located between the rearranged VDJ segments and the μ constant region is the intronic enhancer (Eμ) which contributes to B cell-specific activity and is involved early in B cell development where it regulates V to D-J joining and μ heavy-chain gene expression. However, processes late in B cell differentiation, such as upregulation of heavy chain expression and secretion, as well as class switching, occur normally in the absence of Eμ and appear to be regulated by another regulatory element(s) located 3' of the α constant region. Within this region, four separate enhancer domains, hs3A, hs1,2, hs3B and hs4 were identified and are collectively termed the 3'α enhancer. Activity of these enhancer domains is dependent on the developmental stage of the B cell with hs3A, hs1,2 and hs3B primarily active in activated B cells or plasma cells. In contrast, hs4 is active from a pre-B cell to the plasma cell stage. Expression of the light chain and J chain is also regulated through the activation of a 5' promoter and a 3' enhancer. Appropriate modulation of the above regulatory elements during B cell activation and differentiation results in Ig expression or class switch. However, exposure to therapeutic drugs, environmental compounds, or industrial chemicals may alter the activity of these regulatory elements, perhaps resulting in the suppression or enhancement of Ig expression and secretion.

**Preclinical Relevance**

Due to the similarity between the mouse and human immune systems and the availability of biological reagents, mouse models have primarily been utilized to understand the basic mechanisms of B cell function as well as the impact of potential therapeutic and toxic compounds on these mechanisms. Preclinical studies provide the necessary information to evaluate the potential hazard to humans from occupational, inadvertent, or therapeutic exposure to drugs, environmental compounds, or industrial chemicals.

**Relevance to Humans**

The B cell is a vital component of the immune system. Maintaining immunocompetence is essential to human survival and well-being. Therefore, immunotoxicologic studies are essential in identifying potential modifiers of B cell function which might be inhibited, leading to increased infections, or enhanced, leading to autoimmune and/or hypersensitivity reactions. Either situation could lead to morbidity and even mortality.

**Regulatory Environment**

Leukocyte phenotyping by flow cytometric analysis is routinely conducted in immunotoxicology testing to determine whether exposure to an agent produces a change in the number of cells in leukocyte-specific subpopulations. Changes in the number of B lymphocytes in circulation or in secondary lymphoid organs can be quantified by flow cytometry using fluorochrome-conjugated antibodies directed against B lymphocyte-specific surface peptides such as Ig. Similarly, stages of B cell differentiation can be monitored by measurements of cell surface expression of differentiation markers. Specifically, mature B cells express MHC class II, which is downregulated in plasma cells. Conversely, B cells that are differentiating into plasma cells upregulate syndecan.

**References**


**B Memory Cell**

> B Cell Maturation and Immunological Memory

**B7.1 and B7.2**

These are important co-stimulatory molecules on antigen-presenting cells. Their receptors on T lymphocytes are the CD28 molecule and CTL-4 (present only on activated cells). They co-stimulate T cell proliferation and cytokine secretion. Also known as CD80 (B7.1) and CD81 (B7.2).
Bacteremia

The presence of bacteria in the blood.

- Streptococcus Infection and Immunity

Bacteremic Shock

- Septic Shock

Bactericidal

An agent or host defense mechanism capable of causing the death of bacteria.

- Respiratory Infections

B(a)P

Benzo(a)pyrene is the prototype of the polycyclic aromatic hydrocarbon class of compounds. It is an immunosuppressive compound which predominately effects humoral immunity.

- Plaque Versus ELISA Assays. Evaluation of Humoral Immune Responses to T-Dependent Antigens
- Polycyclic Aromatic Hydrocarbons (PAHs) and the Immune System

Bcl-2 Interacting Domain (Bid)

Bcl-2 interacting domain (Bid) is a novel member of the Bcl-2 family of proteins and is critical to the regulation of apoptosis induced by many stimuli including TNF-α. Bid belongs to the BH3-only family of pro-apoptotic regulators and can mediate apoptosis through two interacting pathways. Activation of caspase-8 at the death-inducing complex results in Bid cleavage and release of the truncated form tBid. tBid translocates to the mitochondrial membrane, where it facilitates the release of apoptogenic proteins like cytochrome C. cJun N-terminal kinase can also cleave Bid producing jBid. jBid binds and sequesters inhibitor of apoptosis proteins leading to increased activation of caspases and increased tBid formation and apoptosis.

- Tumor Necrosis Factor-α

Beige Mouse

A mouse that is deficient in NK cells and other cellular immune functions.

- Animal Models of Immunodeficiency

Benzo-e-pyrene

- Polycyclic Aromatic Hydrocarbons (PAHs) and the Immune System

Berylliosis

- Chronic Beryllium Disease

Beryllium Disease

- Chronic Beryllium Disease

Beryllium-Stimulated [or Beryllium-Specific Peripheral Blood?] Lymphocyte Proliferation Test (BeLPT)

This is an in vitro assay in which beryllium-stimulated cell proliferation is measured by tritiated thymidine incorporation. The test is performed on peripheral blood mononuclear cells to support a diagnosis of beryllium sensitization and on bronchoalveolar lavage cells to confirm chronic beryllium disease.

- Chronic Beryllium Disease

bg/nu/xid Mouse

A mouse with deficient function of NK cells, lymphokine-activated killer cells, and T and B cells.

- Animal Models of Immunodeficiency

Bioaerosols

Biological substances that are dispersed in air in the form of a fine mist intended for inhalation.
Biphenotypic Leukemia

Leukemia

Birth Defects, Immune Protection Against

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Synonyms
Immunoteratology (although this term may also be used for structural or functional defects of immune-related etiology)

Definition
Immune protection against birth defects refers to the ability of immune stimulation in mice to reduce the occurrence or severity of birth defects caused by diverse teratogenic exposures.

Characteristics
Female mice are exposed to any of a variety of agents that cause non-specific activation of the immune system, during or shortly before pregnancy. After the immune stimulation procedure, mice are also exposed to a teratogen. The immune-stimulated mice display reduced numbers of fetuses with birth defects, as compared to control mice that experience identical teratogen exposure, but without the immune stimulation. Immune stimulation procedures that have been used to cause reduced birth defects are diverse, and include intraperitoneal injection of attenuated bacilli or inert particles (pyran copolymer); intravascular, intrauterine or intraperitoneal injection of cytokines (i.e. interferon-γ or granulocyte macrophage-colony stimulating factor); footpad injection with Freund’s complete adjuvant; and intrauterine or intravascular injection with splenocytes collected from rats. These immune stimulation procedures reduced several different birth defects caused by a variety of teratogens that included chemical agents, hyperthermia, x-rays, or metabolic disturbances (see Table 1).

The immune stimulation procedures in mice all cause increased production and release of cytokines. Some of these cytokines, including GM-CSF and transforming growth factor (TGF)-β cross the placenta where they may affect cellular proliferation, differentiation, or apoptosis to reduce birth defects (2). Presumably, these cytokines would cause these actions in the fetus by altering gene expression in target tissues of the teratogens. In this regard, immune stimulation in ethyl carbamate-exposed pregnant mice reduced fetal incidence of cleft palate and reversed affects of the teratogen on fetal palate genes that control cell cycle and cell death (3).

For unknown reasons, non-specific stimulation of the immune system in pregnant mice has a broad spectrum of efficacy for reducing birth defects. Immune stimulation procedures that are effective include footpad injection with Freund’s complete adjuvant, intraperitoneal injection with inert particles (pyran) or attenuated bacillus Calmette-Guérin (BCG), intravascular, intrauterine, or intraperitoneal injection with the cytokines GM-CSF or IFN-γ, or intrauterine or intravascular injection with rat splenocytes. Birth defects that have been reduced include cleft palate, neural tube defects, digit defects, tail defects and craniofacial de-
Effects. Inducing factors for these defects include chemical teratogens, x-rays, hyperthermia, and diabetes mellitus.

Preclinical Relevance

Protection against birth defects as a result of maternal immune stimulation is a recently demonstrated phenomenon. Such protection has been demonstrated—and, for that matter, investigated—only in the mouse. The possibility that similar effects may occur in non-mouse rodent species, non-rodent species, or humans, remains uninvestigated.

Relevance to Humans

Women who work with certain chemicals during pregnancy are significantly more likely to deliver children with congenital malformations. Pesticide exposure in pregnant women working in agriculture-related occupations has been associated with orofacial clefts. More than 100 case reports link human birth defects with maternal exposure to toluene or trichloroethylene during pregnancy (4). Both hyperthermia and the antiepileptic drug valproic acid also increase risk of neural tube defects in humans. The incidence of malformed newborns in women with insulin-dependent diabetes mellitus is 6%–10%, approximately five times higher than among non-diabetic women (5). Relatively minor manipulations of maternal dietary conditions (supplementation with vitamins, retinoic acid, or nicotinamide) can reduce spontaneous or induced malformations in experimental animals. More recently, it has been demonstrated that folic acid supplementation during the periconception period reduces neural tube defects in both rodents and humans. The mouse has generally been a reliable predictor of immune responses in humans. Rodent data showing highly significant reduction in birth defects as a result of immune stimulation suggest the possibility of an immune-mediated beneficial effect on development in humans.

**Table 1** Immune protection against teratogenesis

<table>
<thead>
<tr>
<th>Immune stimulant</th>
<th>Birth defect</th>
<th>Litter affected (%)</th>
<th>Teratogen</th>
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<tr>
<td></td>
<td>with stimu-</td>
<td>without stimula-</td>
<td></td>
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<tr>
<td></td>
<td>lation</td>
<td>tion</td>
<td>TCDD</td>
</tr>
<tr>
<td>Pyran</td>
<td>cleft palate</td>
<td>86</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>cleft palate + digit defects</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>cleft palate + digit defects</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>tail defects</td>
<td>55</td>
<td>28</td>
</tr>
<tr>
<td>Splenocytes (rat)</td>
<td>craniofacial + limb defects exencephaly</td>
<td>81</td>
<td>49</td>
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<tr>
<td>Granulocyte macrophage-colony stimulating factor (GM-CSF)</td>
<td>craniofacial + limb defects neural tube defects</td>
<td>78</td>
<td>50</td>
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<tr>
<td>Interferon-γ</td>
<td>cleft palate neural tube defects</td>
<td>70</td>
<td>48</td>
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<td></td>
<td>51</td>
<td>14</td>
<td>ethyl carbamate diabetes mellitus</td>
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<tr>
<td>Freund’s adjuvant</td>
<td>cleft palate neural tube defects</td>
<td>70</td>
<td>26</td>
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<tr>
<td></td>
<td>51</td>
<td>23</td>
<td>ethyl carbamate diabetes mellitus valproic acid</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BCG (bacillus Calmette-Guérin)</td>
<td>digit defects</td>
<td>19</td>
<td>0</td>
</tr>
</tbody>
</table>

TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin.
All data shown represent significant decreases in birth defects, \( P \geq 0.05 \).
Modified from Holladay et al. (1).


**Regulatory Environment**

At present no guidelines exist regulating maternal immune stimulation procedures that have been used in mice to reduce birth defects. However, it must be considered that immune stimulation in pregnant women may induce or exacerbate pathologic immune responses in genetically predisposed women, including autoimmune diseases. Also, increased levels of some cytokines, such as IFN-γ, during early pregnancy may increase risk of pregnancy loss.

**References**

2. Sharova LV, Gogal RM Jr, Sharov AA, Crisman MV, Holladay SD (2002) Immune stimulation in urethane-exposed pregnant mice causes increased expression of genes for cytokines, including TGFβ and GM-CSF, that have previously been suggested as possible mediators of reduced birth defects. Internat Immunopharmacol 2:1477–1489

**Blastogenesis**

Conversion of small lymphocytes into larger cells that are capable of undergoing mitosis. 

**Blood Cell Formation**

- Bone Marrow and Hematopoiesis

**Blood Clotting**

- Blood Coagulation

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**Blood Coagulation**

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**Synonyms**

Hemostasis, blood coagulation and fibrinolysis, blood clotting.

**Definition**

Upon vascular injury, the dynamic hemostasis system engages platelets and cell-derived microparticles, the stationary vessel wall, as well as humoral and cell-associated factors of blood coagulation and fibrinolysis to ensure a proper wound-healing response under the conditions of continuous and variable blood flow. These spatiotemporally regulated reactions prevent life-threatening bleeding and initiate wound healing and tissue repair mechanisms.

**Characteristics**

**Definition of Components Involved**

As the innermost monolayer of cells, the endothelium covers all blood vessels. Disturbance of its integrity initiates platelet adhesion and aggregation and the onset of blood clotting. Endothelial cells are actively and dynamically integrated in the control and regulation of hemostasis, since they express, bind and endocytose several of the factors involved. Blood platelets are the smallest circulating cellular corpuscles (derived from megakaryocytes in the bone marrow) and serve to provisionally seal the wound in the initial phase of hemostasis. Platelets are devoid of a nucleus and are rich in different storage granules, which contain adhesive proteins, growth factors and low molecular weight agonists—indispensable for the vascular repair process. Humoral factors, including coagulation and fibrinolytic proteins/proenzymes, circulate in their inactive form, and interactions with newly exposed surfaces at the site of vascular injury lead to their activation and accumulation into temporary multicomponent enzyme complexes. These are the backbone elements of the dynamic hemostasis system.

**Initiation, Amplification, and Propagation of Blood Clotting**

Following vascular injury or endothelial cell denudation, the exposed collagenous subendothelial extracel-
lular matrix containing von Willebrand factor and other adhesive proteins serves as a homing area for adhering platelets under conditions of varying blood flow. Deficiency in von Willebrand factor or its cognate receptor GPIb complex on platelets results in impaired platelet adherence at this stage and is associated with critical bleeding tendency. Upon platelet activation and fibrinogen-mediated platelet aggregation, the negatively charged phospholipid membrane areas of platelets become exposed. These serve as new recognition sites for circulating blood clotting factors. Together with the exposure of tissue factor (constitutively expressed in deeper cell layers of the vessel wall) towards plasma components in this initial phase, the assembly of surface-bound multicomponent enzyme complexes leads to initiation and propagation of the blood clotting cascade, culminating in the generation of initial amounts of thrombin.

At this stage, thrombin further amplifies the hemostasis system by enhancing platelet activation/aggregation, and by elevating further thrombin formation through activation of protein cofactors V and VIII, as well as by inducing activation of factor XI. These amplification reactions eventually lead to the generation of sufficient thrombin to induce fibrin formation in association with the temporary platelet plug.

Finally, stabilization of the fibrin clot by covalent cross-linking mediated by thrombin-activated factor XIII (transglutaminase) protects the wound site against unwanted bleeding, invasion of microbes or inflammatory reactions. During wound closure, previously secreted platelet components, such as growth factors and cytokines, promote proliferation and migration of vessel wall cells necessary for proper wound repair to regain a patent vessel wall.

**Intrinsic Control of Blood Clotting**

At the onset of blood clotting, both circulating and cell-associated tissue factor pathway inhibitor (TFPI) provide a stoichiometric threshold for tissue factor-dependent reactions. This is because TFPI reacts with both factors VIIa and Xa in order to prevent the initiation of blood clotting.

Protein cofactors (including tissue factor, factor V, factor VIII, and thrombomodulin) on different levels of the coagulation cascade are essential for triggering the enzymatic efficiency of each multicomponent enzyme complex in a spatiotemporal manner (Table 1). Specifically, diffusible thrombin loses its procoagulant activity by binding to the endothelial cell receptor thrombomodulin, and together with receptor-bound protein C this proenzyme becomes efficiently activated. Subsequently, activated protein C (APC) together with its cofactor protein S inactivates the procoagulant cofactors Va and VIIIa, thereby blocking further thrombin generation. In parallel, diffusible thrombin, and other serine proteases of the clotting cascade, are complexed and inactivated by circulating serine protease inhibitors (such as antithrombin) which thereby serves a low but progressive inhibitory control to prevent systemic thrombin action.

**Fibrinolysis: Initiation, Amplification and Control**

After the major events of wound sealing have occurred, the produced thrombus has to be removed by plasmin degradation in a controlled manner in order to regain the appropriate blood flow conditions of the patent vessel and to complete tissue regeneration. As soon as a fibrin clot surface is established, circulating fibrinolytic factors with affinity for fibrin, such as tissue plasminogen activator (t-PA), and plasminogen bind to the fibrin clot, and plasmin generation is induced. In order to ensure stabilization of the fibrin clot
and to prevent too early an onset of fibrinolysis, thrombin in complex with thrombomodulin activates a circulating procarboxypeptidase B known as TAFI (thrombin-activated fibrinolysis inhibitor). This regulates t-PA and plasminogen binding to the fibrin clot. Since fibrin itself serves as a promoting cofactor for t-PA-mediated plasmin formation, its subsequent degradation serves to limit fibrinolysis. Furthermore t-PA, in addition to plasmin, is controlled by serine protease inhibitors PAI-1 (plasminogen activator inhibitor-1) and $\alpha_2$-antiplasmin in order to prevent bleeding.

**Preclinical Relevance**

**Hemostasis and Cell Functions**

In addition to their "classical" functions, most of the cofactor proteins and enzymes of the hemostasis system exhibit activities that are related to cell proliferation, migration or differentiation. These are all mediated by unrelated receptors on a variety of cells in the body. For example, thrombin constitutes a potent mitogen for vascular smooth muscle cells and has been implicated in the pathogenesis of atherosclerosis. Although the entire functional repertoire of hemostatic factors in this regard has not been uncovered yet, essential functional links are apparent between hemostasis and angiogenesis, inflammation, vessel degeneration, tumor progression or neurological processes.

**Mouse Models and Hemostasis**

The genetic manipulation of mice resulting either in the overexpression of a particular gene for a hemostatic protein or its complete or partial knock-out lead to a variety of important insights into the biology of hemostatic factors and their receptors during embryonic development or during the challenge with pathologies in the adult phase. Here, almost any knock-out of a clotting factor or its respective receptor resulted in an embryonically lethal phenotype or the death of the affected mice perinatally. Based on these discoveries on the role of hemostasis "beyond" blood clotting, new therapeutic regimen for various vascular pathologies may become available in the future.

**Relevance to Humans**

Based on our understanding of the activation, amplification, progression and control of blood coagulation and fibrinolysis in vivo (also from knock-out and transgene animal experiments), the contribution of this system under pathological conditions for the risk of thrombotic, as well as bleeding complications and therapeutic consequences thereof, is obvious. Both acquired and hereditary deficiencies of blood clotting and fibrinolytic factors predispose the affected patients. Moreover, the diagnostic evaluation of hemostasis parameters that fall outside the normal physiological range are indicators and prognostic markers of disease conditions. These include the following factors:

- deficiency in vitamin K: reduction of active vitamin K-dependent clotting factors
- prothrombin F1/F2 fragment: increased production of thrombin
- fibrinopeptides A, B: increased production of fibrin
- fibrin degradation products: increased thrombus formation and dissolution
- fibrin D-dimer products: increased thrombus formation and dissolution
- plasmin/$\alpha_2$-antiplasmin complex: increased thrombus formation/fibrinolysis
- increased lipoprotein(a): less efficient fibrin-dependent thrombolysis
- prolonged clotting times of in vitro global clotting tests: deficiency or dysfunction of the blood coagulation cascade.

The following acquired or hereditary deficiencies will lead to or predispose for a significant disturbance of the blood coagulation and fibrinolysis systems in patients.

**Blood Coagulation. Table 1 Multicomponent enzyme complexes in hemostasis**

<table>
<thead>
<tr>
<th>Function (complex)</th>
<th>Enzyme</th>
<th>Substrate</th>
<th>Cofactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor IXa generation (intrinsic)</td>
<td>XIa</td>
<td>IX</td>
<td>Kininogen</td>
</tr>
<tr>
<td>Factor IXa generation (extrinsic)</td>
<td>VIIa</td>
<td>IX</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>Factor Xa generation (intrinsic tenase)</td>
<td>IXa</td>
<td>X</td>
<td>VIIa</td>
</tr>
<tr>
<td>Factor Xa generation (extrinsic tenase)</td>
<td>VIIa</td>
<td>X</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>Thrombin generation (prothrombinase)</td>
<td>Xa</td>
<td>Prothrombin</td>
<td>Va</td>
</tr>
<tr>
<td>Protein C activation</td>
<td>Thrombin</td>
<td>Protein C</td>
<td>Thrombomodulin</td>
</tr>
<tr>
<td>Factor Va/VIIla inactivation</td>
<td>Protein Ca</td>
<td>Va/VIIla</td>
<td>Protein S</td>
</tr>
<tr>
<td>Plasmin generation</td>
<td>t-PA</td>
<td>Plasminogen</td>
<td>Fibrin</td>
</tr>
</tbody>
</table>
Defects in γ-Carboxylation, Defects in Biosynthesis, Isolated Deficiencies of Clotting Factor
Examples are functional deficiencies in vitamin K-dependent clotting factors, deficiency in protein cofactor VIII (haemophilia A) or factor IX (haemophilia B) which are associated with bleeding complications. Other defects include mutations in the prothrombin gene (thrombotic complications) or deficiency in plasminogen or t-PA (hypofibrinolysis), and mutations in fibrinogen (mostly asymptomatic but some associated with impaired wound healing).

Deficiency of Hemostasis Inhibitors
While antithrombin deficiency is associated with an impaired control of thrombin, α2-antiplasmin deficiency results in hyperfibrinolysis and bleeding complications. Increased PAI-1 levels are associated with an increased prothrombotic tendency, as well as a poor prognosis for atherothrombotic complications.

Gene Defects or Deficiencies of Protein C, Protein S, or Factor V
These defects are associated with an impaired intrinsic control of thrombin formation, whereby the Leiden mutation in factor V (known as APC resistance) is associated with the highest prevalence of thromboembolic complications in affected patients.

Therapeutic Interventions
Different therapeutic interventions exist in order to interfere with or prevent unwanted thrombotic or bleeding complications in patients. Platelet aggregation—and to a certain extent platelet activation—is inhibited by antagonists of the glycoprotein IIb/IIIa integrin, inhibitors of ADP-receptors or by aspirin (an inhibitor of cyclooxygenase). Bleeding complications due to impaired platelet reactivity/function or deficiency/dysfunction of von Willebrand factor may be corrected by substitution therapy. Similarly, deficiency in factor VIII or other coagulation factors can be corrected by supplementing the respective (recombinant) factor. A decrease in thrombin formation and activity can be induced by oral vitamin K antagonists (such as warfarin), by heparin, or by substitution with natural inhibitors of the clotting system (such as TFPI, antithrombin or inactivated factor VIIa) as well as with hirudin (a natural anticoagulant from leech). Acute thrombolysis therapy with natural plasminogen activators (t-PA, urokinase) or streptokinase results in elevated (systemic) plasmin formation. In cases of hyperfibrinolysis, low molecular weight inhibitors that interfere with fibrin binding of t-PA and plasminogen can be applied.

Regulatory Environment
The potential toxicity in patients with hereditary or acquired disorders of hemostasis is brought about by the appearance of, for example, alloantibodies against mutated hemostatic factors or adhesion molecules, via drug-induced pathologies or interference with the inflammatory or immune systems. Conversely, in severe septic shock syndrome, bacterial infection followed by multiple cell activation and a massive consumption of hemostatic factors may lead to life-threatening situations.

References

Blood Coagulation and Fibrinolysis

Blood Group System

A blood group is an inherited character of the surface of the red cell detected by a specific alloantibody. A blood group system consists of one or more blood group antigens encoded by a single gene or cluster of closely linked homologous genes. 

ABO Blood Group System

Blotting

The transfer of protein, RNA, or DNA molecules from an acrylamide or agarose gel to a membrane (usually nylon or nitrocellulose) by capillarity or an electric field. Immobilized molecules can be detected by hybridization to a sequence-specific probe (DNA and RNA), or antibody labeling (protein).

Southern and Northern Blotting

Blotting Membrane

The blotting membrane, usually consisting of nitrocellulose, polyvinylidene difluoride (PVDF), or nylon, is a membrane support for the electrophoretic transfer of proteins out of polyacrylamide gels.
Bone Marrow and Hematopoiesis

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Synonyms
haemopoiesis, hemopoiesis, blood cell formation

Definition
Hematopoiesis is the process of new blood cell formation. It is a continuous process, comprising the regeneration of all different blood cell lineages from a limited number of hematopoietic stem cells (HSC) and hematopoietic progenitor cells (HPC), and is capable of a fine-tuned adaptation to need.

Characteristics
Hematopoietic cells, as harvested from the bone marrow, include cells which belong to a continuum of different stages of a maturation hierarchy, starting from very primitive and undifferentiated, to fully mature and terminally differentiated cells (Table 1). The most primitive hematopoietic cells, stem cells, are able to self-renew; that is, to undergo cell division resulting in at least one daughter cell which maintains the stem cell status. Primitive cells which are not capable of maintaining undifferentiated status, but still have the potential to undergo extensive (though finite) proliferation, are generally termed progenitor cells (Table 1). Towards increased differentiation status, the commitment to a particular cell lineage takes place.

Hematopoietic Growth Factors
Hematopoietic growth factors (HGF) are glycosylated polypeptides of a molecular weight between approximately 22 kD and 60 kD. They regulate the growth and differentiation of the various individual hematopoietic lineages. For example, granulocyte-macrophage colony stimulating factor (GM-CSF) stimulates growth and development of granulocytes and macrophages from precursor bone marrow cells in semisolid culture medium. Similarly, G-CSF stimulates the growth of granulocytic colonies, M-CSF those of macrophages, and multi-CSF of colonies containing multiple myeloid cell lineages (granulocytes, macrophages, erythrocytes and megakaryocytes). Erythropoietin (EPO) and thrombopoietin (TPO) were discovered by their ability to support erythroidic or megakaryocytic development, respectively (1).

HGF are responsible for a regulated and adaptive response to need within the hematopoietic system. Principally, in this system, HGF-induced proliferation and differentiation of progenitor and immature hematopoietic cells are coupled, but HGFs can serve to increase the number of cell doublings and thus the number of mature cells produced from a precursor cell, thus providing a mechanism of fine-tuned regulation of mature cell production in the bone marrow. The commitment of undifferentiated progenitor cells to a single cell lineage is also ascribed to the effect of HGFs. It is irreversible and confines the further development of this cell. HGF withdrawal, on the other hand, results in apoptosis of progenitor cells which express the cognate receptor for a given HGF in a certain differentiation state. Apoptosis is continuously taking place to a certain degree in steady-state hematopoiesis, and inadvertent programmed cell death due to HGF withdrawal provides a negative regulating tool to demand-adapted blood cell maturation.

Bone Marrow and Hematopoiesis. Table 1 Characteristics of immature and mature hematopoietic cell populations

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Self-renewal</th>
<th>Characteristic morphology</th>
<th>Numbers/frequency</th>
<th>Lineage commitment</th>
<th>Proliferation potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem cell</td>
<td>Yes</td>
<td>No</td>
<td>Very few (&lt; 1 in 10 000)</td>
<td>No</td>
<td>&gt; Life-long</td>
</tr>
<tr>
<td>Progenitor cell</td>
<td>No</td>
<td>No</td>
<td>Few (about 1 in 1000)</td>
<td>1–6</td>
<td>Extensive</td>
</tr>
<tr>
<td>Immature cell</td>
<td>No</td>
<td>Yes</td>
<td>Majority of bone marrow</td>
<td>1</td>
<td>Limited</td>
</tr>
<tr>
<td>Mature cell</td>
<td>No</td>
<td>Yes</td>
<td>Frequent</td>
<td>1</td>
<td>None</td>
</tr>
</tbody>
</table>
In addition to the HGFs which stimulate selective cell lineage cell development, additional cytokines such as the interleukin IL-1\(\beta\) or IL-6, stem cell factor/c-kit ligand (SCF) or FLT3 ligand (FL) were identified as synergistic molecules, which on their own cannot stimulate hematopoietic colony growth, but which strongly support the growth and development of progenitor cells initiated by CSFs (2). This is achieved both by shortening cell cycle times and by amplifying the numbers of cell divisions between an immature precursor and a finite differentiation stage. In particular when multiple factors are present, HGFs also regulate the survival of very primitive cells/stem cells.

**Stromal Cell Regulation of Hematopoiesis**

Important survival and differentiation-inducing but also cell adhesion signals for developing hematopoietic cells are provided by the hematopoietic microenvironment. It consists mainly of stromal cells and deposited extra-cellular matrix. The main known stromal cell types are bone marrow fibroblasts, adipocytes, osteoblasts, endothelial cells, and macrophages. These have been extensively characterized after the establishment of long-term bone marrow cultures (LTBMC) which allow the maintenance of HSC and a continuous in vitro hematopoiesis over a period of up to 8 weeks (3). Fibroblasts provide a mesh within the bone marrow cavity, and together with endothelial cells give hold to islands of developing hematopoietic cells which are located in islands. Macrophages play important roles in providing iron for erythropoietic cells in the process of hemoglobinization, and likely also nourish other maturing cell types ("nurse cells"). Adipocytes are the sign of well-proliferating long-term bone marrow cultures, and osteoblasts have been ascribed a role in the maintenance of quiescent HSC. Stromal cells provide the separation of areas of very primitive hematopoietic cells ("cobblestone areas") in LTBMC, and thus divide primitive cell development from islands of maturing cells (Figure 1).

Megakaryopoiesis is associated with sinusoidal endothelium, and release of platelets into the circulation can be seen to occur through egress via sinusoidal lining endothelial cells by electron microscopic preparation of bone marrow. A variety of extracellular matrix substances are produced by stromal cells, which can influence survival and development of hematopoietic cells in conjunction with HGFs; these included fibronectin, laminin, and collagen IV. Soluble HGFs have been shown to be bound to stromal cells by specific proteoglycans, such as GM-CSF and G-CSF, or are expressed as membrane-integral proteins in stromal cells (SCF). LTBMC have allowed detailed studies of the role of stromal components in hematopoiesis.

**Ontogenetic Development and Sites of Hematopoiesis**

Embryonal hematopoiesis arises from a small group of cells which emerge from the dorsal aorta in the aorto-gonado-mesonephros region. The earliest cells with hematopoietic capacity are termed hemangioblasts, and the endothelial cell differentiation potential remains associated with HSC during later stages of ontogenetic development. Following this, HSC are found in the yolk sac, and during the fetal period HSC immigrate into the liver. Before the fetal liver stage, a so-called primitive hematopoiesis prevails (as in mammals with nucleated erythrocytes and a macrophage-like population of leukocytes), whereas after this stage, in most higher organisms, definitive hematopoiesis develops and already bears the features of multilineage differentiation from HSC into lymphoid and myeloid precursors cells. During birth, the cord blood contains a substantial number of HSC in man, and therefore cord blood has been established as a transplant source which is especially well suited for children. In humans, adult hematopoiesis finds its place within the bone cavities, whereas in mice due to the relative restriction of caval bone, hematopoiesis often expands also to the spleen. In states of bone marrow fibrosis, hematopoiesis re-locates to the liver and spleen also in humans.

**Preclinical Relevance**

Toxicity to human hematopoietic and hematopoietic-supportive cells can be assayed using several in vitro test systems. Colony-forming unit (CFU) assays for progenitor cells in semisolid medium give data on the direct effects of hematotoxic compounds on hema-
topoietic progenitor cells. They can be performed using murine or human progenitors. CD34 antigen-positive cells from human cord blood plated at 5000 cells per ml will give rise to approximately 100 hematopoietic colonies; dependent on the choice of added HGF, both granulocyte-macrophage and erythrocytic colonies are developing. Enriched progenitor cell populations are preferred over unselected cell populations, since especially mature macrophages display a source of metabolic activity towards many organic compounds. Stromal cells can be grown as cell lines which are of fibroblastic morphology, or as underlayers of LTBMC which then will include different stromal cell types. The stroma can be irradiated with 30 Gy to eliminate endogenous hematopoiesis, treated with immunotoxins, and then overlaid with HSC to assess toxic effects to the stromal cell compartment. In addition, of course, entire cultures can be treated to assess damage to the system in its entire complexity. The hematotoxic damage exerted by busulfan or cyclophosphamide is detected using exposure of LTBMC stromal layers to the substances. Interestingly, a concomitant depletion of the colony-forming stromal cell precursor cells (CFU-F) is detected. In vivo models have been established for the detection of altered hematopoietic cell turnover by test compounds. The mouse bone marrow micronucleus test serves as a relatively sensitive and easy-to-handle test for detecting alterations in bone marrow cell turnover, and possible genotoxic damage to immature cells. Readout is confined to erythrocytic cells (reticulated and young polychromatic erythrocytes). Mouse models have also been validated to detect long-term hematopoietic stromal cell damage as observed after bone marrow irradiation. In the bone marrow of rats, stromal cells deteriorate and decrease in numbers about 3 months after bone marrow transplantation, indicating that stromal damage follows different kinetics as HSC damage, most likely resulting from a much slower turnover of stromal cells.

**Relevance to Humans**

Human HSC have been transplanted as a curative treatment for patients with a variety of hematologic malignancies for more than 20 years, since the possibility to test for histoincompatibility by anti-HLA antibodies, and the development of improved immunosuppressive medication (4). These patients have been observed closely, and found to have a number of specific alterations in their hematopoietic systems, most likely relating to the toxic effects of their intensive chemotherapy and/or irradiation. In the patient's bone marrow, the numbers of stromal cells and also stromal precursor cells are substantially reduced. Also, numbers of progenitor cells are reduced concomitantly, and the proportion of progenitors which are in cell cycle is highly elevated. Still, numbers of circulating blood cells are normal, as is the adaptive response of hematopoiesis, for example with increased production of neutrophils during states of infection. Also, development of leukemia as a consequence is increased during the first 5 years after transplantation, yet spontaneous rates of leukemia development return to normal levels thereafter in the transplanted patients. Therefore, it is not very likely that changes in numbers or behavior of human hematopoietic progenitor cells will reflect or predict bone marrow insufficiency states or malignant development from HSC. Changes in bone marrow CFC numbers and cellularity have been reported from workers heavily exposed to hematotoxic compounds, which parallel findings with the same compounds in animal or in vitro tests (5).

**Regulatory Environment**

So far, except for the bone marrow micronucleus test, standardized test systems have not been included in the routine investigation of potential HSC toxic compounds. However, the Declaration of Helsinki and national legislation for animal experimentation must be respected.

**References**


**Bootstrap**

A resampling technique in which multiple random samples (with replacement) are obtained from the empirical data and a test statistic is calculated on each new sample. The distribution of the test statistic is thought to reflect the characteristics of the underlying population from which the original sample was drawn.

➤ Statistics in Immunotoxicology
BPDE

- Polycyclic Aromatic Hydrocarbons (PAHs) and the Immune System

BP-7,8-diol

- Polycyclic Aromatic Hydrocarbons (PAHs) and the Immune System

BPQ

- Polycyclic Aromatic Hydrocarbons (PAHs) and the Immune System

Bronchitis

- Respiratory Infections
- Trace Metals and the Immune System

Bronchus-Associated Lymphoid Tissue

Bronchus-associated lymphoid tissue (BALT) refers to secondary lymphoid tissue in the respiratory tract.
- Mucosa-Associated Lymphoid Tissue

Buehler Test

- Guinea Pig Assays for Sensitization Testing

Buffy Coat

The thin, white, leukocyte-rich band that separates the separated serum from the mass of erythrocytes in a centrifuged whole blood sample.
- Lymphocytes

Burkitt’s Lymphoma

- Lymphoma