



Current view

Complement and immune defense: From innate immunity to human diseases

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ABSTRACT

The human organism is constantly exposed to microbes and infectious agents and consequently has developed a complex and highly efficient immune defense which is aimed to recognize and eliminate such infectious agents. The response of the human host to infectious agents forms a double edged sword of immunity. The immune system has to keep a tight balance between attack on foreign surfaces and protection of host surfaces. In its proper function the immune response is aimed to recognize, attack and eliminate invading infectious agents and this response is beneficial for the host. However when the activated immune response like the complement system is not properly controlled and deregulated, effector compounds can attack and damage self-surfaces and this results in disease. In addition pathogens which cause infections and disease protect themselves from the damaging and harmful host immune weapon and use specific immune escape strategies. The complement system forms the first defense line of innate immunity and aids in the elimination of microbes and modified self-cells. Defective regulation of this cascade type system results in infections and in pathology. This can result in diseases, like severe renal diseases hemolytic uremic syndrome (HUS) and dense deposit disease (DDD), in age related macular degeneration a common form of blindness and also in other forms of autoimmune diseases.

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1. Introduction

The human organism is constantly exposed to microbes and infectious agents and consequently has developed a complex and highly efficient immune defense which provides immunity and which is aimed to recognize and eliminate such infectious agents. In order to destroy a foreign invading microbe, the host generates immune effector compounds and delivers these toxic and cytotoxic agents specifically to the surface of the invader. A proper immune defense must guarantee that such toxic immune effector products are precisely targeted to the surface of the invader. At the same time the action of these effector compounds must be inhibited at the surface of self-cells and in particular at the surface of bystander cells located at the site of infection and immune activation. Thus proper immune function requires differentiation between foreign and self-surfaces, recognition of invaders as foreign, and at the same time the guarantee, that self-cells and tissues are not attacked, i.e. are protected from the deleterious effects of the activated immune system. Therefore host cells use regulators to inhibit both the generation and the action of toxic effector products at their surface.

Innate immunity is rather efficient and a large number of infectious microbes are recognized, attacked, killed and eliminated already at this first level of immediate immune response [1]. Pathogens, in contrast to microbes have developed specific means to block and avoid host immune recognition and attack [2]. Apparently every human pathogen uses several and multiple escape mechanisms to cross the multiple layers of host immunity and to inhibit the action of the various immune effector compounds [3,4].

Infectious microbes represent a diverse source of species and include Gram negative and Gram positive bacteria, eukaryotic fungi, multicellular parasites, helminthes, as well as viruses. Each pathogenic microbe has developed multiple and rather efficient means to inactivate the various layers of host defense and to cross innate as well as adaptive immune defense. Despite their diverse origin and nature any human pathogen is challenged by the same human immune defense and always faces the same type of innate immune response in form of recognition and attack. The understanding of such innate immune escape has substantially increased during the last years and escape strategies of a large number of diverse pathogens are now understood in molecular terms. This situation allows to define common and general features of immune and in particular complement escape which are actually shared by numerous pathogens.

The activated complement system generates toxic activation products. And the proper delivery of these compounds is a central aspect for its action. The damaging agents must be targeted to the surface of the invader and at the same time host surfaces

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must be protected. If this system is not properly controlled and out of balance misdelivery of toxic effector compounds to self-surfaces occurs and such reactions can lead to autoimmune disease. A defective host immune response results in attack on host surfaces and leads ultimately to autoimmune diseases [5]. Such defects can be caused by (i) inappropriate immune activation, i.e. activation at the wrong time, (ii) inappropriate delivery of immune effector compounds to the wrong surface, in particular the surface of self-cells or biomembranes, (iii) defective recognition of self or foreign surfaces and also (v) mutated and/or defective regulators. Such a damaging effect on the surface of self-cells or biomembranes can result in autoimmune disease. Numerous examples exist by which, e.g. a deregulated complement activation results in pathology and in disease. Such examples include the severe renal diseases hemolytic uremic syndrome (HUS) and dense deposit disease (DDD). Work on these rare diseases has formed an important basis for understanding the pathomechanisms of additional related disorders, like the retinal disease age related macular degeneration (AMD) which is a common form of blindness in the western world. The rare kidney disorders and also the frequent retinal disease show highly related pathophysiological principles, and are caused by defective local complement regulation.

Thus the response of the human host to infectious agents forms a double edged sword of immunity. The immune system has to keep a tight balance between attack to foreign surfaces and protection of host surfaces. In its proper function the immune response is aimed to recognize attack and eliminate invading infectious agents and this response is beneficial for the host. However when this action is not properly controlled the same effector compounds can attack and damage self-surfaces and biomembranes. Thus defective regulation of the activated complement cascade can cause disease. In addition pathogens which cause infections and disease protect themselves from the damaging and harmful host immune weapon and use specific immune escape strategies.

2. The host site: host immunity and complement as important immune barriers

The host immune response is formed by three central layers, the innate immune system with two major immediately acting effector parts: the complement system and the cellular system, which is composed of macrophages and neutrophils. In addition the adaptive immune system is formed by antigen specific T- and B-lymphocytes.

2.1. Host immunity: a layered defense system

Host immune response represents a multilayered system that, on the first contact with an infectious agent, induces a set of sequential reactions, which are separated in time. The innate immune system is formed by the immediately acting complement system and is followed by the directly acting cellular response mediated by immune effector cells such as macrophages and neutrophils [6]. This response is followed by the antigen specific adaptive immune response, by T- and B-lymphocytes that utilize antigen specific T-cell receptors and immunoglobulins or antibodies. Upon the first contact with an infectious agent the innate immune system acts within seconds and the adaptive antigen specific response requires several days to develop (Fig. 1).

2.2. Complement the central barrier of innate immunity

Complement represents as first defense line of innate immunity [7,8]. This cascade type system forms an immediately acting barrier for invading microbes, and does also coordinate innate as well as adaptive immune functions [3,4,9]. As the first barrier of the innate immune response, new activation products are gener-

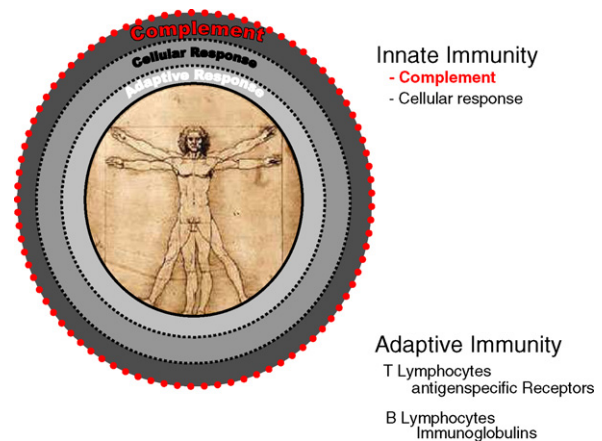


Fig. 1. Multiple layers of human immune response. The human immune defense is a multi layered system which is upon the first contact with a infectious agent represents the immediately and directly acting innate immune response and the antigen specific adaptive immune response. Complement as the first part of innate immunity response is immediately activated within seconds and the cellular part of innate immunity which is mediated by infiltrating macrophages and neutrophils is directly activated and requires minutes to hours. The adaptive response which is mediated by antigen specific T- and B-cell lymphocytes and takes several days to develop.

ated which bind to the surface of infectious agents, such as Gram negative and Gram positive bacteria, fungi, multicellular parasites and helminthes, as well as viruses. This binding of effector components ultimately results in the damage of the invading microbe and in its removal. Therefore complement contributes significantly to the healthy status of a host organism. Based on its powerful and efficient effects the activated complement system actually eliminates the vast majority of the foreign invaders already at this early stage/state of infection.

2.2.1. History

The term complement was first used by Paul Ehrlich (1854–1915) when he described a heat labile system which *complements* the action of immunotoxic serum [10]. Particles which are marked with complement activation products, which are opsonized with C3d are cleared by phagocytosis. The process of phagocytosis was first described by Ilya Ilyich Mechnikov (1845–1916) for the cellular uptake and removal of foreign particles. Mechnikov coined the term Makrophage, for cells which take up and degrade foreign material and particles. He was also the first to show that phagocytosis is relevant for immune function [11]. Complement was initially identified as a blood component which mediates the activity of antibodies or immunoglobulins (which Ehrlich termed 'Seitenketten'; 'side chains'). This assisting activity can now be defined on a molecular level. The complement proteins C1q, C1s and C1r bind to immune complexes, and antigen bound IgG/IgM proteins initiate and mediate effector function in the classical complement pathway. Binding results in a conformational change and in activation of the classical complement pathway. This step initiates the complement cascade and upon progression of the cascade activation products are generated which attack foreign surfaces and cause the elimination of a tagged particle or microbe. The complement system is also active in the absence of antigen specific immunoglobulins and this defense system can – by an intrinsic mechanism – specifically label the surface of, e.g. foreign bacteria or infectious agents. In this case activation occurs via the alternative pathway and the activation product C3b is deposited onto the foreign surfaces. Such C3b coated particles are then recognized by macrophages which express specific surface receptors for C3b or iC3b (complement receptors: CR1–CR4) and C3b deposition enhances uptake, activation and finally the clearance of the invading agent by phagocytosis.

2.3. Complement activation and control

Complement as a central defense system is immediately activated, within seconds upon entry of a microbe into the human host. This powerful defense system is composed of multiple components (>60 different proteins and activation products) that trigger in a cascade type system. Activation of this cascade generates highly reactive and powerful activation products with chemotactic, inflammatory, cytotoxic and – in some cases – also antimicrobial and antifungal activity. The effector functions of the activated complement system include [6,12]:

- (i) release of inflammatory mediators, i.e. C3a and C5a which act as anaphylatoxins,
- (ii) the anaphylatoxins C3a and C4a also display antimicrobial activity,
- (iii) opsonization of (foreign) surfaces with the activation product C3b. C3b opsonized particles are directly recognized and removed by phagocytosis,
- (iv) generation of the membrane attack complex (MAC) also referred to as terminal complement complex, which forms pores in the membrane of the target and which displays cytotoxic activity,
- (v) aiding and directing the adaptive immune response, and
- (vi) complement also coordinates the non-inflammatory, silent removal of cellular debris and modified self-cells including, apoptotic and necrotic cells and of immune complexes.

2.4. Complement activation and regulation

Complement is activated either spontaneously or is activated by antibodies. Activation of the alternative pathway is a spontaneous and continuous process which is initiated by the conformational change of C3. The activated complement system sets in motion a cascade of sequential reactions, which initiate several effector functions, and which generate and deliver immune activation products, that are aimed to destroy and remove a foreign particle or an infectious agent [13].

Complement is further activated by the lectin pathway, which is triggered by carbohydrates on the surface of bacteria [14] and recently a thrombin mediated activation pathway that bypasses the C3 activation step was described [15].

The alternative pathway forms an important immune surveillance system. The initial steps of this reaction are spontaneous and indiscriminatory, as they do not differentiate between self and foreign. Alternative pathway activation is initiated in the fluid phase and newly formed C3 components can deposit in an indiscriminatory manner onto nearby surfaces [16–18].

Once set in motion activation proceeds by default and is amplified on any surface. However these first and initial reactions progress rather slowly and allow ample time for regulation and control. Consequently at this stage self-specific regulators block further progression of the cascade. Control and inhibition start immediately in the fluid phase, but are more efficient on the surface of intact, undamaged self-cells. Self-cells are equipped with integral membrane inserted regulators and in addition use acquired inhibitors which are attached to the cell surface. Thus a combination of membrane bound and surface attached regulators inhibits the AP cascade, blocks progression of the cascade and thus blocks generation and deposition of activation products on the self-surface. The inhibition of the complement cascade at a surface of intact self-cells is important for homeostasis of the host. However – at the same time – no inhibition or control should occur on surfaces of foreign particles and infectious agents and on modified self-cells, which lack appropriate complement regulators. Consequently the complement cascade progresses and complement effector functions are

induced which ultimately result in opsonization, cause damage and are removed by phagocytosis.

Based on their potent damaging capacity the generation and targeting of complement effector compounds is tightly regulated. In a physiological setting this results in damage and elimination of infectious agents, but simultaneously, at the same time and the same site protection of host cells and surfaces. Pathogenic microbes evade complement recognition and inactivate the action of complement effector compounds. Such a defective control can enhance and amplify local complement action and can ultimately cause damage to host surface and structures.

3. The microbial site

3.1. Complement and immune escape: microbe vs pathogen

Microbes aim to infect and propagate in a human organism. Host immune response is responsible for recognition and elimination, of the infectious agent. The complement system has a rather broad specificity and the alternative complement pathway, the evolutionary oldest activation loop, is able to recognize and inactivate invading microbe. Thus in order to establish an infection and to replicate any microbe must cross this initial and central immune barrier. An infectious agent that has developed means to overcome host complement attack as well as the additional layers of host immune response and thus survives in an immunocompetent host becomes a pathogen. Generally speaking, microbes are recognized by the host immune system as foreign and are eliminated. In contrast pathogens use specific escape mechanisms to evade host immune recognition [19].

Despite their diverse source and nature any human pathogenic microbes which enter the human body face the same innate immune response and must cross and respond to these immune defense layers. During the last years the escape mechanisms of several pathogens have been identified. Several complement evasion proteins or virulence factors were cloned and are characterized on a molecular level. Thus allowing to define common features of the pathogen escape strategies [4,5,19].

3.2. Pathogens generate a complement shield

Each human pathogen, independent of the enormous diversity of special origin, faces the same host immune barrier in form of complement attack. Thus each single pathogen must find means to block the activity and the damaging complement effector compounds in order to cross this highly structured and powerful host complement barrier [4,19].

Pathogenic microbes evade host complement attack by avoiding deposition and attachment of opsonic complement products, by inactivating anaphylatoxins, by interfering antigen driven B-cell stimulation, and also by attaching host complement regulators to their surface.

The evasion mechanisms are striking in their diversity and apparently each individual pathogen uses a combination of multiple escape strategies. The full list of the various pathogen encoded proteins which bind host complement regulators is addressed in recent reviews [4,5,19] and a comprehensive or complete list of strategies used by various pathogens is beyond the scope of this overview. However a brief summary of the complement escape of the human pathogenic yeast *Candida albicans* illuminates the basic principle and sets the scenario for other pathogens.

3.3. Complement evasion of the human pathogenic yeast *C. albicans*

C. albicans is an important fungal pathogen that can live as commensal and can also cause life threatening infections and sep-

ticaemia. Infections with *C. albicans* are among the fourth most frequently acquired hospital infections [20].

C. albicans uses sophisticated complement escape mechanisms. The candida surface mimics features of host cell surfaces. Upon contact with human plasma *C. albicans* binds several host plasma proteins and uses the attached host proteins for immune disguise [21,22]. This type of immune camouflage where a foreign surface mimics the features of the host surface is rather efficient and consequently the host immune system cannot further recognize this modified surface as foreign. This form of immune camouflage allows the candida and other pathogens to escape host immune surveillance (Fig. 2).

Candida binds several host complement regulators to its surface, such as Factor H, complement Fcceptor H related protein 1 (CFHR1), FHL1 and C4BP and in addition also the human serum protease plasminogen [22]. During the last years binding and function of these surface attached host regulators was characterized. Four CRASP proteins (complement regulator acquiring surface protein) have been identified from this pathogenic yeast (CRASP1, CRASP2, CRASP3 and CRASP4) and the various proteins are being characterized on the molecular level (Fig. 2)[23]. Bound to the individual yeast CRASP proteins the human complement regulators are functionally active, they block complement activation and the generation of damaging activation products directly at the surface of the pathogen.

Several pathogens bind soluble host proteins to their surface, including Factor H, FHL1, CFHR1, C4BP and plasminogen [24,25]. At the surface the attached host complement regulators are accessible and maintain their complement regulatory functions and further aid in the interaction with host extracellular matrixes and host immune cells. As a consequence, the foreign surface is not accessible and not recognized by host immune surveillance. Consequently such a modified surface is not properly attacked, the pathogen survives and can spread within the organism.

Such immune evasion mechanisms appear common to all human pathogens that are in contact with the host body fluids and represent a rather general mechanism of immune escape. CRASP proteins with the same or highly related binding features have been identified in numerous pathogens including the Gram negative bacteria *Borrelia burgdorferi* (CRASP1, CRASP2, CRASP3/ErpP, CRASP4/ErpC and CRASP5/Erp A), *Pseudomonas aeruginosa*

(CRASP1/tuf), *Neisseria gonorrhoea* (sialylated lipooligosaccharide, LOS and Porin 1a (por)), *N. meningococci* GNA1870, for Gram positive bacteria like *S. pyogenes* (M Protein, M-like Protein, Fba and SCL1), *S. pneumoniae* (HIC, PspC), Viruses Herpes Simplex virus, West Nile Virus and multicellular organisms [26]. However simultaneously and at the same time rather common inhibitory mechanisms and functionally related proteins are identified in diverse microbes and common, shared mechanisms exist between Gram negative, Gram positive bacteria, fungi, multicellular parasites and even viruses. These surface proteins or complement evasion molecules are interesting candidates for vaccine and for drug development. The characterization of these various complement evasion proteins has developed that the Factor H binding protein of *N. gonorrhoea* GNA1870 surface proteins is a unique vaccine antigen that forms multiple interactions with host immune defense [26].

4. Defective recognition and regulation results in diseases

Understanding the temporal action, local distribution and targeting of the various complement effector functions is essential to understand the role of complement in its normal physiological setting, which is essential to maintain homeostasis and to keep the organism intact and healthy. Inappropriate timing, deregulation, misdirected delivery of complement effector products results in pathology and in diseases.

Defective complement action or misdirected targeting of the activation compounds results in numerous diseases such as ischemia reperfusion injury, Systemic Lupus Erythematosus (SLE), Anti-phospho lipid syndrome and others. Thus any effect that disturbs the delicate balance and this complex network of interacting proteins can result in autoimmune disease.

4.1. Hemolytic uremic syndrome (HUS)

Hemolytic uremic syndrome (HUS) is a severe and rare kidney disease which was first described by Gasser et al. in 1955 [27]. This disease is characterized by acute renal failure, microangiopathic anemia and thrombocytopenia [28,29]. Three different forms of HUS are currently defined [30]: (i) the typical form, the diarrhea associated form (D+ HUS) is in most cases associated with infections with shiga toxin like producing *Escherichia coli* (e.g. of the

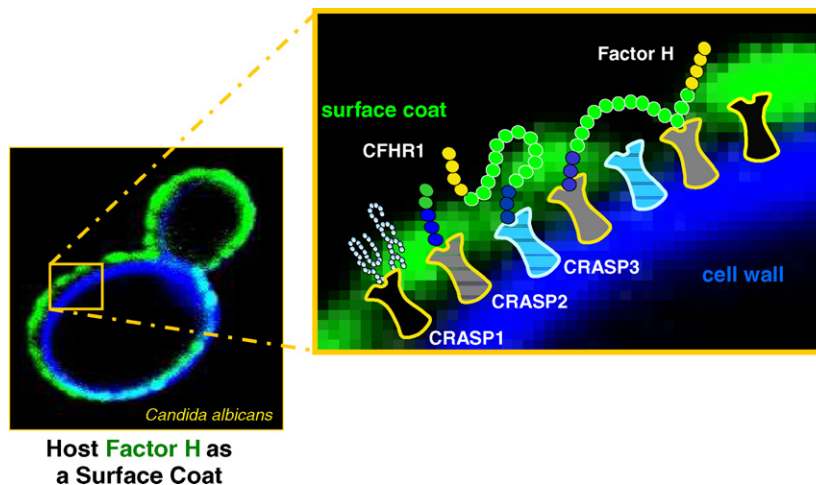


Fig. 2. Immune evasion of the human pathogenic yeast *Candida albicans*. *C. albicans* is a human pathogenic yeast that can live as commensal and also causes life threatening infectious and septicemia. Candida evades host complement attack by acquiring a surface coat which inactivates host complement attack. Candida binds soluble host proteins to its surface. The left image shows the surface coat of *C. albicans* upon incubation in human serum. Bound complement Factor H (green color) is visualized by immunofluorescence microscopy using specific antibodies. The cell wall of the yeast is shown in blue color. Candida uses several surface proteins which due to their related function are referred to as CRASP (complement regulator acquiring surface proteins) which attach multiple host plasma proteins to the surface of the yeast. Acquired host proteins include the complement regulators Factor H, FHL1, CFHR1 and C4BP as well as plasminogen. Bound to the surface of candida the host regulators are functional active and inhibit complement attack at the surface of the pathogen.

Table 1

Common or related genetic defects translate into different diseases.

Disease	Genes affected			Autoantibodies
	Components	Regulators	Associated regulators	
HUS ^a	C3, Factor B	Factor H, MCP/CD46, Factor I	Δ CFHR3-CFHR1 ^b	Autoantibodies to Factor H ^c
DDD/MPGN ^d		Factor H		Autoantibodies to C3Nef and Factor H ^e
AMD	C3, Factor B	Factor H, C2, C4	Δ CFHR3-CFHR1 ^b	

^a In HUS most of the mutations are heterozygous.^b In HUS chromosomal deletion of Δ CFHR1-CFHR3 on human chromosome 1q32 is a risk factor and in AMD the same deletion apparently has a protective effect.^c Autoantibodies against Factor H develop on the background of chromosomal Δ CFHR1-CFHR3 deletion.^d In DDD/MPGN most Factor H mutations are homozygous or compound heterozygous.^e In DDD/MPGN Autoantibodies termed C3 nephritic factor (C3Nef) bind to a neoepitope of the C3 convertase and are frequent in this disease.

serotype O157:H7) and is frequent in young patients, (ii) the second form, the atypical HUS has a genetic cause and occurs in adults, and also in juvenile patients and is not associated with diarrhea (D–HUS). In this atypical form genetic mutations of complement genes are observed, and (iii) the third form termed DEAP-HUS (Deficient for CFHR1/CFHR3 and Autoantibody Positive) affects young patients and shows a combination of a genetic deficiency and an acquired factor. These patients have a deletion of a 84 kbp chromosomal fragment on human chromosome 1q32 which results in the absence of genes coding for the complement Factor H related proteins CFHR1 and CFHR3. Plasma of these patients is also positive for autoantibodies which bind to the C-terminus of Factor H [26].

About 60% of patients with the atypical form of HUS show genetic mutations of complement genes (Table 1)[31]. The vast majority of these mutations occur in a heterozygous set up, thus patients have typically one defective and one intact allele. The affected genes include C3 and Factor B, which form the alternative complement convertase C3bBb. In addition genes coding for regulators or effectors of this enzyme Factor H, MCP/CD46 and Factor I are mutated in aHUS. Thus aHUS forms an important example that multiple genes can be defective in one disease. However a functional comparison shows that the products of these various genes are linked to the same physiological step, i.e. the alternative complement amplification convertase C3bBb. Mutant gene products affect either the components of the C3 convertase or regulators that control the stability and determine the activity of this enzyme (Fig. 3). (Table 2).

Homozygous deletion of CFHR1 and CFHR3 genes represents an additional risk for HUS. In HUS the CFHR1/CFHR3 deficiency is frequently associated with the presence of autoantibodies [32].

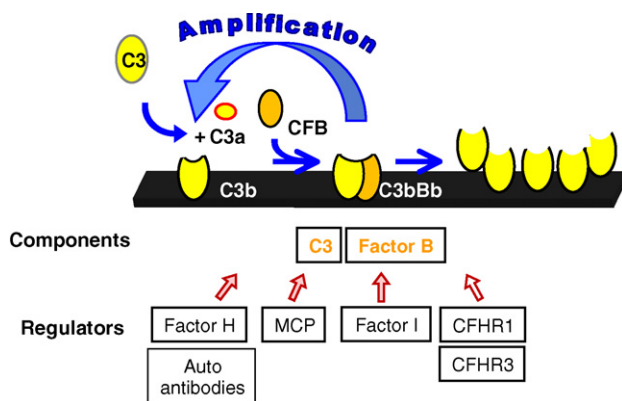


Fig. 3. The alternative complement amplification convertase C3bBb. The C3 convertase of the alternative complement pathway is central to initiate this pathway and also to amplify the activated complement system. Multiple genes were identified to be associated with HUS and AMD and are shown in Table 2. Several of the corresponding genes products encode either the central components of the convertase, i.e. C3 and Factor B, as well as regulators Factor H, Factor I, MCP/CD46 and the putative regulators CFHR1 and CFHR3.

The chromosomal deletion and the absence of the two proteins in plasma is an apparent risk factor for the development of autoantibodies. For the Jena as well as the Newcastle HUS cohorts the frequency of CFHR1/CFHR3 deletion in combination with autoantibodies is between 11 and 13%, in healthy control groups CFHR1/CFHR3 deficiency is close to 5% (4.8%). At present the analyzed cohorts are too small to define whether CFHR1/CFHR3 deficiency alone – in the absence of autoantibodies – represents a risk for HUS [33,34].

In HUS platelet dysfunction and damage of the endothelial cell lining of kidney blood vessels are primary events that results in microvascular lesions and in the formation of microthrombi that occlude the blood vessels in the kidney. Based on the associated genes, microthrombi formation and endothelial cell damage in the kidney are associated with complement deregulation and complement dysfunction in form of a deregulated C3 convertase. This shows that an efficient control and regulation of the alternative complement pathway on the surface of self-cells and biomembrane are essential.

4.2. Dense deposit disease (DDD/MPGN)

The glomerular disorder dense deposit disease (DDD, also termed membranoproliferative glomerulonephritis type II) is characterized by thickening of glomerular basement membranes (GBMs) and dense intramembranous deposits [35]. These pathological deposits are a hallmark for this type of glomerulonephritis. Glomerular dense deposits are formed in the mesangium and along the glomerular basement membrane and are positive for the complement activation product C3 but do not stain for immunoglobulins. Recent proteome analyses of glomerular dense deposits revealed the presence of complement protein and in particular proteins of the terminal complement pathway, as well as the terminal pathway regulators clusterin and vitronectin, com-

Table 2

Proteome analyses of glomerular dense deposits and of retinal drusen.

	Protein	Dense deposits (DDD/MPGN) Crabb et al. [42]	Drusen (AMD) Sethi et al. [36]
1	C3	+	+
2	C4	+	–
3	C5	+	+
4	C6	+	+
5	C7	–	+
6	C8	+	+
7	C9	+	+
8	CFHR1	+	–
9	CFHR5	+	–
10	Clusterin	+	+
11	Vitronectin	+	+

Accumulation of identical complement components, primarily of the terminal pathway in renal dense deposits derived from patients with dense deposit diseases (DDD, also termed MPGN) or in retinal drusen in patients with age related macular degeneration (AMD).

plement Factor H related protein 1 (CFHR1) and CFHR5 [36] (Table 1).

Genetic causes for DDD/MPGN include Factor H gene mutations. Most of the identified mutations affect both alleles and present as homozygous or compound heterozygous mutations [37](Table 1). The majority of the identified mutations result in defective protein secretion and in the absence of the protein in the circulation. In addition two sisters show deletion of one single amino acid residue (Δ K224) which is positioned in the complement regulatory region of Factor H within SCR4 [38]. Both the absence of Factor H in plasma or the presence of a functional defective protein results in uncontrolled progression of the spontaneously activated alternative pathway.

In DDD AP deregulation can be associated with the presence of C3 nephritic factor (C3Nef), an autoantibody that binds to a neoepitope of the newly formed C3 convertase (C3bBb). Binding of this autoantibody stabilizes the convertase, increases enzymatic turnover and enhanced C3 generation results in persistent alternative pathway activation [39]. Based on these findings it is postulated that DDD is caused by both local and systemic dysregulation of the alternative complement pathway (AP).

4.3. Age related macular degeneration (AMD)

Age related macular degeneration (AMD) is the most common cause of blindness in the elderly population of developed countries and represents a severe and growing health problem [40]. About 10 million cases occur in the US and based on the growing age of the population the incidence of the disease is increasing. Macular degeneration is characterized by the breakdown of the macular and with age the development of drusen. Drusen represent debris material that accumulates below the retinal pigment epithelial cells and the Bruch's membrane and the presence of drusen in the macula is considered a major risk factor for AMD. The mechanism of drusen formation is largely unclear [41].

Proteome analyses of drusen showed that these deposits contain numerous complement components in form of C3, C5, C6, C7, C8 and C9, and also include regulators of the terminal complement pathway such as clusterin and vitronectin [42](Table 1). The proteome of retinal drusen and glomerular dense deposits reveals that very similar proteins are contained in these deposits and suggests similar or related mechanisms of formation.

Recently a polymorphism in the Factor H gene (Y402H) was reported to strongly correlate with AMD. Additional genetic variants of Factor B, C3 and C4 and C2 are associated with the disease [43,44]. The association of these additional complement genes with AMD (Table 1) indicates that defective complement activation can cause AMD and suggests also that an imbalance or deregulation of the physiological complement function at the local site, i.e. at the interface of the retinal pigment epithelial cells and the Bruch's membrane causes pathology. Thus defective complement regulation and likely also inflammatory reactions contribute to AMD. The AMD associated variations of the Factor H protein which cause a change from a Tyrosine (Tyr) residue in the protective form to a Histidine (His) residue in the risk variant in domain 7 of the protein does affect interaction with heparin, C-reactive protein and surface binding to retinal pigment epithelial cells [45–47]. This likely affects and reduces the protective functions of Factor H at retinal surfaces particular during inflammatory responses.

In addition the homozygous deletion of CFHR1 and CFHR3 reduces the risk for AMD. The epithelial lining of the cells above the Bruch's membrane is disrupted. In particular, variants in the gene for the complement factor H (CFH) and the genes, Factor B, and complement component C3 and C2 as well as deletion of the CFH1/CFHR3 chromosomal locus have been implicated as major risk or protective factors for the development of AMD.

4.4. Common links and therapy

The last years have witnessed a remarkable development in understanding the role of defective complement regulation in three different diseases, i.e. in hemolytic uremic syndrome (HUS), the glomerular disorder dense deposit disease (DDD) and also for the retinal disease age related macular degeneration (AMD). Genetic evidence and molecular diseases mechanisms were identified which show that:

- a set of related genes is associated with each of the three disorders and genetic defects in form of non-sense and miss-sense mutations were identified in various diseases,
- mutations of the same gene manifests in different organs, like the endothelial lining of the glomerulus in the kidney or the epithelial lining of the retina in the eye. Thus common pathophysiological principles manifest in different organs,
- the concept established for the two rare disorders HUS and DDD was directly translated to the common retinal disease, AMD,
- translation of the experimental results in clinic and therapy and diagnostics. The identification and characterization of new genes and novel molecular mechanisms resulted in the definition of new sub groups of diseases. This allowed to define diagnostic parameters and to directly translate these results into appropriate and successful treatment and in addition prevent kidney damage and prevented young patients from long-term dialysis and kidney transplantation.

A common feature of these three diverse diseases is that the multiple genes are affected, which correspond either for components that form the alternative pathway C3 convertase, i.e. C3 and Factor B or regulators of this enzyme Factor H, Factor I, membrane cofactor protein (MCP/CD46) as well as chromosomal CFHR1/CFHR3 deficiency is associated with these distinct diseases (Fig. 3).

Defining the exact mechanisms of local defective complement control is thus essential to understand pathophysiology of disease and to initiate a proper therapy. The lessons learned during the last years have been directly translated into the clinic, as they improved both diagnosis and therapy of the patients. In addition antibody directed therapy with either Rituximab or Eculizumab (Soliris) a C5 recognizing humanized mAb were initiated [48,49].

5. Conclusions-outlook

Immune defense is central to keep the vertebrate organisms free from infections, to maintain cellular homeostasis and integrity. Complement as a rather ancient defense system forms the first innate immune barrier for invading microbes. In order to cross this first and central immune barrier, pathogens block and avoid recognition and subvert complement effector functions. The fact that the complement binding surface proteins of pathogens, like the GNA1870 of *N. gonorrhoeae* represent candidates for vaccine development shows that blockade of such surface proteins interferes with microbial virulence.

Several complement genes are associated with various diseases as discussed here for HUS, DDD/MPGN II and AMD. Thus diverse diseases can be caused by related pathologic scenarios. But the defects however manifest in different organs and sites, the endothelial lining or the glomerular basement membrane in the kidney and the Bruch's membrane in the retina. These features show a central role of complement in homeostasis and physiology [50]. Results obtained by basic research were directly translated into the clinic for the benefit of the patients. Diagnosis has been improved, now subforms were defined which allowed to initiate new and effective therapy. In addition the situation progression of a kidney transplant

and allowing more appropriate measures to enhance transplant survival.

These examples show that targeting of complement proteins, complement components or and inhibition of activation products is of therapeutic benefit not only for the described diseases, but most likely also for a wide range of additional clinical scenarios.

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