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Review

Characterisation of the properdin-deficient immune phenotype

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ABSTRACT

Properdin (complement factor P) is a conserved serum glycoprotein of the immune defence. It plays a role in strengthening the activation of complement, a system of proteins important in the first line defence against infection. Properdin is active in the alternative pathway of complement within the innate immune system. It is the sole regulator of complement activation and plays a major role in regulating the alternative pathway by binding to and stabilising the inherently labile C3 convertase enzymes, C3bBb and C3bBbC3b. The properdin-deficient mouse line was used in various studies in order to investigate the role of properdin in disease models of immunity, infection and inflammation. It was shown that properdin controls the strength of immune responses by affecting both humoral and cellular phenotypes during acute bacterial infection and resulting inflammation. A clear overview of the measurements for which properdin-deficient and wild-type mice are similar or different in their unstimulated state is lacking. This review shows the cumulative analysis in mouse regarding the effect of properdin-deficiency on baseline immune measurements, and will consider all studies on properdin-deficient and wild-type mice, where inflammatory cellular or humoral mediators, activities and metabolism were measured. The analysis lends support to the concept that systemic therapeutic targeting of properdin may influence properdin-dependent cellular crosstalks within tissues.

KEYWORDS: complement, properdin, properdin-deficient mouse, mouse model, targeting.

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INTRODUCTION

The complement system plays an essential role in host defense against invading foreign pathogens. It is an important part and a major component of the innate immunity and also contributes to acquired immunity [1]. Complement was identified more than 100 years ago and is a cascade of carefully regulated enzymatic reactions. It consists of a series of glycoproteins; found as inactive precursors in plasma, they are synthesised mostly in the liver. Receptors are found on cells as varied as monocytes, dendritic cells, mast cells, T-cells, endothelial cells, and adipocytes. In normal, stable-state conditions the complement system has a role in the silent clearance of apoptotic cells, of thresholding the adaptive immune response, of securing immune privilege and fertility.

Complement is activated in the fluid phase and occurs *via* three pathways, classical pathway, lectin pathway and alternative pathway. Each pathway responds to a different set of activators and each pathway leads to the formation of C3 convertases that cleave the central protein C3 to C3a and C3b. The C3 convertases are central to the activity of the cascade.

The classical pathway (C1, C4, and C2) is activated by antigen-antibody complexes and other molecular patterns such as histones and DNA. The lectin pathway (MBL, Ficolins, CL-11, MASP-1, MASP-2, C2, C4) is activated by recognition and binding of pathogen-associated molecular patterns with lectin, such as mannose, acetyl-glucosamine and others. Both pathway activations lead to the assembly of a C3 convertase. The C3 convertase enzyme of the classical and lectin pathways is C4b2b whereas C3bBb cleaves more C3b from C3 as part of alternative pathway activation. The alternative pathway (C3, factors B, D and properdin) forms an amplification loop of complement activation driven by C3 convertase, C3bBb. It is initiated by spontaneous hydrolysis of C3 with the Factor B to form $C3(H_2O)B$ complex, causing constant low-level auto-activation in the fluid phase (so-called tickover).

The activation of complement promotes inflammation by generating anaphylatoxins (C3a, C4a and C5a) and facilitates phagocytosis and lysis of certain microorganisms, especially gram negative, by membrane attack complex formation [2, 3].

Recent main advances in our understanding of complement have resulted from the use of genetically engineered mouse lines. These for instance have shown the role of complement in cellular integrity and tissue homeostasis [2]. Its discovered roles are becoming more and more varied; however, its agreed function is in host defence by recognising changed surfaces and pathogens and removing pathogens *via* direct killing, opsonisation and lysis, activation of phagocytosis, and initiation of local inflammatory reactions.

Since the generation of the properdin-deficient mouse line about ten years ago [4], a wealth of studies analysed its response to infectious and inflammatory challenges, recording baseline measurements in comparison with its congenic wild-type controls to identify the elicitation of a response. This paper will contextualise the purpose of generating a mouse line deficient of properdin, the only positive regulator of complement activation, discuss the knowledge obtained from t_0 measurements across all studies, relate this to data obtained from properdin-deficient individuals, and identify open questions.

Function of properdin

Properdin is secreted by leukocytes and activated endothelial cells, and has a designated role in the alternative complement pathway of the innate immune system. It is measurable in serum of healthy controls and patients with sepsis, for whom depressed levels at admission related to longer hospital stay [5]. Properdin was first discovered as an important component of an antibody-independent complement activation pathway in 1954 by Dr. Louis Pillemer and collaborators [6], and then in the 1970s it was shown to be a stabilising component of the alternative pathway C3 convertase. The name properdin is derived from the Latin word *perdere; pro-perdere* meaning prepare to destroy [7]. Properdin is also necessary in the amplification of the classical and mannose-binding lectin pathways [8]. Contrary to its hypothesised role as a pattern-recognition molecule in its own right, a recent study demonstrated that C3b was essential for properdin to bind to zymosan, a component of yeast cells, or *Escherichia coli* [9].

A time line of properdin-related discoveries is compiled below (Figure 1) and shows e.g. the year of description of hereditary deficiency as a risk factor in meningococcal infection (*Neisseria meningitis* W135, X, Y, Z) and the cloning and characterization of the cDNA encoding guinea-pig properdin, the year when the oligomeric structure of properdin was resolved, and lastly the targeting of mice properdin gene for in *vivo* and in *vitro* research.

Properdin binds to a surface ligand of C3b alone or to iC3b, C3bB or C3bBb complex by means of one of its thrombospondin subunits. Properdin, which is bound to C3bB and C3bBb, enables more C3b to bind because of its oligomeric structure. The C3bB is a short-lived complex which is cleaved by Factor D in the presence of Mg^{2+} at a single site in the Factor B subunit, resulting in the release of the Factor B amino-terminal fragment (Ba) and the activation of the serine protease domain. Properdin substantially extends their half-lives and protects the alternative pathway convertases

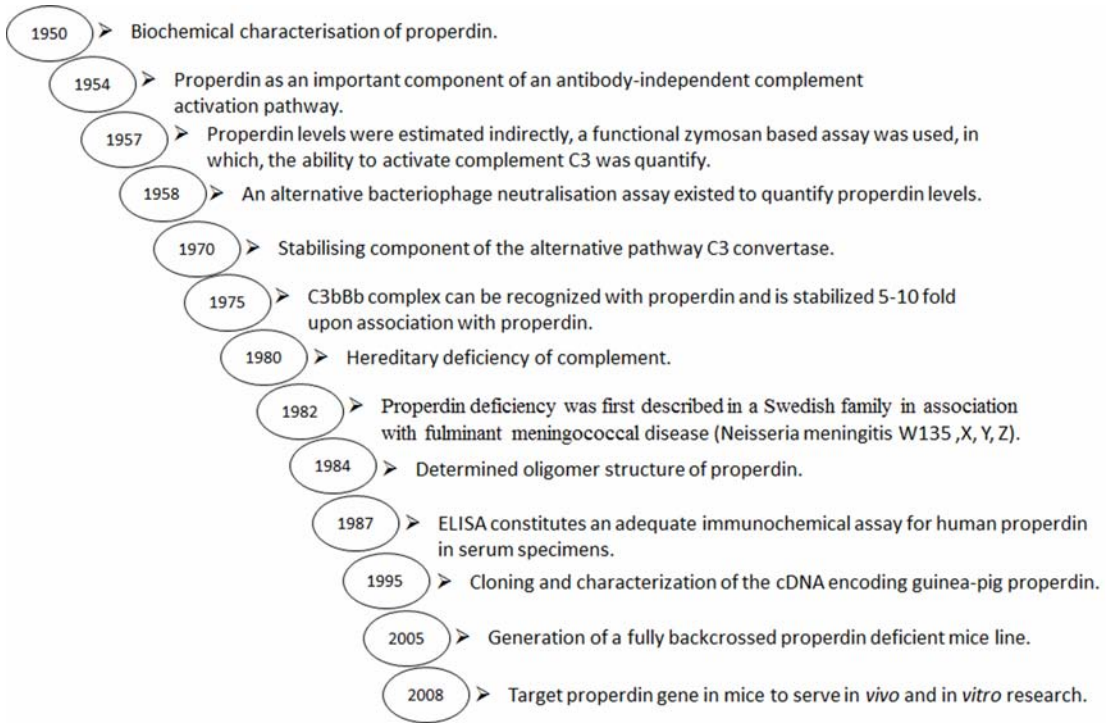

Figure 1. A diagram of a time line of properdin discovery [6, 10-16, 4].

(C3bBb and C3bBb5b) from rapid inactivation [11]. Properdin attains its full stabilising effect on C3bBb when it binds to more than one C3b or more ligands at a time on the surface [17]. It also stabilises the C5 convertases by binding to C3b in C3bnBb and C3b2b4b. This interaction inhibits the deactivation of the C3 convertase by Factors I and H. By stabilising the C3 convertases, properdin amplifies and deposits more C3b on the activating surfaces of pathogens and generates more anaphylatoxins C3a and C5a and opsonin C3b; hence the alternative pathway is called an amplification loop of complement activation.

Properdin binds to glycosaminoglycans on early apoptotic T cells. This is thought to enhance complement activation and phagocytosis by macrophages and dendritic cells using CR3, an integrin composed of CD11b and CD18 [7]. Furthermore, a study by Xu *et al.* (2008) [18] demonstrates that properdin is able to bind to late apoptotic and necrotic cells prior to C3 deposition on the cell surface but not to early apoptotic cells. This proposes that early apoptotic cells are cleared by anti-inflammatory macrophages. Moreover, properdin binds to late apoptotic and necrotic cells *via* ligands such as DNA, which is one of the main autoantigens exposed on apoptotic cell surfaces. Properdin, throughout apoptosis, can bind to small fragments of DNA, although it does not bind to the complete nucleosomal units of DNA. This happens in the absence of C3 or C3b. Properdin binds to the eukaryotic cell surface by glycosaminoglycans (GAGs) and to the changed surfaces of apoptotic cells; however, the physiological relevance is uncertain [19]. Properdin in serum does not bind to apoptotic T cells compared to properdin expelled *via* degranulating neutrophils [7]. This difference may be related to the oligomerisation state of properdin impacting on its function [20]. Similarly, local release of properdin is important for properdin-initiated complement opsonization and killing. However, this describes an effect of properdin outside traditional complement activation [21].

Properdin deficiency in man

Properdin deficiency is the most common deficiency for a component of the alternative pathway. However, it remains unknown why it is linked to an increased susceptibility of individuals to severe fulminant meningococcal disease caused by certain

serotypes of *N. meningitidis* [22-24], as properdin itself is not thought to bind directly to these bacterial surfaces.

There are three types of properdin deficiency phenotypes, type I, type II and type III. Type I complete deficiency is the most common type with no properdin in serum (less than 0.01 mg/l) in spite of a normal mRNA level. Type II is an incomplete deficiency with less than 10% of the normal level of properdin in serum. Type III is characterised by a normal serum concentration but a dysfunction in properdin protein. All three types are associated with an increased susceptibility to meningococcal disease.

Properdin deficiency in man was first reported in a Swedish family in 1982 with 3 males affected and is associated with a high susceptibility to meningococcal meningitis with high mortality [13]. This emphasises the important role of the alternative pathway in controlling the growth of meningococci [19]. Since then, properdin deficiency has been described in over 100 individuals of about 30 families [25], and type I properdin-deficiency was first described in the family with properdin deficiency. Patients with a complement deficiency of properdin or C3 or late complement component of alternative pathway have a great risk of meningococcal disease than normal individuals. Properdin deficiency is the most common genetic defect of the alternative pathway components, and is inherited in an X-linked manner, with all index cases for properdin deficiency being male. Since 1982 further studies have reported people who have been diagnosed as properdin-deficient. The majority of cases are of European extraction, suggesting a relatively recent mutation, and the most common type observed was type I deficiency. Properdin deficiency is typically detected in patients suffering from infections with *N. meningitidis* and subsequently in their families.

Individuals with properdin deficiency have several infections in the early years of life. However, not all of individuals with properdin deficiency get meningococcal infections, and it has been suggested that a high concentration of subclass IgG2 is a contributory susceptibility factor. Moreover, a lack of the subclass IgG2 has previously been associated with reduced immune responses to T-independent antigens like the meningococcal antigen [26]. This disease is often complicated by sepsis, frequently caused by uncommon serogroups W-135 and Y and it occurs especially at young age. The alternative pathway has been shown to play a very important role in anti-capsular antibody-dependent immunity to *N. meningitidis in vitro* and this may explain the association of properdin deficiency with meningococcal disease [27] together with the fact that recurrent infections are rare. In addition, properdin deficiency is connected with recurrent otitis media and pneumonia [28]. A study by Fijen *et al.* in the Netherlands showed that 30% of complement-deficient people were properdin-deficient and 33% of the persons who developed a meningococcal disease because of an uncommon serogroup of *N. meningitidis* were complement-deficient [22].

A few studies of complement deficiencies investigated the impact of these deficiencies on vaccine success and showed that most of the complement-deficient patients are able to mount specific antibody responses. Therefore to protect properdin-deficient individuals from infections with *N. meningitides* a vaccination with a tetravalent ACYW meningococcal capsular polysaccharide vaccine is advised every 3 years.

Properdin binds differently to different types of lipopolysaccharide (LPS) and lipooligosaccharide (LOS) modifications. Whereas it was observed to bind strongly to *Salmonella typhosa* LPS, its binding to *Salmonella minnesota* and *Escherichia coli* LPS was weak [29]. Properdin's ability to identify and bind to certain bacterial surfaces enhances the alternative pathway activation, while other bacterial surfaces that do not bind properdin activate the alternative pathway more slowly [24]. This may indeed be the basis of the observation that a deficiency of properdin coincides with fulminant *N. meningitidis* sepsis. Complement activation with an intact alternative pathway is necessary for complement activation in response to *Neisseria*. However, research shows that properdin is not necessary for all microbial activators that activate the alternative pathway, because zymosan was able to activate the alternative pathway of a mouse with properdin deficiency [29].

Properdin-deficient mice

To investigate in depth the role of properdin in the immune response, two groups independently produced mice genetically engineered to be deficient of properdin, to use specifically for *in vivo* or *in vitro* experimentation [29, 4]. Because the mouse properdin gene is syntenic to the human gene (chromosome X)

and human and mouse properdin sequences show a high degree of identity with almost complete protection of the relatively large number of Cys (44) and Trp (20) residues (about 76%) [30, 16], the expectation was that the deficient mouse line, by comparing it to the wild-type, would provide additional important insight into the role of properdin in complex conditions of relevance to man. Importantly, it was found that the role of properdin extended beyond meningococcal sepsis as patient data had long implied.

Properdin-deficient mice were reduced in their survival after cecal ligation and puncture (CLP), a model of acute polymicrobial septic peritonitis. Over an observation period of 14 days, mice deficient of properdin were significantly impaired in their survival compared with wild-type littermates [4]. Moreover, in the absence of properdin, mice showed significantly higher mortality in nonseptic shock models of LPS injection [31], but properdin-deficient mice were more resistant to zymosan-induced shock than wild-type mice [31]. Furthermore, important roles of properdin have been revealed in disease models of arthritis, for example K/BxN model of arthritis [32, 33, 8] and of abdominal aortic aneurism (AAA) [34]. Because mice deficient of properdin are protected from the severity of particular diseases [31, 8, 34], efforts are being evaluated to understand the consequences of therapeutically inhibiting properdin [35, 8] and the contribution of locally synthesized properdin in the disease pathogenesis.

In the absence of properdin, mice have deranged normal cellular function in response to stimuli. For example, in the absence of properdin, bone marrow-derived dendritic cells had less numbers of intracellular *Listeria monocytogenes* compared to cells from wild-type mice and showed impaired maturation to function as antigen presenting cells. *In vivo*, properdin-deficient mice had greater disease severity, consistent with impaired activation of M1 type, and poorer prognosis than wild-type mice. These findings added to the knowledge that properdin is important to help the complement system as part of a protection mechanism against infection, extending to infection with primarily intracellular pathogens. In properdin-deficient mice, infection may lead to lower phagocytic function, and reduced T helper cell type 1 (Th1) cytokine release. Macrophages and dendritic cells isolated from wild-type mice maintain the M1 phenotype, while, macrophages and dendritic cells isolated from properdin-deficient mice act like M2 polarised cells, which is characteristic of C57BL/6 background [36].

In an *in vitro* study, it was shown that stimulation of macrophages differentiated from bone marrows of properdin-deficient and wild-type mice using conditioned tumour cell medium leads to a more immune suppressive phenotype in properdin-deficient mice. The deficiency of properdin again showed greater M2 skewing, including mRNA expression for genes involved in arginine metabolism, production of type 2 cytokines, and lower surface expression of molecules needed for antigen presentation. Properdin deficiency might promote a tumour environment that helps the tumour evade the immune response.

From this work, an all-encompassing take-away message emerged from the role of properdin in health and disease and is summarised in Figure 2.

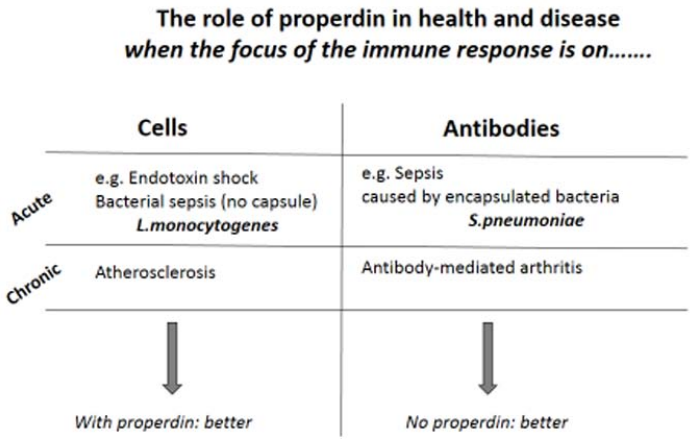

Figure 2. Role of properdin in health and disease [36, 33, 37].

Properdin in the cellular innate immune response

As part of a study to understand the role of properdin in murine infection with *L. monocytogenes* [36], it was found that the expression of IL-17A mRNA of splenic was decreased in unstimulated properdin-deficient mice compared to the wild-type controls (Table 1). IL-17A is necessary for host defense against extracellular and intracellular pathogens. Therefore, IL-17 protein was determined by enzyme-linked immunosorbent assay (ELISA) from supernatants of control splenocytes from wild-type and properdin-deficient mice after culturing for 48 hours. The level of secreted IL-17 was observed to be the same between wild-type and properdin-deficient mice [33]. Likewise, when investigating the level of cytokine IL-17A in synovial fluid and mediastinal lymph nodes cells supernatant, the levels of cytokine IL-17A in properdin-deficient were found to be the same as in wild-type mice.

IL-17A is released from the cells of the innate immune system such as neutrophils, natural killer T (NKT) cells and γδT cells and highly specialised epithelial cells during acute inflammatory response despite the fact that IL-17A is known to be released from Th-17. It has been reported that the level of complement activity affects IL-17 production by T cells and macrophages [38]. IL-17 also plays significant role in activating and recruitment of neutrophils in the innate immune system [39].

Properdin in the cellular adaptive immune response

Although IFN-γ production by blood $CD4^+$ T cells from control properdin-deficient mice was same as compared to that from wild-type mice, as shown by, flow cytometry, IFN-γ secretion as determined by ELISA in supernatants from splenic $CD4^+$ T cultures, bone marrow derived dendritic cells and macrophages was same in properdin-deficient and wild-type mice [33].

Bone marrow-derived macrophages from wild-type and properdin-deficient mice showed similar gene expressions for IL-6, IL-1β, iNOS, TNF-α, IL-10, arginase-1 and MCP-1. Moreover, IL-10 and IL-12 in supernatants of bone marrow-derived macrophages, as well as the number of CD11b+CD206+ cells among Granulocyte Monocyte Colony-Stimulating Factor (GM-CSF)-differentiated bone marrow-derived macrophages cells and sC5b-9 serum showed no difference between the genotypes. However, CCL2 levels in serum were higher in properdin-deficient than wild-type mice, which may go along with the relative M2 skewing in the absence of properdin [40].

Table 1. Arrows indicate an increase (↑) or decrease (↓) in inflammatory mediators, cell phenotypes or activities in unstimulated properdin-deficient compared to wild-type mice.

Category	KO (knock out)	Methods	Samples	*Vitro/Vivo*
Inflammatory mediators	IL-17↓	qPCR	Spleen	*In vivo*
	FcγRIIb (307 bp)↓	qPCR	Spleen	*In vivo*
	FcγRIV (461 bp)↑	qPCR	Spleen	*In vivo*
	CCL2↑	ELISA	Serum	*In vivo*
	IFN-γ ↓	ELISA	Spleen supernatant	*In vivo*
	FcγRIIb↓	Flow cytometry	On a gated population of B220+ cells spleen	*In vivo*
Cells	TLR2 (548 bp)↑	RT-PCR	Dendritic cells and macrophage	*In vitro*
Activities	AP activity ↓	Fctl ELISA	Serum	*In vitro*
	CP activity ↓	Fctl ELISA	Serum	*In vitro*
	LP activity ↓	Fctl ELISA	Serum	*In vitro*

In the absence of properdin, the production of cytokines IL-4 and IL-22 was the same as in wild-type. The receptor activator of nuclear factor κB ligand (RANKL) levels in synovium (determined using ELISA) was also the same in both genotypes. Nitric oxide production by macrophages isolated from untreated properdin-deficient and wild-type mice, as well as surface expressions of CD11c and CD40, CD80, CD86 and MHCII dendritic cell were the same in both genotypes.

Levels of C4c in sera as well as of C5a and C3 proteins were the same in the absence of properdin compared to the wild-type. The activities of classical and lectin pathways were impaired in properdin-deficient compared to wild-type mice due to the lack of the properdin-mediated stabilisation of the amplification loop. The alternative pathway activity showed a clear impairment due to the dependence of the activator surface to bind to properdin in order to initiate detectable activation [4, 41].

Inflammatory mediators

Immunoglobulin Fc receptors (FcR) are an important player in regulating both innate and adaptive immune responses as they provide a link between antibody-antigen complexes and cellular effector mechanism ending in phagocytosis, endocytosis of IgG-opsonised particles and release of inflammatory mediators.

The mRNA expression levels for Fcγ receptors FcγRIIb and FcγRIV on splenic and bone marrow-derived macrophages from wild-type and properdin-deficient mice were previously determined [42] by reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative RT-PCR (qPCR) [42] and showed a reduction in the level of FcγRIIb mRNA expression in splenocytes from properdin-deficient mice compared to wild-type mice. The mRNA expression of FcγRIV determined by qPCR was higher and significantly different in splenocytes from properdin-deficient mice compared to splenocytes from wild-type mice (Table 1). Therefore, FcγRIIb expression was decreased in splenocytes from properdin-deficient mice which showed an inverted relationship of FcγRIIb/FcγRIV compared to wild-type.

The level of FcγRIIb expression on splenocytes prepared from properdin-deficient and wild-type mice was measured in [36, 42] using flow cytometry; the splenocytes were analysed with PE-labelled B cell-specific rat anti-mouse CD45R/B220 and FITC-labelled rat anti-mouse CD16/CD32 (FcγIII/II receptor). Flow cytometric analysis of FcγRIIb expression was conducted on a gated population of $B220^{+}$ cells and the results were expressed as mean using MFI (mean fluorescence intensity) value in both genotypes in parallel. B-cells were used because FcγRIIb is the only FcγR which is expressed by B cells [43].

The FcγRIIb are inhibitory receptors controlling cytokine release, antibody production, and phagocytosis by balancing the inflammatory response to infection. FcγRIV receptors are activating receptors favoring phagocytosis and the release of inflammatory mediators. We have interpreted [42] this altered ratio of FcγRIIb and FcγRIV in the absence of properdin as a contributing factor in the outcome of primarily antibody driven pathologies [36].

Cell and tissue mediators

TLRs detect pathogen-associated molecular patterns (PAMPs) in the extracellular or vacuolar spaces and promote transcriptional responses. Properdin-deficient mice showed higher expression of TLR2 mRNA in control bone marrow-derived dendritic cells and macrophages compared to wild-type mice [42].

Bone mineral density in properdin-deficient and wild-type mice was measured in [44] by Micro-CT analysis (μ-CT) scan. The whole bone mineral density, as determined from lumbar vertebrae, was not altered [44].

Quantitative values of mRNA expression levels of C3 in uninfected bone marrow-derived macrophages from wild-type and properdin-deficient mice were determined in an earlier study [42] under normoxia and hypoxia. The expression was higher in wild-type compared to properdin-deficient mice in normoxic condition. But in hypoxic condition, the expression of C3 in bone marrow-derived macrophages was higher in properdin-deficient compared to wild-type mice. This difference in hypoxia induced modulation of gene expression between properdin-deficient and wild-type mice points to an impact of properdin expression on the cellular susceptibility to hypoxia. Currently, the importance of an intact “complosome” [45] on cell

activities has become a novel focus in the research area around complement and properdin [46].

qPCR values of mRNA expression levels of C3 in bone marrow-derived macrophages from properdin-deficient mice in hypoxic condition were higher compared to properdin-deficient mice in normoxic condition [42].

The number of neutrophils, as counted by flow cytometry in peripheral blood from properdin-deficient and wild-type mice, showed no differences between both genotypes, meaning that bone marrow production and cell mobilisation (which requires complement) were not impaired in the absence of properdin [47].

Dendritic cell populations derived from bone marrows of wild-type and properdin-deficient mice using GM-CSF and IL-4 showed no obvious difference in ultrastructure when analysed by scanning and transmission electron microscopy. The reader is referred to Table 2 for additional immune measurements that showed no difference between wild-type and properdin-deficient mice.

Cumulative analysis of the effect of engineered properdin deficiency in mouse on baseline immune measurements is shown in Table 2.

Limitations of mouse models

Mice are model organisms widely used in research to study the function of human genes and human

Table 2. The humoral and cellular phenotype of unstimulated properdin-deficient mice.

Category	Measurement	Methods	Wild-type (WT)/Properdin -deficient (KO)	References
Inflammatory mediators	C4c levels in sera	ELISA	WT=KO	[36]
	Splenic IL-17 mRNA	Quantitative analysis qPCR	WT>KO	[36]
	C5a plasma and peritoneal lavage	ELISA	WT=KO	[31]
	C3 protein in serum	ELISA	WT=KO	[48]
	C5a , TNF-α, IL-6 and RANKL levels in synovium	ELISA	WT=KO	[32]
	mRNA expression levels of C3	qPCR	WT=KO	[42]
	TNF-α cytokines in immature bone marrow-derived and spleen-derived dendritic cells culture	ELISA	WT=KO	[49]
	IFN-γ from dendritic cells and macrophages.	ELISA	WT=KO	[36]
	Splenic FcγRIIb expression on a gated population of $B220^{+}$ cells	Flow cytometry	WT>KO	[36]
	Splenic FcγRIIb	qPCR	WT>KO	[36]
	Splenic FcγRIV	qPCR	WT<KO	[36]
	Surface expressions of CD11c and CD40, CD80, CD86 and MHCII dendritic cell.	Flow cytometry	WT=KO	[36]
	C3a and C5a in colon explant culture supernatants	ELISA	WT=KO	[50]
	Pulmonary C3 activation (iC3b/C3dg) using Lung homogenates	Western blot	WT=KO	[36]
	TNF-α and IL-10 Plasma and peritoneal lavage	ELISA	WT=KO	[31]
	TNF-α alveolar perineal macrophages	ELISA	WT=KO	[31]

Table 2 continued..

	IFN-γ production from popliteal lymph nodes and splenocytes.	ELISA	WT=KO	[32]
	IL-4 production from popliteal lymph nodes	ELISA	WT=KO	[32]
	Serum CCL2	ELISA	WT<KO	[51]
	Levels of cytokines (IL-6, IL-12, IL-22, IL-17A, TNF, and IL-10) produced by epithelial cells.	ELISA	WT=KO	[50]
	Supernatants from splenic $CD4^+$ T cells secreting IFN-γ.	ELISA	WT=KO	[33]
	IFN-γ in serum	ELISA	WT=KO	[42]
	Serum IL-6	ELISA	WT=KO	[52]
	Cytokines (IL-17, IL-6) and C5a in synovial fluid, plasma	ELISA	WT=KO	[33]
	IL-10 and IL-12 secreted by bone marrow-derived macrophages	ELISA	WT=KO	[40]
	cDNA bone marrow macrophages (GM-CSF) to quantify gene expression of IL-6, IL-1β, iNOS, TNF-α, IL-10, arginase-1 and MCP-1	qPCR	WT=KO	[40]
	Nitric oxide production from peritoneal macrophages	Standard Griess reaction	WT=KO	[31]
	Plasma levels of intact C3, and C3 activation	ELISA	WT=KO	[53]
	Cytokines (IL-4, IL-5, IL-17A and IFN-γ) secreted from mediastinal lymph node (MLN) cells	ELISA	WT=KO	[54]
Cells	mRNA expression of TLR2 in dendritic cells and macrophages	RT-PCR	WT<KO	[42]
	Dendritic cell and macrophages populations derived from bone marrows using GM-CSF and IL-4	Scanning and transmission electron microscopy	WT=KO	[42]
	% of M-MDSCs (CD45+Gr-1 +CD11b+) in bone marrow	Flow cytometry	WT=KO	[51]
	mRNA expression of FcγRIIb and FcγRIV on bone marrow-derived macrophage	qPCR	WT=KO	[42]
	mRNA expression of TLR2, CD11b and C5aR from spleen	qPCR	WT=KO	[42]
	TNF-α level in dendritic cells and macrophages.	ELISA	WT=KO	[42]
	Pulmonary neutrophils in lung homogenates	Cytological quantification	WT=KO	[36]
	$CD4^+$ $RANKL^+$ T cells	Flow cytometry	WT=KO	[33]
	$CD4^+$ $CD69^+$ T cells	Flow cytometry	WT=KO	[33]

Table 2 continued..

	$Ly6G^+C5aR^+$ cells	Flow cytometry	WT=KO	[33]
	$Ly6G^+$ $RANKL^+$ cells	Flow cytometry	WT=KO	[33]
	Differential blood counts	Blood smear	WT=KO	[48]
	IL-17 and IFN-γ production Blood $CD4^+$ T cells	Flow cytometry	WT=KO	[33]
	Number of mature osteoclasts differentiated from bone marrow cells for 7 days	Specific TRAP staining	WT=KO	[33]
	Spleen $CD4^+$ T cells	Flow cytometry	WT=KO	[33]
	$Ly6G^{low}CD11b^+$ and $Ly6G^{high}CD11b^+$ cells were determined in spleen	Flow cytometry	WT=KO	[33]
	$Ly6G^{high}CD11b^+$ BM cells expressed C5aR	Flow-cytometry	WT=KO	[33]
	Frequencies of $Ly6G^{low}CD11b^+$ and $Ly6G^{high}CD11b^+$ cells in bone marrow	Flow cytometry	WT=KO	[33]
	CD11b+CD206+ cells in GM-CSF-differentiated bone marrow-derived macrophages cells	Flow cytometry	WT=KO	[51]
	Polymorphonuclear leukocyte (PMN) count of experimental mice lungs	H and E slides stained	WT=KO	[49]
	Differential cell count for monocytes, macrophages, lymphocytes and mast cells from peritoneal lavages	Cytospun-Giemsa-stained cells	WT=KO	[49]
	% of PMN-MDSCs (CD45+Gr-1 +CD11b+) in bone marrow	Flow cytometry	WT=KO	[51]
	% of $CD206^+$ $F4/80^+$ in spleen	Flow cytometry	WT=KO	[51]
	The mean of granule positive peritoneal mast cells	Cells stained with Wright's and Bismarck brown stain under light microscopy	WT=KO	[55]
	Colonic sections for apoptosis and cellular infiltration	Slides with a TUNEL staining kit	WT=KO	[50]
	Neutrophil counts (neutrophils from the bone marrow in response to GMCSF)	Flow cytometry	WT=KO	[47]
Tissues	Colon sections revealed numbers of $Ly6G^+$ neutrophils and F4/80 macrophages	Immunohistochemistry and quantification	WT=KO	[50]
	Bone density	Micro-CT analysis	WT=KO	[44]
	Colon	Histology	WT=KO	[50]
	Kidney histology	Analysis after staining with Periodic acid-Schiff (PAS)	WT=KO	[54]

Table 2 continued..

	Liver histology	H and E	WT=KO	[42, 31]
	Determination of anti-PPS2, PPS3, PPS6B IgM antibodies	Specific ELISA	WT=KO	[49]
	Serum IgM	ELISA	WT=KO	[36, 48]
	Natural antibodies	ELISA	WT=KO	[32]
Activities	Classical pathway (CP) activity	Fctl ELISA	WT>KO	[52, 56]
	Alternative pathway (AP) activity	Fctl ELISA, rabbit RBC lysis	WT>> KO	[52, 4]
	Lectin pathway (LP) activity	Fctl ELISA	WT>KO	[56]
	sC5b-9 serum	ELISA	WT=KO	[51]
	The reaction time (r), clotting time (k) kinetics of clot formation (α) and clot using blood.	ROTEG analysis	WT=KO	[48]
Metabolism	Liver function tests (AST, ALT serum)	AST, ALT assay	WT=KO	[52]
	Glucose in blood	Glucose meter	WT=KO	[52]
	Lipid measurements, Triglycerides	Triglyceride quantification assay for mouse liver	WT=KO	[52]
	Nonesterified fatty acids (NEFA)	Extraction methods used for the colorimetric determination of NEFA in serum.	WT=KO	[52]
	Insulin and adiponectin in serum	ELISA method	WT=KO	[52]

diseases, for example cancer, cardiovascular diseases and diabetes as mice genome is comparable to the human genome (about 99%). Presently different studies are in progress to generate a complete map of mouse gene functions. Different strains are used. It is essential to know about the origin of the laboratory mouse and evolutionary history of its genome to understand the nature and importance of genetic background on phenotypic expression of specific genes [57].

The C57BL/6 mouse is the most commonly used strain in biomedical research [58]. Using the background mouse strain in controls is critical to the result of the experiment, and should be carefully considered in the interpretation of the experimental results. There are many genetic and phenotypic differences between for example, C57BL/6 and DBA/2 strains [59-63]. Hence, it is essential to have a statistically important number of transgenic and nontransgenic animals of mixed genetic backgrounds to differentiate the phenotype of the transgene in disease models from differences in the genetic backgrounds.

Mice are being used in experimental research as a tool for immunologists to study immune responses, and this produced understanding of the human immune system. However, there are major differences between mice and human immunology. These differences must be taken into account when using mice as preclinical models of human disease as they affect both innate and adaptive immunity, for example, Toll receptors, cytokines and cytokine receptors, Th1/Th2 differentiation, inducible NO synthase etc. [64]. Another limitation is that mice are used to respond to human pathogens or develop diseases of significance for man, which follow a

different time pattern in humans [65]. The use of genetic engineering of animals engenders ethical concerns, which need to address animal welfare issues that might be raised in a specific study. A cost-benefit evaluation becomes necessary, namely an analysis that focuses on the justification of animal use, the justification of numbers and species to be used, and the essential of balancing relevance and investigation design with the cost to the animal [66]. Sharing a compilation of data achieved with a specific mouse line of genetic deficiency takes a step towards minimising animal use and working out targeting strategies whilst measuring relevant parameters.

The overall objective will be to translate the knowledge gained to understanding human diseases. Only the venue of genetic engineering made it possible to expose roles for properdin that could never have been found using the biochemical methods that pioneered the area by originally functionally characterising the protein and then purifying it.

CONCLUSION

Complement is an essential component of the innate immune system that can lead to inflammation, but can also modulate the developing adaptive immune response. Properdin is a crucial component of an amplifying pathway of complement activation. Properdin knockout mice have been generated and have been used to evaluate the role of properdin in the alternative pathway of complement activation *in vitro* and *in vivo*. Based on our cumulative analysis, properdin has a subtle influence on baseline immune responses overall, but shapes significantly distinct systems in a manner that is outside its characteristic fluid phase properties. Herein lies the challenge for the future, to gauge the effect of fluid phase *vs* diffusible targeting as the cues for cells change. Intriguing questions remain for the role of properdin in cancer immunology, in ischemic or non-ischemic tissue injury and repair, and lastly, the extent of selection pressure exerted by the properdin system on populations of invasive or commensal organisms.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

REFERENCES

1. Menges, M., Baumeister, T., Rossner, S., Stoitzner, P., Romani, N., Gessner, A. and Lutz, M. B. 2005, Journal of Leukocyte Biology, 77, 535-543, doi: 10.1189/jlb.0804473.
2. Zipfel, P. F. and Skerka, C. 2009, Nature Reviews Immunology, 9, 729-740, doi: 10.1038/nri2620.
3. Walport, M. J. 2001, The New England Journal of Medicine, 344, 1058-1066, doi: 10.1056/NEJM200104053441406.
4. Stover, C. M., Luckett, J. C., Echtenacher, B., Dupont, A., Figgitt, S. E., Brown, J., Mannel, D. N. and Schwaeble, W. J. 2008, Journal of Immunology (Baltimore, Md.: 1950), 180, 3313-3318.
5. Stover, C., McDonald, J., Byrne, S., Lambert, D. G. and Thompson, J. P. 2015, Frontiers in Immunology, 6, 24, doi:10.3389/fimmu.00024.
6. Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W. and Wardlaw, A. C. 1954, Science (New York, NY), 120, 279-285.
7. Kemper, C., Mitchell, L. M., Zhang, L. and Hourcade, D. E. 2008, Proceedings of the National Academy of Sciences of the United States of America, 105, 9023-9028, doi.org/10.1073/pnas.0801015105.
8. Kimura, Y., Zhou, L., Miwa, T. and Song, W. C. 2010, The Journal of Clinical Investigation, 120, 3545-3554, PMCID: PMC2947218.
9. Harboe, M., Garred, P., Lindstad, J. K., Pharo, A., Muller, F., Stahl, G. L., Lambris, J. D. and Mollnes, T. E. 2012, Journal of Immunology, 189, 2606-2613, doi: 10.4049/jimmunol. 1200269.
10. Robert, A. and Nelson, Jr. 1958, Journal of Experimental Medicine, 108(4), 515-535.
11. Fearon, D. T. and Austen, K. F. 1975, Journal of Experimental Medicine, 142(4), 856-63.
12. Lepow, I. H. 1980, Journal of Immunology, 125, 471-475.
13. Sjoholm, A. G., Braconier, J. H. and Soderstrom, C. 1982, Clinical and Experimental Immunology, 50, 291-297.
14. Smith, C. A., Pangburn, M. K., Vogel, C. W. and Muller-Eberhard, H. J, 1984, Journal of Biological Chemistry, 259, 4582-4588.
15. Nielsen, H. E. and Koch, C. 1987, Clinical Immunology and Immunopathology, 44(2), 34-9.

16. Maves, K., Guenthner, S. T., Densen, P., Moser, D. R. and Weiler, J. M. 1995, Immunology, 86, 475-479.
17. Hourcade, D. E. 2006, The Journal of Biological Chemistry, 281, 2128-2132, doi: 10.1074/jbc.M508928200.
18. Xu, W., Berger, S. P., Trouw, L. A., de Boer, H. C., Schlagwein, N., Mutsaers, C., Daha, M. R. and van Kooten, C. 2008, Journal of Immunology, 180, 7613-7621.
19. Kemper, C., Atkinson, J. P., Hourcade, D. E. 2010, Annual Review of Immunology, 28, 131-155.
20. Pedersen, D. V., Roumenina, L., Jensen, R. K., Gadeberg, T. A., Marinozzi, C., Picard, C., Rybkine, T., Thiel, S., Sørensen, U. B., Stover, C., Fremeaux-Bacchi, V. and Andersen, G. R. 2017, EMBO Journal, 36(8), 1084-1099. doi:10.15252/embj.201696173.
21. Kouser, L., Paudyal, B., Kaur, A., Stenbeck, G., Jones, L. A., Abozaid, S. M., Stover, C. M., Flahaut, E., Sim, R. B. and Kishore, U. 2018, Frontiers in Immunology, 9, 131, doi: 10.3389/fimmu.2018.00131.
22. Fijen, C. A., van den Bogaard, R., Schipper, M., Mannens, M., Schlesinger, M., Nordin, F. G., Dankert, J., Daha, M. R., Sjöholm, A. G., Truedsson, L. and Kuijper, E. J. 1999a, Molecular Immunology, 36(13-14), 863-7.
23. Fijen, C. A., Kuijper, E. J., te Bulte, M. T., Daha, M. R. and Dankert, J. 1999b, Clinical Infectious Diseases, 28, 98-105
24. Spitzer, D., Mitchell, L. M., Atkinson, J. P. and Hourcade, D. E. 2007, Journal of Immunology (Baltimore, Md.: 1950), 179, 2600-2608.
25. Linton, S. M. and Morgan, B. P. 1999, Clinical and Experimental Immunology, 118(2), 189-191, doi: 10.1046/j.1365-2249.1999.01057.x.
26. Fijen, C. A., Bredius, R. G. and Kuijper, E. J. 1993, Annals of Internal Medicine, 119, 636.
27. Sjoholm, A. G., Jonsson, G., Braconier, J. H., Sturfelt, G. and Truedsson, L. 2006, Molecular Immunology, 43, 78-85.
28. Schejbel, L., Rosenfeldt, V., Marquart, H., Valerius, N. H. and Garred, P. 2009, Clinical Immunology (Orlando, Fla.), 131, 456-462.
29. Kimura, Y., Miwa, T., Zhou, L. and Song, W. C. 2008, Blood, 111, 732-740.
30. Goundis, D. and Reid, K. B. 1988, Nature, 335, 82-85.
31. Ivanovska, N., Dimitrova, P., Luckett, J., Lonnen, R., Schwaeble, W., Stover, C. 2008, Journal of Immunology, 180(10) 6962-6969, doi.org/10.4049/jimmunol.180.10.6962
32. Dimitrova, P., Ivanovska, N., Schwaeble, W., Gyurkovska, V. and Stover, C. 2010, Molecular Immunology, 47, 1458-146, doi:10.1016/j. molimm. 2010.02.007.
33. Dimitrova, P., Ivanovska, N., Belensk, L., Milanova, V., Schwaeble, W. and Stover, C. 2012, Arthritis Research and Therapy, 14, R173.
34. Zhou, H., Yan, H., Stover, C., Fernandez, T., Rodriguez. De., Cordoba, S., Song, W., Wu, X., Thompson, R., Schwaeble, W., Atkinson, J., Hourcade, D. and Pham, C. 2012, Proceedings of the National Academy of Sciences USA, 109(7), E415–E422, doi: 10.1073/pnas.1119000109.
35. Gupta-Bansal R., Parent, J. B. and Brunden K. R. 2000, Molecular Immunology, 37, 191-201.
36. Dupont, A., Mohamed, F., Salehen, N., Glenn, S., Francescut, L., Adib, R., Byrne, S., Brewin, H., Elliott, I., Richards, L., Dimitrova, P., Schwaeble, W., Ivanovska, N., Kadioglu, A., Machado, L., Andrew. P. and Stover, C. 2014, Medical Microbiology and Immunology, 203, 257-271, doi: 10.1007/s00430-013-0324-z
37. Steiner, T., Francescut, L., Byrne, S., Hughes, T., Jayanthi, A., Guschina, I., Harwood, J., Stover, C. and Francis, S. 2014, PLOS, 9(3), e92404, doi.org/10.1371/journal.pone.0092404.
38. Grailer, J., Bosmann, M. and Ward, P. A. 2012, Frontiers in Immunology, 3, 387
39. Kolls, J. K. and Linden, A. 2004, Immunity, 21, 467-476.
40. Al-Rayahi, I., Browning, M. and Stover, C. 2017, Immunity, Inflammation and Disease, 5(1), 68-77, doi: 10.1002/iid3.142.
41. Kotimaa, J. P., van Werkhoven, M. B., O'Flynn, J., Klar-Mohamad, N., van Groningen, J., Schilders, G., Rutjes, H., Daha, M. R., Seelen, M. A. and van Kooten, C. 2015, Journal of Immunological Methods, 419, 25-34, doi: 10.1016/j.jim.2015.02.010.
42. Mohamed, F. 2014, PhD thesis, University of Leicester.

43. Smith, K. G. and Clatworthy, M. R. 2010, Nature Reviews Immunology, 10, 328-343.
44. Saeed, A. 2018, PhD thesis, University of Leicester.
45. Arbore, G., Kemper, C. and Kolev, M. 2017, Molecular Immunology, 89, 2-9, doi: 10.1016/ j.molimm.2017.05.012.
46. Reichhardt, M. P. and Meri, S. 2018, Seminars Immunology, pii: S1044-5323(18), 30002-2, doi: 10.1016/j.smim.2018.03.003
47. Freeley, S. J., Popat, R. J., Parmar, K., Kolev, M., Hunt, B. J., Stover, C. M., Schwaeble, W., Kemper, C. and Robson, M. G. 2016, Journal of Pathology, 240(1), 61-71, doi: 10.1002/path.4754.
48. Dupont, A. 2008, PhD thesis, University of Leicester.
49. Salehen, N. 2011, PhD thesis, University of Leicester.
50. Jain, U., Cao, Q., Thomas, N., Woodruff, T., Schwaeble, W., Stover, C. and Stadnyk, A. 2015, The Journal of Immunology, 194, 3414-3421, doi:10.4049/ jimmunol.1401814.
51. Al-Rayahi, I. 2017, PhD thesis, University of Leicester.
52. Kheder, R. 2017, PhD thesis, University of Leicester.
53. Lesher, A., Nilsson, B. and Song, W. 2013, Molecular Immunology, 56(3), 191-198, doi:10.1016/j.molimm.2013.06.002.
54. Wang, Y., Miwa, T., Ducka-Kokalari, B., Redai, I., Sato, S., Gullipalli, D., Zangrilli, J., Haczku, A. and Song, W. 2015, Journal of Immunology, 1401819; doi: https://doi.org/ 10.4049/jimmunol.1401819
55. Siad, S. 2014, PhD thesis, University of Leicester.
56. AlAridhee H. 2018, PhD thesis, University of Leicester.
57. Yoshiki, A. and Moriwaki, K. 2006, ILAR Journal, 47(2), 94-102.
58. Zheng, Q. Y., Johnson, K. R. and Erway, L. C. 1999, Hearing Research, 130, 94-107.
59. Belknap, J. K., Phillips, T. J. and O'Toole, L. A. 1992, Brain Research Bulletin, 29, 337-344.
60. Klein, R. F., Mitchell, S. R., Phillips, T. J., Belknap, J. K. and Orwoll, E. S. 1998, Journal of Bone and Mineral Research, 132, 1648-1656.
61. Peleg, L. and Nesbitt, M. N. 1984, Journal of Heredity, 75, 126-130.
62. Plomin, R., McClearn, G. E., Gora-Maslak, G. and Neiderhiser, J. M. 1991, Behavior Genetics, 21, 99-116.
63. Zidek, V., Musilova, A., Pintir, J., Simakova, M. and Pravenec, M. 1998, Mammalian Genome, 9, 503-505.
64. Mestas, J. and Hughes, C. 2004, Journal of Immunology, 172(5), 2731-8.
65. O'Sullivan, G., O'Tuathaigh, C., Clifford, J., O'Meara, G., Croke, D. and Waddington, J. 2006, Technologies, 3(2), 173-180.
66. Brown, M. and Murray, K. 2006, ILAR Journal, 47(2), 118-23.