Molecular mechanisms of complement activation by damaged cells

Ciurana, C.L.F.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Download date: 06 Dec 2018
INTRODUCTION
Immunity

Immunity distinguishes two types of responses to pathogen infection, the innate and adaptive immunity. Both types involve cellular as well as humoral mediators. Innate immunity constitutes a first line defense mechanism against pathogens using ubiquitous and non-specific cells, such as macrophages and neutrophils, and soluble mediators such as complement proteins. Adaptive immunity on the other hand uses antigen specific tools provided by lymphocytes, such as antibodies or cytotoxic T cells.

The innate immune system is required for defense against pathogens but also is involved in the maintenance of tissue homeostasis. Dying cells may become abundant in human body as a result of normal growth and ageing but also in response to internal or external stimuli. Either induced via the so-called programmed cell death, apoptosis, or via necrosis when aberrantly induced, a dying cell in general is a waste product that has to be removed from the circulation and the tissues to prevent inflammation.

Cell death

Apoptosis is characterized by chronological morphological changes such as membrane flip-flop and blebbing, nuclear and cytoplasmic condensation, and nucleosomal DNA fragmentation [1;2]. With the exception of the latter, these events occur in the cells when the plasma membrane is still intact, since in general apoptotic cells and bodies are meant to be engulfed by phagocytes before intracellular constituents leak into the environment and induce inflammation. Necrosis rather consists of cellular swelling and disruption of internal and external membranes leading to the rapid release of intracellular proteins, DNA fragments and other cell debris which subsequently trigger inflammation. Although necrosis and apoptosis constitute very distinct forms of cell death that in typical cases can be easily discriminated by their morphological attributes, the distinction between apoptotic and necrotic cells sometimes is not so clear [3;4]. Also the general view that apoptosis and necrosis constitute anti- or pro-inflammatory forms of cell death, respectively, does not seem to hold for every situation. For instance, loss of membrane integrity occurs as a later event during apoptosis, therefore cells dying by apoptosis may also trigger inflammation, particularly during the later stages.
Complement is a part of the innate immune system and as such is an important effector system of adaptive immunity since it “complements” the function of antibodies. The complement system, as blood coagulation, is composed of a number of inactive precursor proteins that circulate in the blood. Interaction with an activator leads to an organized cascade reaction in which proteins activate each other in a specific order by limited proteolysis [5]. Thus, during this activation process some complement proteins are converted from a zymogen into an active enzyme. Ultimately, the membrane attack complex (MAC) is formed which leads to a pore formation and subsequent cell lysis. Activators of this enzymatic cascade may vary and induce activation via three known pathways that differ from each other by the initial or upstream proteins of the cascade, the final phase from C3 on to the MAC formation being common to all three pathways. The classical activation pathway can be triggered by direct recognition of the pathogenic surface of certain viruses [6], gram negative bacteria [7] or apoptotic cells [8] by the complement protein C1q, or by interaction of this protein with immune complexes [9;10] or adaptor molecules bound onto pathogenic surfaces, such as C-reactive protein [11]. Recognition of microbial mannose-containing polysaccharides activates complement via the mannan-binding lectin pathway (MBL) [12]. Finally, the alternative pathway can be triggered by spontaneous cleavage of native C3 subsequently stabilized by binding to pathogenic surfaces.

Biological activity of the complement system is not restricted to MAC formation and cell lysis. Other activated complement fragments, such as the anaphylatoxins C3a and C5a, have inflammatory properties as well. To prevent uncontrolled activation of complement and damage to self cells, fluid phase and membrane-bound regulatory mechanisms stand by. However, unrestrained complement activation may occur and has been involved in the development of several clinical conditions, including ischemia/reperfusion injury [13] and rheumatoid arthritis [14]. The mechanisms of activation of complement and their regulation are further discussed in chapter 2 (figure 2).

Adaptor molecules

The classical activation pathway of complement sometimes uses bridging molecules between the pathogenic surface and the recognition unit of the first complement protein, C1q, which together with 2 C1r and 2 C1s molecules constitutes the C1 complex. Binding of C1q
to these bridging molecules fixes a conformation of C1q that allows auto-activation of the pro-enzymes C1r and C1s to form the active C1 complex [15]. Active C1 then activates C4 and C2 that together form a C3-convertase, the C4b2b complex, which in its turn activates C3 and the rest of the cascade. Immunoglobulins M or G as well as molecules from the pentaxins family, such as C - reactive protein, serum amyloid P-component and PTX3 are known to have such a bridging function and can be considered as adaptor molecules of the classical complement pathway. Notably, pentaxins have specificity for the head groups of phospholipids such as phosphocholine and phosphoethanolamine, and can bind to hydrolyzed or oxidized phospholipids [16; 17]. A similar specificity at least for phosphocholine has been described for some natural antibodies as well [18].

**Cell clearance and complement**

Outer and inner leaflet of the membranes of normal cells have a somewhat different composition of phospholipids, which is known as membrane asymmetry [19]. During apoptosis this asymmetry is lost with subsequent exposition of phospholipids of the inner leaflet, such as phosphatidylserine, in the outer leaflet. These newly exposed phospholipids allow a direct recognition of apoptotic cells by specific macrophages receptors [20-22]. Altered membrane phospholipids, however, may also serve as recognition molecules for plasma proteins [23], such as complement proteins [24] and adaptor molecules [25; 26]. Presumably, these plasma proteins are also involved in cell clearance by binding of apoptotic cells to complement or Fc receptors on macrophages [25]. A critical role of complement in the clearance of apoptotic cells has been demonstrated in complement knock-out mice that developed systemic autoimmunity as a result of circulating self antigens [27; 28].

**Complement in ischemia reperfusion injury**

Ischemia reperfusion (I/R) injury may occur when ischemic tissue is reperfused. In particular after a prolonged period (few hours) of ischemia, reperfusion will cause additional damage to the tissue rather than to limit damage. This paradoxical effect of reperfusion has been shown to result from an inflammatory response elicited in the ischemic tissue following reperfusion, and will be discussed in more detail in chapter 2. Among the mediators involved in I/R-injury are reactive oxygen species (ROS), activated complement fragments and polymorphonuclear neutrophilic granulocytes (PMN) that together will plug the capillaries...
and cause a so-called no-reflow phenomenon [29;30]. Ultimately these phenomena lead to cell death, either by apoptosis or necrosis, depending on the remaining cellular energy supply [31-35].

Though complement is known to play a role in I/R injury, the mechanism of activation is not yet clear. Several activation mechanisms have been proposed, which are discussed in some detail in chapter 2. Yet these mechanisms have not been fully elucidated in humans. In this thesis we focused on the molecular mechanisms of complement activation by either apoptotic or necrotic cells as models for I/R-induced complement activation.

Scope of this thesis

Complement cascade plays a key role in I/R-injury. To investigate the mechanism of activation in this condition we decided to investigate the molecular mechanism(s) of activation by damaged cells in vitro, and to establish whether the identified mechanisms are relevant for I/R-injury. The literature on the possible molecular mechanisms of complement activation during ischemia and reperfusion is reviewed in chapter 2, which also contains a description of the complement system in detail. In chapters 3 and 4, studies on the mechanisms of complement activation by apoptotic and necrotic cells, respectively, are described. In these studies cells were incubated with recalcified plasma in vitro, and complement activation was studied using neutralizing monoclonal antibodies against C1q and mannan binding lectin, together with absorption of adaptor molecules. Chapter 5 further investigates the possible binding sites for adaptor molecules, identified in the previous chapters as being involved in complement activation, on apoptotic cells. Chapter 6, describes studies on an Elisa developed to measure natural anti-phosphorylcholine IgM, which are antibodies among the IgM antibodies that bind to apoptotic cells. The relation of IgM antibodies binding to apoptotic cells to inflammatory responses in patients with acute myocardial infarction is discussed in chapter 7, in which the co-localisation of IgM antibodies and complement proteins in infarcted myocardial tissue is demonstrated. Chapter 8 investigates the association between levels of IgM that binds to apoptotic cells, including anti-phosphorylcholine IgM, and clinical and inflammatory parameters in patients with acute myocardial infarction. In chapter 9, we explored the possibility to use cells, Jurkat cells as well as endothelial cells, exposed to metabolic inhibitors as an in vitro model for ischemia-reperfusion. Finally, chapter 10 contains a general discussion and a summary of the work.
Reference List


