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Review

Pathogen clearance and immune adherence "revisited": Immuno-regulatory roles for CRIg

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ABSTRACT

Rapid elimination of microbes from the bloodstream, along with the ability to mount an adaptive immune response, are essential for optimal host-defense. Kupffer cells are strategically positioned in the liver sinusoids and efficiently capture circulating microbes from the hepatic artery and portal vein, thus preventing bacterial dissemination. *In vivo* and *in vitro* studies have probed how complement receptor of the immunoglobulin superfamily (CRIg), also referred to as Z39Ig and V-set and Ig domain-containing 4 (VSIG4), acts as a critical player in pathogen recognition and clearance. While recent data suggested that CRIg may bind bacterial cell wall components directly, the single transmembrane receptor is best known for its interaction with complement C3 opsonization products on the microbial surface. On Kupffer cells, CRIg must capture opsonized microbes against the shear forces of the blood flow. *In vivo* work reveals how immune adherence (IA), a process in which blood platelets or erythrocytes associate with circulating bacteria, plays a critical role in regulating pathogen capture by CRIg under flow conditions. In addition to its typical innate immune functions, CRIg was shown to directly and indirectly influence adaptive immune responses. Here, we review our current understanding of the diverse roles of CRIg in pathogen elimination, anti-microbial immunity and autoimmunity. In particular, we will explore how, through selective capturing by CRIg, an important balance is achieved between the immunological and clearance functions of liver and spleen.

1. Introduction

The liver has an important role in the immediate elimination of pathogens from the blood stream [1,2]. The portal vein collects blood that drains from the intestine and the liver is the first organ to encounter commensal and enteric pathogens released into the circulation. The liver is also perfused with blood from the hepatic artery. Both sources of blood mix in the liver sinusoids where the Kupffer cells reside. Liver Kupffer cells have a critical role in capturing microbiota that have overcome the intestinal barrier or pathogens that have reached the systemic circulation from peripheral sites [3]. Thus, harboring the vast majority of all resident phagocytes in the body, a healthy liver captures the bulk of circulating pathogens. Still, the infected host relies on the induction of adaptive immune defenses in the spleen to help eliminate pathogens that escaped liver clearance and remain disseminated [4,5]. In addition to their phagocytic capacity, splenic dendritic cells efficiently present antigen to mount cellular adaptive immune responses to eliminate disseminated pathogens [6]. Initiation of effector CD4 and/or CD8 T-cell responses in the spleen helps eliminate intracellular pathogens that escaped clearance by liver Kupffer cells [7,8]. In addition, CD4+ follicular helper T cells facilitate splenic antibody responses that directly limit unimpeded systemic spread of pathogens and assist the liver in capturing antibody-coated pathogens [9]. By comparison, liver Kupffer cells are poor antigen presenting cells that maintain a tolerized state and avoid immune (over)activation upon exposure to and clearance of enteric bacterial products [10]. The complement receptor of the immunoglobulin superfamily (CRIg) is an important phagocytic receptor that aids KC and other tissue resident macrophages in clearance. Recent data indicates that CRIg plays a central role in balancing the rapid elimination of circulating pathogens by specialized phagocytes in liver and spleen, ensuring both short and long-term protection from pathogens [11]. CRIg succeeds in this balancing act with aid of immune adherence (IA), the process of binding complement-opsonized pathogens in the circulation first described in 1953 by Robert Nelson for human erythrocytes and later confirmed for rodent platelets [12,13]. In the following, we will outline our current understanding how the physiological location, expression pattern and binding properties of CRIg go hand-in-hand with the dynamic processes of

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intravascular complement opsonization and IA, to generate a balanced immune response to systemic bacteria.

2. Expression of CRIg

2.1. Origin of CRIg+ macrophages

CRIg is expressed on subsets of tissue resident macrophages. The largest subset of these are Kupffer cells which originate from fetal liver-derived erythromyeloid progenitors and rely on self-renewal for their maintenance rather than on infiltrating bone marrow-derived monocytes [14,15]. After colonization of the liver, yolk-sac derived progenitor cells activate a distinct transcriptional program that leads to the development of Kupffer cells that self-sustain throughout life. This self-renewal capacity is tightly controlled by repressive transcription factors including MAFB and specific enhancers [16]. Kupffer cells constitute ~90% of all macrophages in the body, implying that the largest pool of CRIg+ expressing cells is self-renewing and does not depend on bone marrow-derived hematopoietic precursors for its replenishment and maintenance. The yolk-sac origin of CRIg-positive macrophages in mice is also consistent with the absence of CRIg on *in vitro* generated bone marrow-derived macrophages or DC's or its absence on murine monocyte-derived macrophages that infiltrate tissues *in vivo* as part of the inflammatory response [15,17]. Besides KCs as the largest population of CRIg-expressing macrophages in humans and rodents, in humans CRIg is also expressed on synovial macrophages (joints), foam cells (atherosclerotic plaques), Hofbauer cells (placenta), adrenal gland macrophages, large intestinal macrophages and alveolar macrophages [18–21]. Their inflammation-independent strategic localization in tissues are suggestive of an anti-inflammatory, homeostatic and/or clearing role for CRIg-expressing macrophages, as opposed to a pro-inflammatory role. In line with this notion, CRIg transcript levels show a strong correlation with the hemoglobin scavenger receptor CD163 (GTEx Portal, https://www.gtexportal.org, Broad Institute), a marker of alternatively activated (M2) macrophages associated with homeostasis and healing, rather than with pro-inflammatory responses [22]. In human cirrhotic patients, high numbers of CRIg+ peritoneal macrophages positively correlate with improved liver status and better prognosis, consistent with their superior capacity to ingest complement C3-opsonized targets [23,24], as discussed further in Section 3.1. This important role of CRIg+ macrophages in immune surveillance is equally emphasized by the impaired circulatory clearance seen when CRIg is ablated on KC [11,25], which we will review in greater detail in Section 5.

2.2. CRIg+ macrophages in immune surveillance

The protein sequence encoding the CRIg ectodomain has a low (~20%) homology with known B7 family of co-stimulatory molecules, with the sequence similarity confined to a conserved stretch of residues forming the Ig domain fold [26]. Human CRIg contains an additional membrane-proximal IgG domain and more closely resembles members of the B7 family than mouse CRIg. The existence of structural features of CRIg in common with B7 family members prompted investigators to probe CRIg's effect on immune modulation. Administration to mice of soluble CRIg ectodomain reduced the induction of T-cell responses *in vivo* and the production of Th cell-dependent IgG responses [27], in line with a role for CRIg in maintaining T cell unresponsiveness in healthy tissues. Consistent with a role in immune tolerance, CRIg is expressed on a subset of macrophages in the pancreas and CRIg expression is inversely correlated with the development of insulitis in a mouse model of autoimmune type-1 diabetes [28,29]. In this model, CRIg attenuated early T cell activation and promoted the differentiation of FoxP3+ regulatory T cells by enhancing their responsiveness to IL-2. [29]. As CRIg also attenuates T-cell effector response in the context of carcinomas [30,31], defining relevant T-cell ligands may afford important new insights with implications for cancer immunotherapy. More work needs to be done to define the molecular basis for CRIg's T-cell modulatory activity.

Taken together, various lines of evidence indicate an important function for self-renewing, CRIg-expressing macrophages in continual immune surveillance, clearance of particles and tissue homeostasis: 1) Their positioning at strategic locations in tissues for optimal control of systemic infections at an early stage, 2) Their efficient yet quiescent (non-pro-inflammatory, tolerogenic) ability to phagocytose complement opsonized targets, and 3), Their ability to promote immune tolerance and homeostasis in tissues vulnerable to autoimmune attack or infection.

3. CRIg's role in phagocytosis and complement regulation

3.1. CRIg as a complement receptor

The complement system contributes to homeostasis by marking potentially harmful "foreign" (pathogens, microbiota) and "self" (apoptotic debris, immune complexes) substances for clearance from the circulation and periphery. In doing so, complement plays a central role in preventing autoimmunity and overwhelming infection. This role is reflected by the broad distribution of complement receptors on phagocytes, including CRIg on KC, and the fact that CD11c (the alpha chain of CR4) and Mac1 (synonymous for CR3) are defining markers on DC and macrophage populations, respectively [32]. Conversely, many successful pathogens employ refined strategies to evade complement opsonization and lysis, something they would not evolutionarily "invest in" if not essential for their survival [33]. Indeed, individuals with complement deficiencies are prone to develop prototypic "immune complex diseases" like SLE and systemic vasculitis or suffer from recurrent infections [34]. Thus, if complement-mediated capture and phagocytosis of "unwanted substances" is impaired, ineffective and/or aberrant (auto)immunity can be the result.

CRIg's ability to bind the activated but not the native form of complement protein C3 [35] makes it an ideal pathogen recognition receptor (PRR) that recognizes C3-opsonized gram positive and gram negative pathogens as well as viruses [11,25,36]. Its selectivity for activated C3 molecules avoids dampening decoy-like activity by abundantly present native C3. The structural basis for this selectivity arises from a slight change in the conformation of the activated C3 beta chain, allowing CRIg to bind active, but not native, C3 [35]. The relatively low affinity (micromolar) of CRIg for its C3b, iC3b and C3c ligands is significantly enhanced by multiple CRIg molecules expressed on F-actin rich filopodial extentions which effectively engage with the many C3b/iC3b molecules that typically coat the surface of an opsonized pathogen [25]. CRIg binds its C3 ligands with its N-terminal IgV domain and subsequently internalizes C3 ligand together with its covalently attached target cargo [23,25]. Avoiding degradation, CRIg is actively rescued from the phagosome prior to its fusion with the lysosome, and diverted to a pool of recycling endosomes from where it is recruited back to the plasma membrane to ensure its continued participation in phagocytic events.

3.2. Structural basis for CRIg's regulation of complement activation

CRIg is unique among known C3 receptors in binding the beta chain within C3b, iC3b and C3c, interacting with a relatively large "key-ring" like surface area. The binding interface between CRIg and (i)C3b or C3c is large, discontinuous, and buries 2670 $Å^2$ of solvent-accessible surface [35]. The C3b binding site also affords CRIg a complement regulatory role: beside being an opsonin, C3b constitutes a central subunit of the alternative pathway C3 and C5 convertases, and its binding to CRIg prevents further complement cleavage. The ability of CRIg to function as negative regulator of complement activation (RCA) fits its association with homeostatic, non-inflammatory processes: it suppresses the