Comment Concerning the Role of CD47 and Signal Regulatory Protein Alpha in Regulating the Clearance of Aged Red Blood Cells

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Freshly isolated human red blood cells (RBC) bind to the signal regulatory protein alpha (SIRPα) on macrophages in a CD47-dependent manner (CD47 = integrin-associated protein). This interaction provides a ‘do not eat me signal’ such that these RBC are not phagocytosed and remain in circulation as exemplified for mouse RBC by Oldenborg and collaborators [1, 2]. Recently, Burger et al. have reported [3] and reviewed in this journal [4] that CD47 can function like a switch and then induces rather than inhibits phagocytosis of ‘experimentally aged human RBC’. In reality the studied RBC were oxidatively damaged by treating with CuSO₄ and ascorbic acid. The ‘eat me signal’ could be induced by blocking CD47 on oxidized RBC with F(ab’)₂ anti-CD47. Data from others [5] suggested that thrombospondin-1, known to interact with CD47, may act similarly. Hence, the authors studied whether a particular peptide of thrombospondin-1 (4N1K) promoted the interaction of CD47 with SIRPα. Experiments with human red pulp macrophages and oxidized RBC showed that phagocytosis increased upon addition of the 4N1K decapptide, but not when supplemented with an irrelevant peptide from thrombospondin-1. The 4N1K peptide was half as effective as blocking CD47 with F(ab’)₂ anti-CD47. The F(ab’)₂ fragment of the CD47-specific antibody prevented CD47 from interacting with SIRPα and this was sufficient to induce an ‘eat me signal’, because the inhibitory signal could not be induced by SIRPα. The authors think that binding of the 4N1K peptide to oxidized CD47 induces an ‘eat me signal’ not by blocking the interaction with SIRPα, but by conveying to oxidized CD47 the ability to interact in a new, so far unknown way with SIRPα which then induces an ‘eat me signal’. The authors suggest that thrombospondin-1 binding to CD47 can switch the role of CD47 to a promoter of erythropagocytosis, which may even be responsible for in vivo clearance of aged RBC. This, however, is highly questionable, because the 4N1K peptide of thrombospondin-1 was applied at 3 × 10⁻⁵ mol/l, a concentration that exceeds the thrombospondin-1 concentration in plasma by a factor of 10³ to 10⁴ [6]. Correspondingly, Head et al. [7] found that the 4N1K peptide at the very same high concentration (50 µg/ml) binds to CD47 without the need to impose a conformational change by e.g. oxidation. Moreover, incubation of RBC with 50 µg/ml 4N1K peptide for 24 h induced phosphatidylserine exposure on these RBC, amounting to an annexin binding that was 5 times higher than in controls and resulted in 40% loss of viable RBC.

The two sets of findings may explain the extra RBC destruction during vaso-occlusive crisis in sickle cell anemia, where local concentrations of thrombospondin may be considerably higher and the plasma concentration is 2–3 times higher than normal [6]. This type of induced RBC destruction is random and does not affect a particular RBC subpopulation. Otherwise the findings of Burger et al. [3, 4] can in no way provide mechanistic details on how aged RBC are selectively cleared in vivo at the end of their life span of 120 days. The CD47/thrombospondin/SIRPα interactions lack the subtleties required to signal a preferential clearance of senescent RBC at a controlled pace. One reason is that about 40% of CD47 are mobile within the plane of the membrane of any cell age [8]. Hence, oxidative damage, aggregation, and the altered conformation of CD47 are induced in an unrestricted manner. Furthermore, binding of thrombospondin-1 or its active peptide stabilizes the new CD47 conformation at any concentration above a minimal dose having sufficient affinity. Thus, the suggested recognition principle lacks means to selectively tag a particular RBC subpopulation. This becomes evident by comparing the properties of the CD47/thrombospondin/SIRPα-induced RBC removal with those operating through a naturally occurring antibody (NAb) to band...
3 protein [for review see 9]. In this system recognition of senescent or oxidatively stressed RBC depends on bivalent binding of anti-band 3 NAbs to band 3 oligomers, but not to preexisting band 3 dimers. Anti-band 3 NAbs [10] have a low affinity and require that their target is presented in form of oligomers. Cross-linkable band 3 oligomers represent a minute fraction of band 3 protein of 1.5 ± 0.3% on young and 1.9 ± 0.3% on senescent RBC (different at a confidence level of 0.06) despite a million copies of band 3 per cell [11]. Band 3 oligomers are formed upon detachment of band 3 protein from the cytoskeleton via selective phosphorylation [12] and binding of oxidatively generated hemichromes to the cytoplasmic portion of band 3 protein, promoting clusterization [13]. Finally, the few anti-band 3 NAbs associating with oligomerized band 3 protein represent an insufficient number to induce phagocytosis [14]. The low number of firmly bound anti-band 3 NAbs is, however, compensated by a massive deposition of C3b induced by bound anti-band 3 NAbs. The reason is that bound anti-band 3 NAbs have a unique affinity for C3 within their Fab arm [15] and therefore preferentially generate C3b-IgG complexes in the presence of active complement [16]. C3b-IgG complexes subsequently stimulate alternative complement pathway C3b deposition because these complexes first bind properdin that greatly enhances factor B binding [17]. This sequence of well controlled processes favors a selective opsonization of in vivo aged and oxidatively stressed RBC and at the same time prevents an excessive opsonization.

References