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 (SIRP α) 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 Oldenburg 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 SIRP α . Experiments with human red pulp macrophages and oxidized RBC showed that phagocytosis increased upon addition of the 4N1K decapeptide, 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 SIRP α and this was sufficient to induce an 'eat me signal', because the inhibitory signal could not be induced by SIRP α . The authors think that binding of the 4N1K peptide to oxidized CD47 induces an 'eat me signal' not by blocking the interaction with SIRP α , but by conveying to oxidized CD47 the ability to interact in a new, so far unknown way with SIRP α 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 erythrophagocytosis, 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 10^3 to 10^4 [6]. Correspondingly, Head et al. [7] found that the 4N1K peptide at the very same high concentration (50 μ g/ml) binds to CD47 without the need to impose a conformational change by e.g. oxidation. Moreover, incubation of RBC with 50 μ g/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/SIRP α 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 RBC 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/SIRP α -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 $1.5 \pm 0.3\%$ on young and $1.9 \pm 0.3\%$ 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 oli-

gomerized 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 C3b₂-IgG complexes in the presence of active complement [16]. C3b₂-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

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