Immune Adherence Revisited: Novel Players in an Old Game

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Erythrocytes bind immune complexes (ICs) composed of antibodies binding their respective antigen (e.g., bacteria, parasites, viruses, or autoantigen) plus complement proteins via complement receptors (immune adherence [IA]). In vivo studies have shown that erythrocytes act as an inert shuttle, targeting ICs to fixed macrophages in liver and spleen. Here we outline established and emerging implications of IA in health and disease.

In 1953, Nelson (14) coined the term “immune adherence” (IA) to describe the antibody (Ab)- and complement-dependent binding of bacteria [i.e., immune complexes (ICs) containing bacteria] to erythrocytes (Fig. 1). In 1979, the receptor on erythrocytes mediating IA was identified and named complement receptor 1 (CR1, CD35) (3). Work on IA in vivo using various model ICs (i.e., ICs consisting of defined antigens reacted with relevant Abs in vitro) later indicated that complement plays a major role in the IA-mediated clearance and splenic localization of ICs in humans (18). Deposition of complement proteins on bacteria, parasites, viruses, or autoantigens allows their interaction with CD35 (Fig. 1). Recently, identification of further contributing mechanisms has refined the molecular understanding of IA (5, 8). Also, IA might regain attention with emerging evidence of an association of the human immunodeficiency virus 1 (HIV-1) with erythrocytes in vivo (7). Here we review the current molecular understanding of IA, discuss established as well as evolving (patho)physiological implications, and speculate on the general significance of these phenomena.

The molecular basis and physiological significance of IA

Targets of IA. In a series of elegant experiments (14), Nelson showed some 50 years ago that bacteria become targeted for IA. In his original work, Nelson incubated bacteria (Treponema pallidum and Pneumococci) with whole serum and autologous erythrocytes. He observed that bacteria became adherent to erythrocytes in the presence of specific antibacterial Abs as well as complement but not after inhibition of complement activity. Subsequently, he showed IA to occur in vivo by using bacteria opsonized with Abs and complement. There is no reason to believe that viruses (if detectable in plasma) can escape IA in the presence of specific, complement-activating Abs. However, until recently there has been no direct evidence for IA of viruses in humans or nonhuman primates, although in vitro data have supported this possibility (13). In addition, in vitro IA has been observed for various parasites (e.g., Leishmania) where in fact IA was demonstrated more than 20 years before Nelson’s work. Not surprisingly, besides whole microorganisms, fragments thereof recognized by specific Abs form ICs capable of activating complement. Such complement-opsonized ICs adhere to erythrocytes in much the same way as whole microorganisms.

Besides infection-associated IA, much interest has been paid to the fact that circulating autoantibodies (autoAbs) recognize their antigens in the bloodstream, forming circulatory ICs in various autoimmune diseases. However, similar to infection-associated ICs, there has to date only been indirect evidence for IA of such autoimmunity-associated ICs. Whether apoptotic bodies or microvesicles released by endothelial or circulating cells are recognized by natural Abs and/or autoAbs, and hence are carried by erythrocytes in the circulation, remains an intriguing speculation.

In summary, the putative targets for IA are numerous, including both “nonself” (infectious and infection-associated antigens) and “self” antigens (autoantigens, apoptotic cells), all to be cleared in a safe way from the circulation.

The IA receptor CD35 and its ligands. CD35 is a single-chain transmembrane glycoprotein (190–280 kDa) expressed on erythrocytes, most white blood cells, tissue phagocytes and glomerular podocytes. The long extracellular domain of the molecule is arranged in four tandems of so-called homologous repeats (LHRs). Except for the LHR proximal to the cell membrane (LHR D), each of the LHRs bears one binding site for the complement proteins C3b and/or C4b (Fig. 2). Recent evidence indicates that CD35 is the receptor for two other opsonins: C1q and mannan-binding lectin (MBL) (9). Through its globular heads, C1q binds the Fc portion of aggregated IgG, whereas MBL binds specific bacterial and viral structures (20). Both molecules appear to attach to the LHR D of CD35 (Fig. 2). C1q as well as MBL may induce/enhance or strengthen IA. It is, however, important to note that, in vivo, no significant IA takes place when C3 activation/deposition is blocked. For instance, on injection of model ICs in humans deficient in classical pathway activation (a prerequisite for the deposition of a relevant amount of C3b on ICs), no IA is observed (19).

Since the affinity of CD35 for monomeric C4b/C3b is low, a relevant avidity depends on the interaction of CD35 with multiple C3b molecules on ICs. The unique clustered distribution of CD35 on erythrocytes, for which the mechanism is
unknown, further increases the avidity of erythrocytes for C3b-coated ICs (15). “High-avidity” (i.e., clustered) expression of CD35 on erythrocytes, combined with their sheer number, is putting this cell type in charge of transporting ICs in the circulation (Fig. 1). This may help to prevent inappropriate activation of phagocytic cells. As mentioned above, activated and covalently deposited C3b is the major ligand for CD35 and is mainly responsible for IA. C4b may play an additional role in IA under specific circumstances. Activation of C3 may occur via one of the three complement pathways: the classical and MBL pathways and the alternative pathway (Fig. 3). In the presence of specific Abs, the classical pathway becomes engaged, whereas binding of MBL to mannose residues initiates the MBL pathway. Both of these pathways lead to the activation and incorporation of the zymogen C2 into an enzymatically active protein complex (C3 convertase) capable of cleaving and activating more C3.

Lack of complement regulation (i.e., absence of complement-regulatory proteins) allows “tick over-activated” (i.e., spontaneously-activated) C3 to establish alternative pathway C3 convertase. This occurs through spontaneous hydrolysis of the thioester bond in C3 to form C3(H2O), which has an altered conformation, supporting binding of the alternative pathway zymogen Factor B. Binding of Factor B by C3(H2O) allows a plasma protease called Factor D to cleave Factor B into Ba and Bb, the latter remaining associated with C3(H2O) to form the C3(H2O)Bb complex. This complex is a fluid phase C3 convertase capable of cleaving many molecules of C3 into C3a and C3b. The thioester bond exposed on C3b is extremely reactive and has no mechanism for distinguishing an acceptor hydroxyl or amine group on a host cell from a similar group on the surface of a pathogen. Deposition of C3b is of central importance in IA, because binding to CD35 depends on the number as well as spatial distribution of fixed C3b. In addition, factors such as size of a given IC, composite complement activity, and genetic background (e.g., different alleles are associated with differing levels of receptor cell surface expression) all influence the efficiency with which IA occurs.

Besides its receptor function, CD35 is also a major cofactor for the inactivation of C3b by the ubiquitous C3b-inactivating enzyme Factor I, a function CD35 has in common with

FIGURE 1. Antibodies are complemented by complement (hence the name) in mediating binding of bacteria to erythrocytes. The model depicts the following sequence: binding of bacteria-specific antibodies, activation of the classical pathway of complement, deposition of activated complement components on bacteria/antibody immune complex (IC), and binding of the IC to complement receptor 1 (CR1, CD35) expressed by an an erythrocyte.

FIGURE 2. Schematic structure of the receptor mediating immune adherence (IA) of CD35. Binding sites for the activated complement fragments C4b and/or C3b were mapped to the distal 3 short consensus repeats (SCRs) of long homologous repeats (LHR) A–C, the binding site for C1q, and mannan-binding lectin (MBL) to LHR D.
example, it has been suggested that HIV-1 binds erythrocytes through interaction with the Duffy antigen receptor for chemokines (DARC) (10). The in vivo relevance of such interaction needs further scrutinizing; e.g., it will be important to test whether DARC-deficient HIV-1-infected individuals differ in their HIV-IC binding capacity (see IA and infectious diseases, below) and, if so, whether a modified IC-binding capacity has clinical implications.

**IA in human disease**

Although the fate of model ICs and their binding mechanism to erythrocytes has been extensively studied, relatively little research has focused on what type of antigens are involved in IA in human diseases. Both autoimmunity and infection-derived ICs are thought to be potentially pathological.

**IA and autoimmunity.** Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of many non-organ-specific autoAbs such as anti-DNA and anti-C1q Abs with the corresponding antigen being preserved in plasma. The inevitable formation of ICs between these autoAbs and their target antigens might be followed by IA and clearance of the complexes by fixed macrophages in liver and spleen. Experimental data in nonhuman primates have clearly shown that ICs unable to bind erythrocytes are more likely to become deposited in small vessels such as the capillaries of the glomerular tuft in the kidney (6). Expression of complement-regulatory proteins in the glomerulus can be changed by many factors, including complement attack itself, and expression levels are affected in various glomerular disorders. Corollary to this, one might think of erythrocytes as a buffer system that, by providing IA, prevents the pathological deposition of autoantigen-containing ICs in tissues as seen in SLE.

Interestingly, IA is deficient in patients with active disease. Indeed, complement becomes activated and depleted by the disease process, and erythrocytes lose their CD35 receptors by an unknown mechanism (19). Both deficits might be related to the continuous formation of ICs. Thus there seems to be some kind of vicious cycle with excessive generation of ICs leading to consumption of the normal clearance system followed by pathological IC deposition, which in turn generates even more complement activation and depletion. Both starting levels of CD35 and pathological amounts of circulating ICs may initiate such a vicious cycle.

**C1q-deficient individuals** provide another angle to look at this assumed pathophysiological process. This deficiency does not allow the classical pathway to function. Hence ICs are not opsonized and consequently cannot be cleared through IA. These individuals almost invariably (>90%) develop a lupus-like disease. Besides the deficient clearance of ICs in these individuals, emphasis has recently focused on the role of complement in the clearance of apoptotic cells and bodies, which express many of the antigens recognized by pathologica autoAb. Bott et al. (1) have shown that C1q knockout mice develop a lupus-like phenotype and accumulate pathological levels of apoptotic bodies within the glomerular tuft of the kidneys. A similar abnormal clearance of apoptotic cells or bodies might also be relevant in humans with C1q deficiency or
those with deficiencies of other components of the classical pathway of complement activation, all prone to autoimmune disease. Hence one might speculate that IA retains apoptotic bodies in the circulation until cleared by sessile phagocytes in the liver or spleen. The appropriate removal from the circulation of apoptotic bodies derived from circulating and endothelial cells by IA might prevent activation of autoreactive B cells recognizing potential autoantigens expressed on the surface of the apoptotic cells (16).

**IA and infectious diseases.** In general, IA-dependent clearance of pathogens from the circulation is thought to be beneficial for the host, but infectious agents may take advantage of being transported to cells susceptible to infection e.g., in the spleen (11).

Chronic viral infections, such as HIV and hepatitis C virus (HCV) or hepatitis B virus infection, are characterized by the simultaneous presence of virions and virus-specific Abs in the bloodstream, forming circulating ICs. In vitro, ICs formed by incubating HIV-1 with envelope-specific Ab and normal human serum as a source for complement have been shown to readily bind to K562 cells expressing recombinant CD35 as well as to erythrocytes (13). Recently, we found evidence that a similar association between HIV-1 and erythrocytes occurs in vivo (7). Interestingly, HIV-1 was found associated with erythrocytes even after prolonged suppression of viral RNA in the plasma. Given the short half-life of erythrocyte-associated ICs (minutes), detection of HIV-1 on erythrocytes after prolonged periods of undetectable HIV-1 RNA in plasma provides direct evidence for ongoing virus replication/release in these individuals. Abnormal handling of HIV-1 ICs by splenic macrophages of chronically infected individuals may further contribute to accumulation of HIV-1 on erythrocytes. Other reservoirs of cell surface-bound HIV-1 include dendritic cells, B cells, and follicular dendritic cells (FDC) (4, 12). Binding of HIV-1 to FDC and B cells also occurs through the interaction of complement fragments on HIV-1 ICs with complement receptors expressed on both cell types (12). Cofactor function of CD35 on erythrocytes has been speculated to promote degradation of C3b fixed on HIV-1 ICs, thereby favoring their subsequent binding to B cells and FDC. In this respect, it will be relevant to compare infectivity of HIV-1 associated with erythrocytes and HIV-1 in plasma. Indeed, the opsonization of HIV-1 with C3b has been shown to facilitate infection of leukocytes expressing complement receptors, and some findings suggest that the tighter the association of the cell-bound virus to sensitive target cells the more efficient the transfer of infectious virus. Various processes must be involved in the maintenance of virus infectivity in the ICs of red blood cells, because complement in plasma has direct antiviral activity and the viruses seem to be protected from neutralizing Abs that can rapidly inactivate HIV in plasma. Thus understanding the role of various cell-surface receptors and adhesion molecules in the interaction and transfer of HIV-1 may allow identification of future targets for therapy. Future work will have to define the pathophysiological role of IA in HIV and other chronic viral infections, because IA may differ between vari-

![Figure 4](http://physiologyonline.physiology.org/)
ous pathogens. In chronic HCV infection, for example, high levels of rheumatoid factors (antibodies often correlating well with the activity of clinically manifested autoimmunity) are present and are known to interfere with IA through depletion of complement and physical hindrance of C3b-coated ICs to bind CD35. ICs formed by virus, antiviral Ab, and rheumatoid factors are thus more likely to become deposited in tissues. One thus may speculate that the high incidence of IC-mediated vasculitis/arthritis/glomerulonephritis seen in HCV infection relates to deficient IA-dependent clearance of ICs in this particular disease.

References


