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Presence of autoantibodies against complement regulatory proteins in relapsing-remitting multiple sclerosis

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Complement was proposed to play an important role in the onset of Multiple Sclerosis (MS) lesions by inducing physical damage to myelin-producing cells. Every somatic cell is however endowed with a repertoire of membrane-bound molecules which normally down-regulate the complement activation cascade (Regulators of Complement Activation, RCA) and therefore protect cells from complement-dependent lysis. We show here that antibodies against two complement regulatory molecules expressed in the membrane of human cells (CD46 and CD59) are present in sera from relapsing-remitting MS patients in the acute phase, that they are directed against the active site of the RCA molecules and that they inactivate their regulatory function, thus providing a mechanism by which cells of the nervous system might be damaged in a complement-dependent fashion during the acute MS phase. Moreover, we found that most of these sera also contain antibodies reacting with an epitope of the transmembrane glycoprotein of HIV which is conserved in most retroviruses; this may support the hypothesis that self-reacting antibodies might have arisen in these patients as an immune response after retroviral infection or expression of endogenous retroviral proteins. *Journal of NeuroVirology* (2000) **6,** S42–S46.

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The complement system and complement regulatory proteins

The complement system consists of several plasma proteins with effector function, soluble regulatory proteins and cell surface-anchored receptors, some of which also display regulatory function. The complement system can be triggered by antigen-antibody complexes (classical pathway) or by cell surface polyanions (alternative pathway). In both cases, the central event in complement activation is the cleavage of the C3 protein: in fact, both the classical and alternative pathways of complement activation lead to the cleavage of C3 in two fragments, C3a and C3b. The C3 protein contains a cryptic and highly reactive thioester ring which becomes exposed in the C3b fragment and mediates C3b fixation on cell surfaces. C3b fixation is the critical step of a process which leads to the formation of a pore in the target membrane (the Membrane Attack Complex, MAC) which eventually results in cell lysis (Figure 1a). The complement system may also be directly activated through the alternative pathway by membrane polysaccharides, a process which does not require antigen-antibody (Ag-Ab) complexes and is therefore potentially harmful to the host cells; however, every human cell expresses different kinds of regulatory molecules which inhibit MAC formation at different stages and is therefore protected from unwanted triggering of the complement pathway. Moreover, soluble regulators of complement are present in high concentration in blood and may bind to cell surface structures, thus providing additional protective mechanisms. Some of these regulatory molecules contain C3b-binding sites and either inhibit C3b formation by C3 convertases (Decay-accelerating function of DAF(CD55), CR1 (CD35) and CFH) or promote C3b cleavage by a specific protease, Factor I (cofactor function of MCP (CD46), CD35 and CFH). Other factors, such as protectin (CD59), block the final step in the

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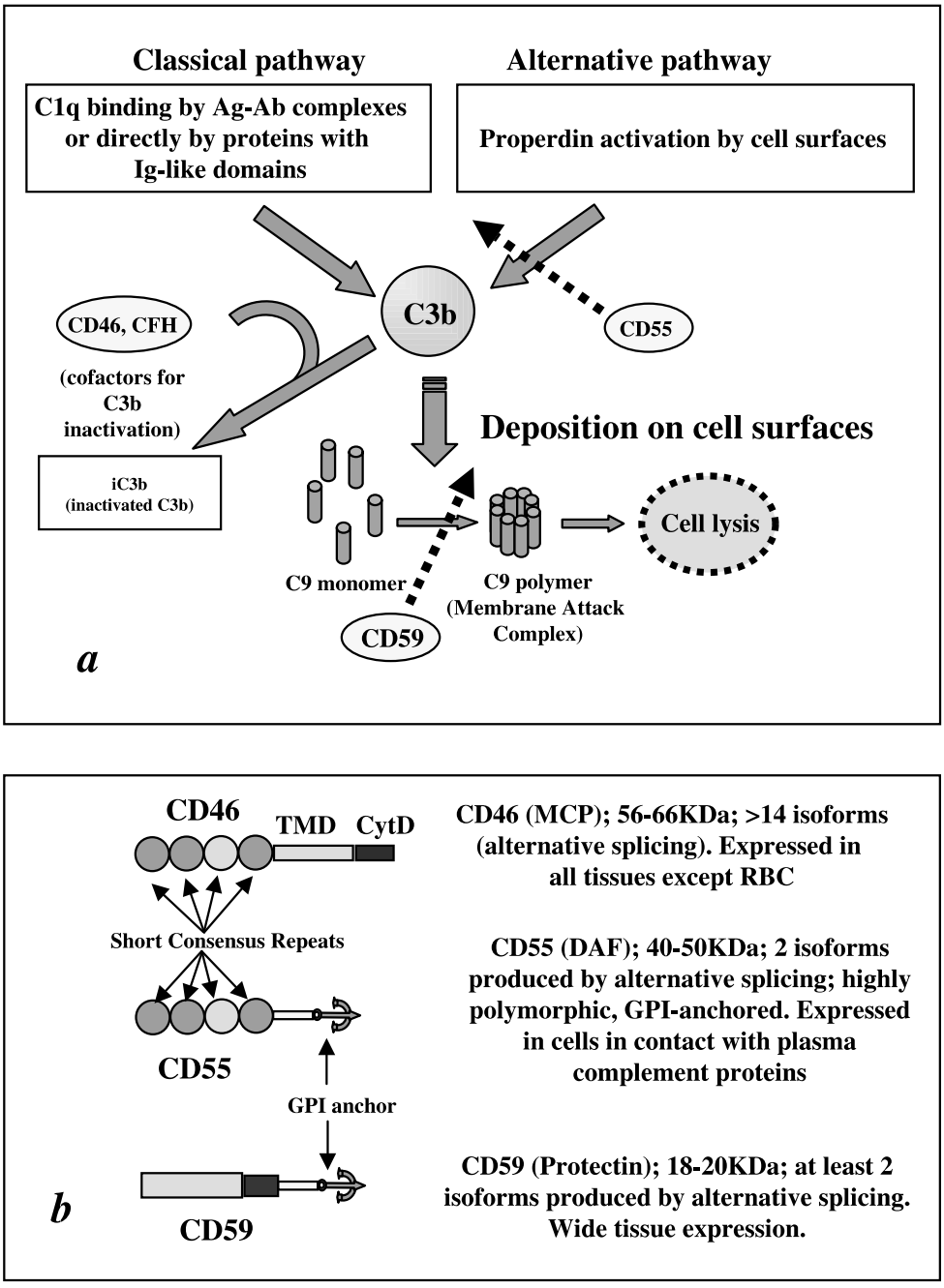

Figure 1 (**a**) Schematic representation of the complement activation pathway and its regulation by RCA proteins. (**b**) Features of the main membrane-bound regulators of complement activation. TMD=Transmembrane Domain; CytD=cytoplasmic domain.

formation of Membrane Attack Complexes and therefore provide an additional mechanism for cell protection. The main features of membrane-bound RCA molecules are shown in Figure 1b.

Possible role of complement in damage to cells of the nervous system

There is increasing evidence for the involvement of complement in the pathogenesis of MS (Storch *et al*, 1998; Trbojevic *et al*, 1998; Vedeler *et al*, 1996). As a consequence of damage to the blood-brain barrier (BBB), an intrathecal immune response to neural antigens is triggered which results in activation and deposition of complement components on cells of the nervous system. As a result of production of antibodies to myelin components, myelin-producing cells may die through a direct (formation of a Membrane Attack Complex, MAC) or/and indirect (triggering of phagocytes) complement-dependent process.

Role of viruses in breaking the immunological tolerance against neural antigens

Normal subjects may contain immunocompetent (IC) cells specific for neural antigens. However, a functional BBB usually restricts the access of these cells to the neural structures they might recognize (peripheral tolerance) (Kumar, 1998; Morgan *et al*, 1999). Upon viral infection, epitopes harboured by viral components cross-reactive with self proteins might trigger a specific clonal expansion. Upon breakage of the BBB, these IC cells may reach the sites where the self antigens are expressed and raise an intrathecal immune response with consequent damage to nervous system components.

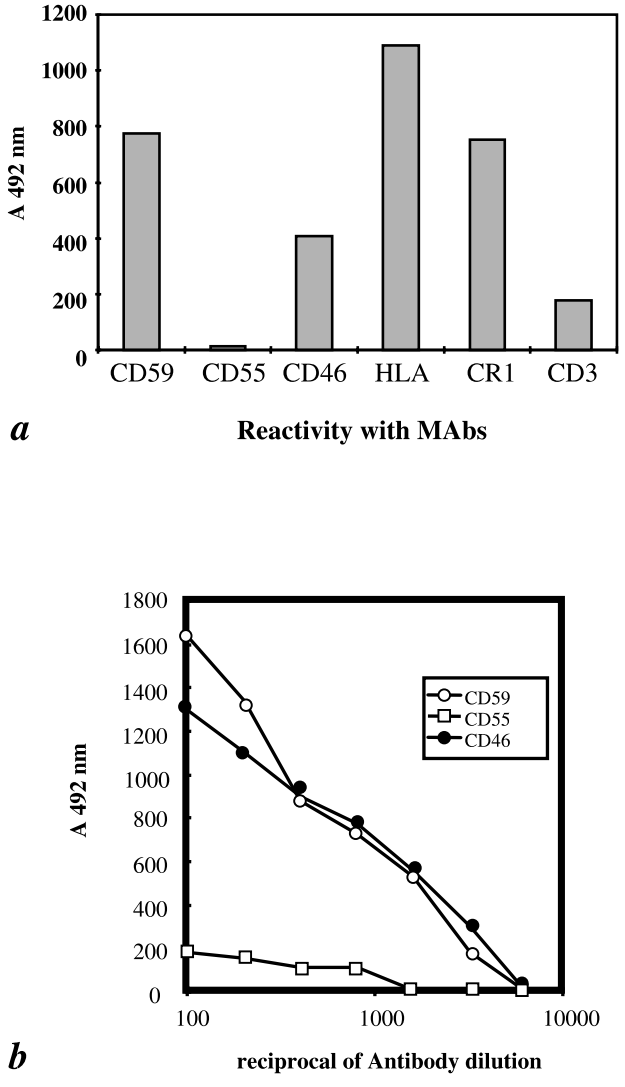

Figure 2 Expression of RCA molecules on human cells. (**a**) SHSY cells were attached to ELISA plates pre-coated with poly-L-lysine and exposed to specific monoclonal antibodies (MAbs). As shown, only CD35 (CR1), CD46 and CD59 are expressed in this cell line. (**b**) U937 myelomonocytic cells were used as described in 2a. In these cells, only CD46 and CD59 were expressed.

We therefore investigated the possibility that antibodies against cell-anchored complement regulatory proteins might be present in MS patients' sera and facilitate complement-dependent cytotoxicity in cells of the nervous system.

Expression of complement regulatory molecules in neural and leukocyte cell lines

The expression of complement regulatory molecules in neural and leukocyte cell lines was detected using an attached cell ELISA: briefly, the cells were seeded on ELISA microplates pre-coated with poly-L-lysine and exposed to specific monoclonal antibodies (MAbs) against RCA molecules (1 h, RT). After washing, a secondary species-specific anti-immunoglobulin antibody labelled with horseradish peroxidase was used for the detection of the bound primary Ab (1 h, RT). The reaction was developed with O-phenylenediamine (OPD) and read in a Microplate Reader (BioRad) at 492 nm. As shown in Figure 2a,b, neural SHSY cells (American Type Culture Collection, ATCC) express CD35, CD46 and CD59 but not CD55, and the same was observed for the monocytic U937 cells (ATCC).

Presence of antibodies reacting with CD46 and CD59 in MS patients' sera

The presence in MS sera of antibodies specific for CD46 was checked using recombinant CD46 (Towbin *et al*, 1979) (rCD46, 0.1 μg/assay, kindly provided by Dr Sarah Russell, University of Melbourne, as the solid phase in ELISA. Eighty per cent of RR-MS patients in the acute phase of the disease showed high levels of anti-CD46 antibodies. The antibody specificity was also confirmed in Western blot assays using cell membrane proteins, as described in Figure 3a,b. The Western blots were

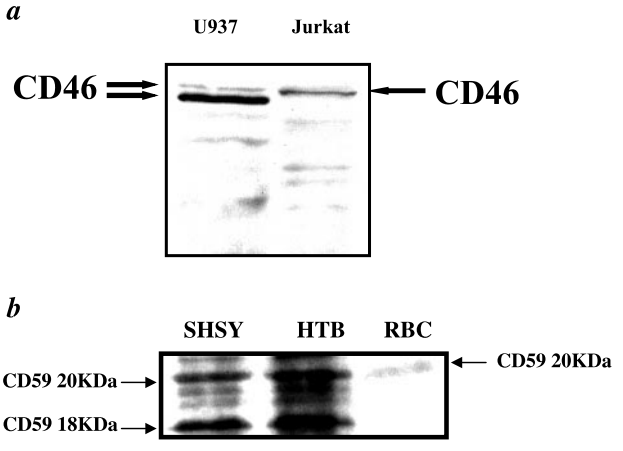

Figure 3 Antibodies against RCAs in patients with MS. (**a**) The results of this Western blot show that RR-MS patient sera contain antibodies which recognise the CD46 molecule. (**b**) The results of this Western blot show that antibodies from RR MS patients recognise the 20–18 kDa form of the CD59 molecule expressed in the neural cell lines SHSY and HTB.

performed according to the method described in (Christiansen *et al*, 1996); briefly, cells were lysed in hypotonic Tris-buffer (10 mM Tris, 40 mM NaCl, pH 7.0) with 2% NP40. Nuclei and unbroken cells were discarded by differential centrifugation and the membrane fraction boiled 5′ in 2.5% SDS. Samples were applied to SDS gels with acrylamide percentages optimized for the protein molecular weights. After the run, proteins were electrophoretically transferred to nitrocellulose filters and exposed to antibodies specific for RCAs and to sera from MS patients.

Binding of antibodies from MS sera to neural cell lines

SHSY cells immobilised on ELISA plates were used as the solid phase in the assays to assess the presence of antibodies in chronic, RR and control sera. We observed that, based on the reaction intensity of the antibodies, sera could be grouped in three different classes: non-binders, medium binders and high binders (data not shown). Control sera from normal subjects and sera from chronic progressive MS did not contain antibodies to SHSY. Only RR-MS sera were shown to

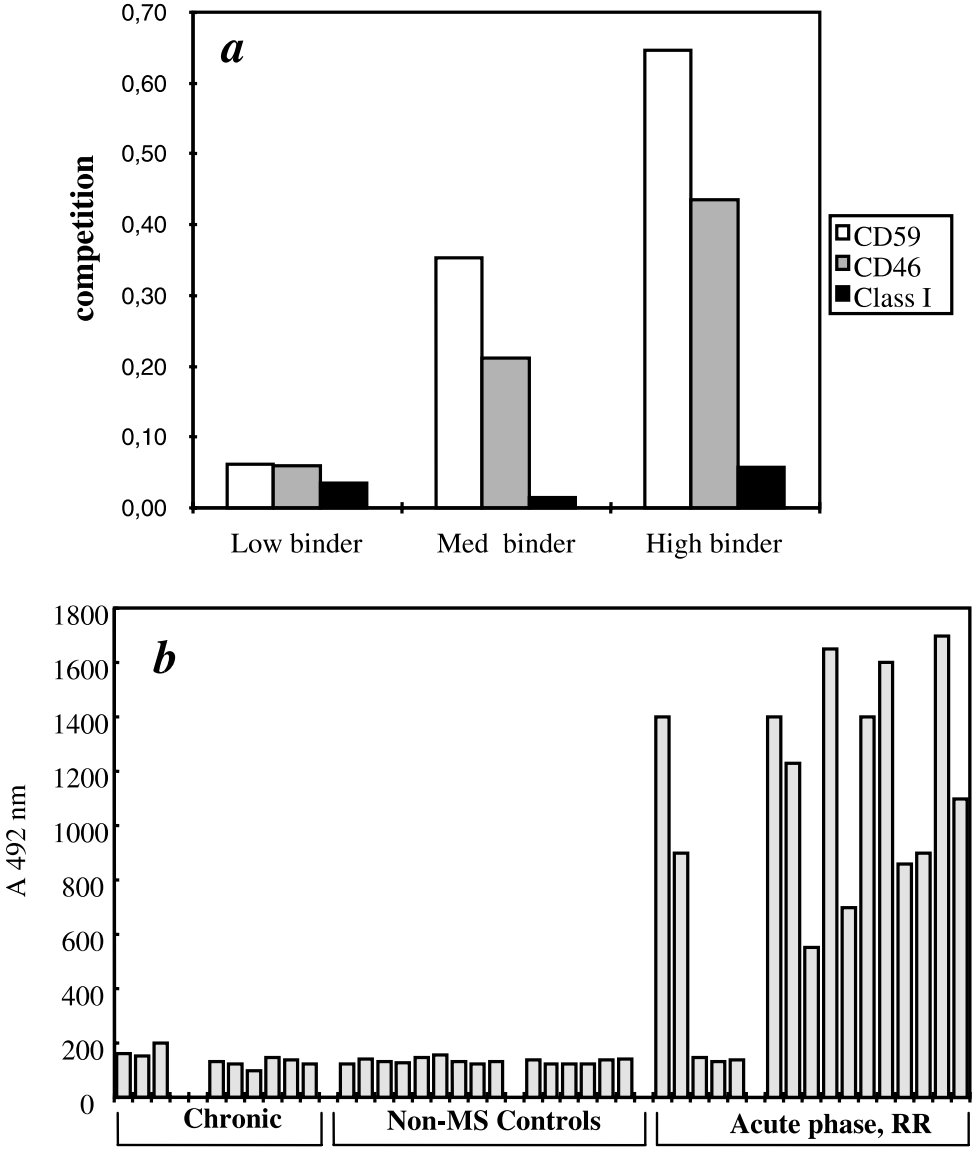

Figure 4 (**a**) Competition of the interaction of specific neutralizing monoclonal antibodies with SHSY by RR-MS sera. Antibodies in MS sera efficiently compete the binding of anti-CD46 and anti-CD59 MAbs to SHSY cells. These results suggest that the patient's antibodies recognise the binding site of the RCA molecules. (**b**) Presence of antibodies against an HIV-derived peptide antigenically homologous to RCAs in RR-MS patients in the acute phase. Peptide number 18 (NKSLEQIVNNMIWMEWDREINNYIS) was used to coat ELISA plates and exposed to sera from control subjects and from chronic progressive and relapsing-remitting patients in the acute phase. As shown, most of the RR patients in the acute phase had antibodies specific for this peptide.

contain antibodies reacting significantly with SHSY cells.

Anti-RCA antibodies in MS sera are directed against the active site of the molecule
MAbs blocking the function of CD46 and CD59 were used as tracers in an attached cell ELISA to check whether or not the Abs contained in MS sera were directed against the active site of the RCA molecules. As shown in Figure 4a, sera from high and medium binders were effective in competing the binding of neutralising antibodies to the cell line SHSY. MAb W6/32, directed against a monomorphic HLA Class I epitope, was used as a negative control of the assay.

Anti-RCA antibodies from MS sera induce complement-dependent killing of SHSY cells
MAb W6/32 was used to activate fresh human complement on SHSY cells. Heat-inactivated MS sera in the acute phase were used as a source of human antibodies against RCA molecules. Cytotoxicity was measured using a Trypan Blue exclusion assay. High and medium binder subjects contained antibodies which induced an enhanced complement-dependent cell cytotoxicity, most probably due to their capacity of blocking the active sites of the complement regulators. Therefore, the presence of antibodies against RCA molecules in these patients might really have a functional relevance.

Acute phase RR MS sera contain antibodies against a gp41 peptide antigenically cross-reactive with CD46
As shown in Figure 4b, antibodies against peptide number 18 (Pintér *et al*, 1995), corresponding to a conserved epitope of HIV-1 gp41 which is cross-reactive with RCA molecules, are also present in sera from RR, MS subjects in the acute phase. This suggests that this epitope might have stimulated the immune system of these subjects, either as a consequence of retroviral infection, or due to activation of endogenous retroviral analogues of the transmembrane protein (Alliel *et al*, 1998). This observation clearly deserves further attention.

Conclusion

We observed the presence of antibodies which neutralise RCA molecules during the acute phase of RR-type MS. These antibodies are in fact able to compete the binding of commercially available MAbs which are directed against the binding site of these proteins, and can also induce *in vitro* complement-dependent killing of cells, provided that complement is activated through the classical pathway. These observations suggest that when complement is activated *in vivo* by antibodies against neural antigens, the high levels of anti-RCA Abs inactivate the regulatory molecules, leading to deposition of activated complement components and, consequently, a higher susceptibility to direct as well as indirect complement-mediated cell damage.

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