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Ian Spendlove · Judith M. Ramage · Richard Bradley
Claire Harris · Lindy G. Durrant

Complement decay accelerating factor (DAF)/CD55 in cancer

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Abstract The complement system is a powerful innate mechanism involved in protection of the host against pathogens. It also has a role in the clearance of apoptotic cells and has been implicated in a range of pathologies including autoimmunity and graft rejection. The control of complement is mediated through the complement regulatory proteins (CRPs). These are present on most cells and protect normal cells from complement-mediated attack during innate activation. However, in a range of pathologies and cancer, these molecules are up or down regulated, sometimes secreted and even lost. We will review the expression of CRPs in cancer, focussing on CD55 and highlight other roles of the CRPs and their involvement in leukocyte function. We will also provide some data providing a potential mechanism by which soluble CD55 can inhibit T-cell function and discuss some of the implications of this data.

Keywords EGF-TM7 · γ-IFN · Complement · Co-stimulation · Extracellular matrix

Introduction

The complement system provides a powerful means of control of both pathogenic organisms and clearance of apoptotic cells. It forms a large component of the innate immune system and through its activation serves to bridge innate and adaptive immune responses. The complement proteins are a heterogeneous mixture of more than 30 proteins found in plasma and on the cell surface. The main components are proteolytic enzymes, which when activated allow activation of the next component in the cascade. The central regulatory components are the active C3 convertases (C4b2a and C3bBb) and C5 convertases (C4b2a3b and C3bBb3b) which serve to amplify the cascade [81, 82]. This results in the deposition of complement components, primarily C3b and iC3b; then, following further cleavage, C3c and C3dg [53, 43]. These act as opsonins for various receptors of the C3 products. These complement receptors are present on different cell subsets and are known to modulate various effector functions on these cells. Left unchecked, the complement cascade leads to the eventual formation of the C5b-9 pore-forming membrane attack complex (MAC). Under pathological conditions, this leads to a rapid depolarisation of the cell membrane and lysis. However, non-lethal MAC formation is also known to result in cellular activation, indicating that tight control of MAC formation may influence regulation of cellular immune responses [58].

The C3 convertase can be activated in one of three ways. The classical pathway is activated by antibodies (in humans, IgG1, IgG3 and IgM). These are recognised by the C1 components which become activated and cleave C4 to C4b/C4a, deposition of 4b onto cells and association with 2a to generate the C3 convertase. The mannose-binding lectin, which recognises terminal mannose residues on a range of microbes, results in a similar activation of the classical pathway C4b2a convertase. The alternative pathway activation follows breakdown of C3 to C3b/C3a, deposition onto cells and association with factor B. Activation of the C3 convertase results in amplification of the cascade and an increase in the production of the soluble anphylatoxins (C3a and C5a) which act as chemokines

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I. Spendlove (✉) · J. M. Ramage · R. Bradley · L. G. Durrant
CR UK Academic Department of Clinical Oncology,
Institute of Infections Immunity and Inflammation,
The University of Nottingham, NG5 1PB, Nottingham, UK
E-mail: Ian.Spendlove@Nottingham.ac.uk
Tel.: +44-115-9628035
Fax: +44-115-9627923

C. Harris
Department of Medical Biochemistry and Immunology,
University of Wales College of Medicine, Heath Park,
CF14 4XN, Cardiff, UK

recruiting a range of cells to sites of complement activation. The amplified cascade also results in the deposition of C3 fragments and the C5b-9 components on the cell surface, resulting in formation of the MAC [60].

The membrane bound complement regulatory proteins (mCRPs)

During complement activation, normal cells in the local environment are susceptible to complement-mediated damage. In order to protect host cells from bystander killing and as a mechanism of regulation of complement, all cells have complement regulatory proteins (CRPs) associated with their cell membranes. This group of proteins, CD35, CD46, CD55 and CD59, all contribute to the inactivation of complement and thus dampen the activated complement cascade. They belong to the regulators of complement activation (RCA) family and are charactrerised by the presence of short consensus repeat (SCR) domains [31].

CD35 (complement receptor 1; CR1) is broadly restricted in expression to haematopoietic cells including monocytes and granulocytes. It acts as a cofactor for a soluble complement regulator (factor I; fI) and together they cleave C3b to iC3b, and then C3c and C3dg, thus regulating C3 convertase activity [5].

CD46 (membrane cofactor protein) works like CD35 in acting as a cofactor for fI, cleaving the C3b and C4b components of complement. It is expressed on virtually all cells except erythrocytes and two splice variants can often be found on the same cell type [70].

CD59 (Protectin) is glycosyl phosphatidylinositol (GPI), anchored to the cell membrane. Its expression is similar to that of CD55 and together they are notable markers of the haematopoietic clonal disorder paroxysmal nocturnal haemoglobinurea (PNH). This condition is characterised by a susceptibility to red blood cell (RBC) lysis due primarily to the lack of CD59. It acts by sequestering the C8 and C9 components, preventing C9 polymerising into the pore-forming MAC. CD59 acts as a last line of regulation of complement prior to MAC formation and along with CD46 and CD55 protects normal cells from bystander complement-mediated damage [57, 15].

CD55 (decay accelerating factor; DAF) is also a GPI-anchored protein that is expressed on the surface of virtually all cells. It functions by dissociating the C3 convertases (C4bC2a and C3bBb) and consequently the C5 convertases into their component parts, after which they are no longer able to reassociate. Unlike CD35 and CD46, CD55 does not act in a proteolytic way but accelerates the decay of the convertases. Recent studies have shown that the kinetics of association/dissociation of C3bBb with CD55 were too rapid for affinity measurements to be made by biacore [25].

Decay-Accelerating Factor and Cancer

The cytoprotective role of mCRPs has made them an attractive target in a number of clinical situations, primarily where the use of monoclonal antibodies has been employed. In recent years, this has included an increasing number of anti-tumour antibodies. Their mechanisms of action vary and are often complex including multiple effector functions. The EGF receptor antibodies can, according to the specific antibody, block ligand binding, receptor dimerisation and induce apoptosis/oncosis. These clinical antibodies also have the potential of activating complement and associated cellular mechanisms. Complement-dependent cytotoxicity (CDC) is mediated by the C1 complex-initiated complement activation. This results in anaphylatoxin production and deposition of C3b fragments on the target cells. The endpoint of this reaction is MAC formation and cellular lysis. Antibody-dependent cellular cytotoxicity (ADCC), where recognition of the Fc portion of the antibody by Fc-receptor expressing NK cells, monocytes/macrophages and granulocytes results in activation of phagocytic or lytic properties of the effector cells. Recently, it has been demonstrated that the membrane-associated C3 fragments, particularly iC3b, can enhance ADCC. This occurs by recognition of iC3b by the CR3 receptor in conjunction with antibody-Fc-receptor (CR3-enhanced ADCC). However, engagement of iC3b alone appears not to be sufficient to activate effector cells. For this to occur requires bacterial polysaccharides such as β-glucan to prime the CR3 receptor (reviewed in [17]). However, many of the clinically used antibodies do not rely heavily on this mechanism due to the presence of mCRPs on the surface of tumour cells that can effectively regulate complement-mediated lysis induced by antibodies to tumour antigens [7]. Since these early studies, the expression of mCRPs has been measured in a range of tumours and variations in expression are found, although it would appear that there is a general increase in expression of the different CRPs and that these vary between tumour types, reviewed in Fischelson [14]. CD55 has been measured in a range of tumour types when an antibody 791T/36, later identified as recognising CD55 [78], was used to image tumours including colon [42, 45, 13, 1, 27], breast [48, 84], lung [80], gastric [33, 27, 45], ovarian [4,79,3], thyroid [61], prostate [16], pancreatic [72,8], melanoma [73], glioma [51], oesophageal [75, 30] and cervical cancer [77], where there generally appears to be a dysregulation of expression with some tumours overexpressing CD55 while others appear to have a reduced expression.

Prognosis

It has frequently been suggested that loss of expression of one of the regulators would be compensated by increase in expression of one of the others. Analysis of

tissue microarrays has begun to address this issue. A large series of both breast ($n = 800$) and colorectal carcinoma tissue ($n = 500$) archives have been used to characterise expression patterns of the CRPs and determine their influences on prognosis. In breast carcinoma, >90 % of tumours expressed both CD55 and CD59; the majority of these only had weak expression and loss of expression for both markers was associated with poor survival [50,48]. This, however, was in contrast to colorectal carcinoma in which overexpression of CD55 was seen to be a marker of worsening prognosis [10], and CD59 followed a similar trend [83]. In both these studies, CD46 showed the least variation in expression levels and was also found on most of the tumours [83,49]. Preliminary analysis of these tumours for expression of all the CRPs indicates that at least one of the CRPs is always expressed and supports the hypothesis that loss of one CRP may be compensated by up-regulation of another (unpublished observation). Whether the variations in expression reflect the activities of ongoing immune surveillance involving complement or are a result of dedifferentiation of the tumours remains unclear.

Soluble CRPs

Soluble complement regulatory proteins (sCRP) have been reported in blood, urine and tears at low levels [54]. Similarly, in a range of chronic inflammatory conditions, there have been reports of elevated sCRPs. sCD55 has been reported in rheumatoid arthritis and in inflammatory bowel disease [54, 45, 32]. Similarly, there are reports of extracellular and sCRPs in a range of tumours including the ascities of ovarian carcinoma [4, 3], gastric and colorectal cancer. These observations originated from the use of a tumour-targeting antibody 791T/36, which was used in immunoscintigraphy studies on a range of tumours. These included osteosarcoma [67, 64, 12], gastric, colorectal, pancreatic [27] and ovarian carcinoma [63] and also metastatic disease in the liver [2]. In each of these, the antibody showed specificity for the tumours. Explant and histological analysis revealed that the antibody localised to both the stromal and cellular components of many of these tumours [65]. During these early studies, there were no adverse effects observed in over 100 patients imaged from the use of an anti-CD55 targeting antibody, which is supportive of the proposals of using CD55 targeting strategies for a range of antibody-mediated immune therapies. The aim of these studies is to enhance the action of these tumour-targeting antibodies by utilising CRP blocking antibodies [17, 76].

The presence of extracellular and soluble CRPs has been reported in a range of pathological conditions. CD46 has been shown to be shed from tumour cells in ascites of ovarian carcinoma by metalloproteinases [20]. Similarly, secretion of CD59 has been observed in a number of cell lines and in ascitic fluid of ovarian cancer [3, 36, 15].

CD55 has been reported in the synovial fluid of rheumatoid arthritis [35] and IBD. In colorectal cancer and IBD, sCD55 is present in stool [41, 37]. This was shown to be released by protease activity, is a marker of poor prognosis and has been proposed as a diagnostic marker [41]. A range of cell lines from different cancers are known to deposit CD59 and CD55 into their extracellular matrix [29, 45, 59, 15]. Similarly, this extracellular deposition of CD55 is seen in colon and gastric [45, 59] cancer.

Alternative roles for CRPs

The presence of extracellular and soluble forms of CRPs has obvious cytoprotective effects, particularly as these forms appear to maintain their regulatory activity [59, 54]. However, there is also increasing evidence that the CRPs have a wider range of activities than regulation of complement. Co-ligation of CD46 and CD3 has been shown to costimulate T cells resulting in increased effector function [85, 38]. In naïve T cells, this has been shown to stimulate the production of a T-regulatory phenotype which can exert their regulatory function by perforin-mediated killing of target cells [19]. Similarly, CD59 has been shown to co-stimulate NK cell activity but only in association with restricted co-receptors and has also been shown to be involved in CD3-mediated signalling of T cells [52, 6, 66]. CD55 has been shown to co-stimulate T cells when engaged and cross-linked by antibodies [74, 9]. Recently, CD55 was demonstrated to be a ligand for CD97, a member of the EGF-TM7 family of receptors. CD97 is an early activation marker on leukocytes that appears absent from thymic cells and is upregulated within 4 hours of activation of T-cell [11]. CD97 was identified as a receptor for CD55 when CD97-transfected cells were shown to interact with RBCs that could be inhibited with antibodies to both CD55 and CD97 [22].

Methods

CD55 was purified as previously described [78]. Briefly, 2×10^9 791 T cells were harvested and lysed (50 mM Tris–HCl pH 8.5, 150 mM NaCl containing 1% octyl glucoside and proteinase inhibitors: 0.1 mM PMSF, 5 mM EGTA 25 mM benzamidin, 10 μg/ml leupeptin (Sigma, UK)) for 1 h at 4°C. Cell lysate was cleared by centrifugation at 13,000*g* for 10 min and 100,000*g* for 30 min. Affinity chromatography was carried out on protein A-Sepharose cross-linked to 791T/36 MAb and the column washed with 50 mM Tris–HCl pH 8.0, 0.3 M NaCl, 0.1% NP-40. Antigen was eluted with 50 mM diethylamine pH 11.5, 0.5% NP-40, neutralized by adding 200 μl of 1 M Tris–HCl pH 8.0 and analysed by SDS-PAGE and silver staining. Protein concentration was measured by bicinchoninic acid (BCA) protein assay kit (Sigma–Aldrich, UK) and dialysed twice against

sterile PBS prior to use. Recombinant CD55-Fc was generated as previously described [24]. Briefly, SCR domains 1–3 and 1–4 were PCR cloned inframe and 5′ to a human IgG1 Fc region. CHO cells were transfected and supernatant collected for CD55-Fc fusion protein purification. Protein was purified on protein-A sepharose, eluted and dialysed against PBS. Recombinant proteins were tested against a range of anti-CD55 antibodies and also for their ability to inhibit complement deposition [24].

T-cell media (TCM) comprised batch-tested RPMI 1640 supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, 2 mM glutamine, 20 mM HEPES, 2 mM sodium pyruvate, 1:100 non-essential amino acids (Sigma–Aldrich, UK.), 5% heat-inactivated (HI) Human Male AB serum (5%)(First Link, UK).

FACS analysis

Cells were harvested and immunolabelled with 1 μg of antibody per 1×10^6 cells for 1 h at 4°C. Cells were then washed and FITC goat anti-mouse used as the detecting antibody for 30 min at 4°C prior to analysis by FACS scan (Becton Dickenson). The adherent monocytes cells were immunolabelled with antibodies to CD55, CD14, MHC class I, MHC class II and CD3 (as an indicator of T-cell contamination). T cells clones were immunolabelled with antibodies to CD3, CD4, CD8, CD16, CD45 RA/RO, CD56 (Dako Ltd, UK) and CD97 (gift from Jorg Hamann).

Generation of T-cell clones

Peripheral blood mononuclear cells (PBMC) were obtained from heparinised blood, separated by centrifugation over histopaque 1077 (Sigma, UK), washed and re-suspended in TCM. T cells were generated by seeding PBMCs at 2×10^6 cells/well on a 24-well plate for 7 days with 20 μg/ml peptide (CD4 peptide was from the Tie-2 receptor tyrosine kinase [GGITIGRDFEALMN QHQDPLEV], the CD8 peptide was also from Tie-2 and contained within this sequence [ITIGRFEAL] [68]. The responding T cells were cloned by limiting dilution at 5, 1 and 0.5 T cells/well in 20 μl terasaki wells using 1×10^6/ml irradiated autologous PBMC as antigen-presenting cells (APC), 50 U/ml recombinant IL2 and 20 μg/ml peptide. Clones were expanded at 21 day intervals by restimulation with 5×10^5/ml irradiated allogeneic (PBMC), IL2 (50 U/ml) and 2 μg/ml phytohaemagglutinin (PHA). Antigen-specific proliferation of clones was examined at least 10 days after restimulation with PHA. The specificity of clones was assessed by co-culture of $(2–4)\times10^4$ T cells, with peptide and either 5×10^4 autologous irradiated PBMC or 1×10^5 PBMC adhered for 2 h before removal of non-adherent cells.

PBMCs (100 μl) were seeded at $(1–2)\times10^6$ cells/ml in a flat-bottomed 96-well plate (Nunc). The cells were incubated at 37°C and 5% CO_2 for 1 h. Wells were washed three times in PBS to remove any non-adherent cells and the PBS replaced with 100 μl of TCM. T cells were washed twice in TCM and 100 μl added to the relevant wells at 5×10^5 cells/ml with peptide. Cultures were incubated at 37°C for three days. ^{3}H-Thymidine (0.5 μCi/well) was added 6 h prior to harvesting and counting on a TopCount (Becton Dickenson) scintillation counter.

Blocking assays were set up as for the proliferation assays with the following modifications; Soluble CD55 was added to the monocyte cultures immediately prior to the addition of T cells. Blocking antibodies were added to the relevant cells at 10–20 μg/ml for 1 h at room temperature. Cells were then washed twice in TCM prior to addition to the cultures. To neutralise CD55, equal amounts of antibody and CD55 were incubated together for 1 h at room temperature prior to inclusion in the cultures.

Cytokine measurement

Proliferation assays were set up as described above. Following 3 days of incubation and prior to thymidine pulsing, 100 μl of culture media was removed and assayed for the presence of IFN-γ using the cytometric bead array (CBA) (Becton Dickenson) according to the manufacturer's protocol. Briefly, cytokine capture beads were added to the samples or cytokine standards in flow cytometry tubes. These were vortexed and fluorescent detection antibody added to each tube. The samples were incubated at room temperature for 3 h. Beads were pelleted by centrifugation, washed twice and resuspended prior to reading by FACScan. Standard curves were plotted from 10 points dilutions of cytokines and data for samples converted to pg/ml cytokine.

Results

Soluble CD55 inhibits T-cell proliferation

We and other authors have demonstrated that CD55 exists in the extracellular matrix of tissues in various pathological conditions. It has also been hypothesised that extracellular CD55 plays an important role in the maintenance of these pathologies; however, direct evidence has not been demonstrated. The expression of both CD55 and CD97 were assessed on well-characterised T cells clones (Table 1) and APCs, respectively (Fig. 1a, b). To investigate the effects of sCD55, proliferation assays were established and purified sCD55 was introduced into cultures of adherent monocytes just prior to addition of T-cell clones. The result was a consistent decrease in proliferation by 20–80%. This was also demonstrated at a range of peptide concentrations (Fig. 2a) with a greater effect being observed at lower levels. Furthermore, increasing concentrations of sCD55 showed a titratable effect on T-cell proliferation

Table 1 T cells were characterised for expression of common markers by FACS analysis. Two antibodies recognising CD97 were used, CLB97/1 (1) to the amino terminal EGF-like domains and CLB97/3 (3) to the membrane-proximal stalk region. Cells (5×10^5) were labelled with primary antibody, washed and stained with FITC-goat anti mouse and analysed by FACScan (Becton Dickenson). The mean fluorescence intensity values are provided (MLF)

Clone	Marker (MLF)							
	CD3	CD4	CD8	CD56	CD45RO	CD16	CD97 (1)	CD97 (3)
CM	450	900	7	8	85	6	980	850
JR	400	4	1007	76	90	5	725	745

(Fig. 2b). The sCD55 was purified from cells by affinity purification and contained an intact GPL anchor. It has been reported that GPL-anchored proteins can re-insert themselves back into membranes, which may have had an effect on the assays. We therefore generated two soluble recombinant CD55-Fc fusion proteins, the first containing SCR domains 1–3 and the second containing all four SCR domains [24]. These were tested and were recognized by a range of CD55 monoclonal antibodies. They were also functional in terms of ability to inhibit complement [26]. Both recombinant forms of CD55 were able to inhibit T-cell proliferation to a level similar to that of purified sCD55 (Fig. 3, 4). However, the inhibitory effects were not observed with Tie-2-Fc, an Fc fused to the extracellular region of the receptor tyrosine kinase Tie-2. This supports the inhibitory effect of sCD55 and indicates that the Fc domain does not influence the assays.

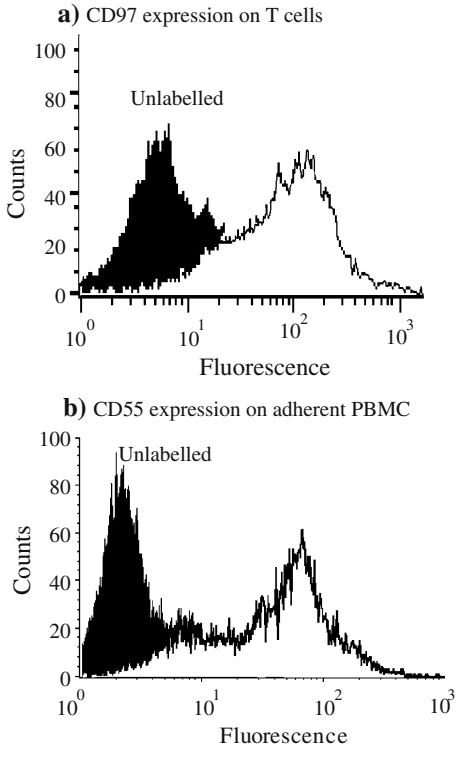

Fig. 1 FACS analysis of (a) T cells for CD97 expression and (b) adherent PBMCs for CD55 expression. Cells (5×10^5) were labelled with primary antibody, washed and stained with FITC-goat anti mouse and analysed by FACS scan (Becton Dickinson)

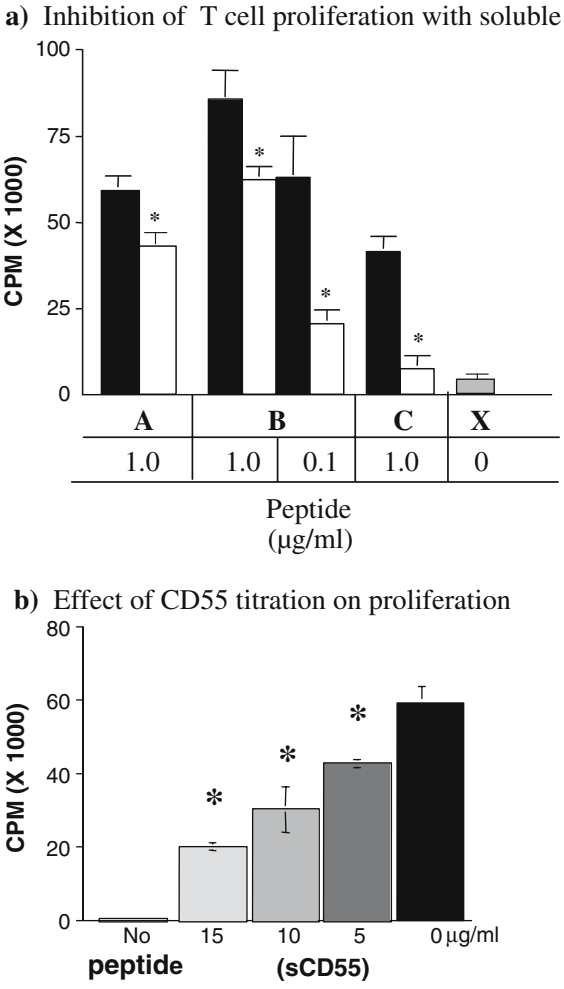

Fig. 2 (a) T-cell assays were set up with autologous adherent monocytes, resting T cells and cognate peptide (*black bars*). In each of three assays (A, B + C), 5 µg of soluble CD55 (*white bars*) was added to the cultures prior to the addition of T cells. X represents background proliferation of T cells cultured with APCs in the absence of peptide. Values represent the means and standard deviations of quadruplicate samples (*P values < 0.02). (b) In a repeated assay, quadruplicate cultures were treated with three different concentrations of soluble CD55 prior to the addition of T cells. Proliferation was measured after 3 days by 6 h thymidine incorporation and analysed by scintillation counting (*P values < 0.0003 to 0.0001)

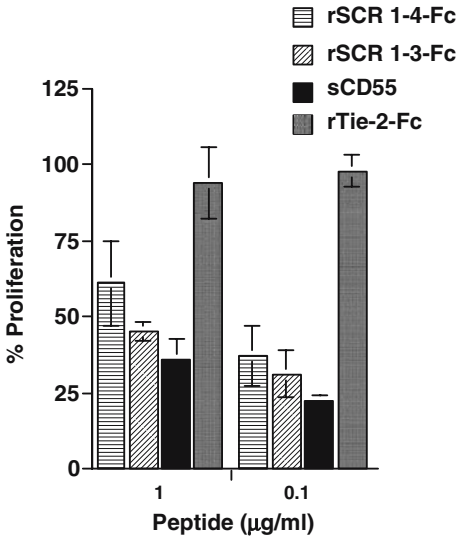

Fig. 3 Proliferation assays were established with autologous adherent monocytes, resting T cells and cognate peptide at 1.0 and 0.1 µg/ml. Soluble CD55 (5 µg/ml) (*black bars*), purified recombinant CD55 SCR 1-3-Fc or CD55 SCR 1-4-Fc (20 µg) (*hatched bars*) or recombinant Tie-2-Fc (20 µg) (*grey bar*) was added to the cultures prior to the addition of T cells. Proliferation was measured after 3 days by 6 h thymidine incorporation and analysed by scintillation counting. Percentage proliferation is presented compared to cells treated with peptide alone

Using the antibody BRIC 216 that is known to block the interaction of CD55 with CD97, we were able to assess the effects of this antibody on neutralising sCD55, confirming the specificity of the interaction. Purified

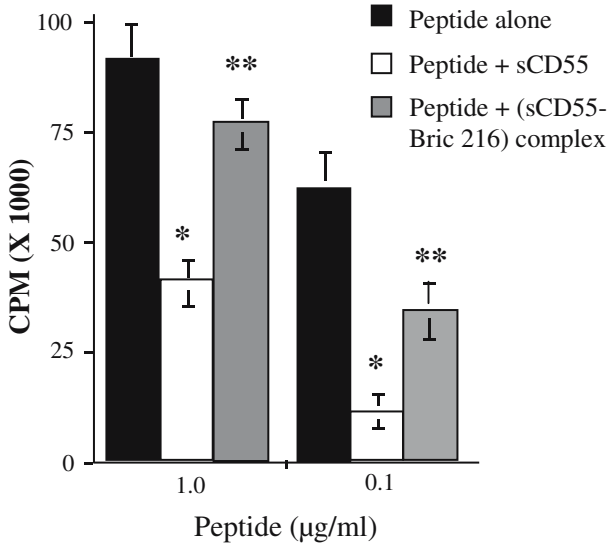

Fig. 4 (a). Proliferation assays were set up with autologous APCs and cognate peptide at 0.1 µg/ml (*black bars*). Soluble CD55 (10 µg/ml) was added to the cultures prior to addition of the T cells (*white bars*). Pre-formed CD55–anti-CD55 complexes were formed from 10 µg/ml of sCD55 and 10 µg/ml of anti-CD55 antibody (BRIC 216) incubated together for 1 h at 4°C (*grey bars*). This was added to the cultures prior to addition of the T cells. Standard errors are plotted from quadruplicate samples

sCD55 was pre-incubated with anti-CD55 antibodies prior to introduction to the T-cell assays. These assays demonstrate that the inhibitory effect of sCD55 could be neutralised, by pre-incubation of the sCD55 with anti-CD55 Mab, restoring proliferation (Fig. 5). These results were also mirrored when IFN-γ was measured from the assays (Table 2). The sCD55 was able to inhibit IFN-γ production by approximately 70%. This was reduced to approximately 40% by pre-incubating the sCD55 with anti-CD55 antibody.

Discussion

CD55 has recently been defined as a ligand for an early activation marker on leukocytes (CD97). The functional consequences of this interaction are now beginning to be explored. Data from two groups working on CD55 knockout mice have demonstrated a significant increase in the magnitude of immune responses in CD55-deficient mice [47, 28]. It was suggested that this might be due to involvement of CD55 expressed on macrophage interacting with CD97 on circulating T cells [55]. More recently, Hamann et al. have demonstrated that the interaction of CD55 with CD97 has an important role in the migration of neutrophils in models of both inflammatory bowel disease and pneumonia [44]. In this study, the migration of labelled, transplanted neutrophils was successfully blocked with anti CD97 antibodies. These studies have great implications for the role of CD55 in a range of immunological conditions. They also highlight the potential role of CD55 in both complement regulation and modulation of adaptive responses. The precise role of CD55 in both these situations requires further clarification. The relationship of these models to human immunity also requires clarification as there are subtle differences in the regulation of complement in both systems, with differences in expression patterns of the CRPs in different species [56, 23].

We have been studying the overexpression of CD55 and its potential role in immune regulation. We have examined the functional role of human CD55 and its interaction with CD97 on human peripheral blood monocytes and T cells, respectively. CD97 is expressed at height levels on activated T cells but has not been detected on naïve or thymic T cell [11]. CD55, however, is upregulated on activated monocytes but expression is lost on differentiation to dendritic cells. These findings confirm histochemical reports of a lack of expression of

Table 2 Culture supernatants were collected (Fig. 4) and assayed for IFN-γ using a Duoset ELISA (BD). The concentrations were calculated from IFN-γ standard curve. The values shown are from four replicates of cultures containing 0.1 µg/ml of specific peptide

	Peptide alone	+sCD55	+ (BRIC 216-sCD55)
IFN-γ (pg/ml)	900	200	400
% Reduction	–	78	45

CD55 on DCs, except follicular DCs [34]. This implies that any functional interaction between CD97-expressing T cells could be with CD55-expressing monocytes. We, and others, have shown that CD55 is present in the extracellular matrix and as soluble forms in a range of pathological conditions, including rheumatoid arthritis and inflammatory bowel disease.

We have demonstrated that purified sCD55 is capable of inhibiting T-cell effector function. Both the purified sCD55 and the recombinant forms maintained their complement inhibitory activity and were also effective in inhibiting T-cell function. The latter was reflected by decreases in both proliferation and IFN-γ secretion. The ability of soluble ligands/receptors to modulate T-cell responses is well documented. Leukocyte functional antigen 1 (LFA-1) interacts with intercellular adhesion molecules, ICAM 1 and 2. Recently, it has been shown that subsequent signaling via LFA-1 is sufficient to lower the threshold for T-cell activation and Th-1 commitment [62]. This function was abrogated by the presence of soluble ligands such as sICAM-1 [18].

Another ligand that has been shown to lower the threshold for T-cell activation is MICA, an MHC-like family member. This interacts with NKG2D on CD8 T cells and NK cells, lowering the threshold for activation in response to stress [69]. Like ICAM-1 and CD55, MICA has also been shown to be present as a soluble form, particularly in chronic inflammatory situations and cancer [71]. It has been proposed that production of soluble forms of those molecules involved in T-cell regulation may be a process by which the immune system attempts to dampen responses that cannot be resolved. In these situations, soluble ligands may act as decoy receptors, preventing both adhesion and any potential downstream signalling leading to cellular activation.

The inhibitory effect of sCD55 was demonstrated with both purified and soluble CD55, reducing the level of proliferation and IFN-γ secretion. This was confirmed by antibody-blocking data that was successfully able to neutralise the sCD55. These results support previous data [22,46,21], while adding a potential functional consequence to this interaction. The expression pattern and behaviour of CD55–CD97 is similar to that shown for a number of other ligand–receptor pairs including CD28–B7.1/7.2, ICAM1–LFA-1 and NKG2D–MICA/B. These molecules play diverse roles in the regulation of immune responses, from the co-stimulation of naïve T cells [40] to the peripheral regulation of regulatory and effector T cells [39]. This was elegantly demonstrated during the analysis of T cells from the epithelium of inflamed gut. The T cells analysed showed an upregulation of the NKG2D receptor in response to IL-15 secreted by stressed gut epithelium. Engagement of this receptor with its stress-induced epithelial ligands, MICA/B, resulted in lowering the threshold for release of effector function. This identified a direct mechanism of peripheral T-cell regulation in vivo [69].

This rapidly evolving field has identified a number of secondary functions for the CRPs including cell adhesion leukocyte co-stimulators stimulation and mediators of regulatory T cells. The mechanisms that result in production of soluble complement regulators and the role they play in the pathogenesis of these diseases remains to be identified. However, their presence and overexpression potentially make them a good therapeutic target.

References

1. Armitage NC, Perkins AC, Pimm MV, Farrands PA, Baldwin RW, Hardcastle JD (1984) The localization of an anti-tumour monoclonal antibody (791T/36) in gastrointestinal tumours. Br J Surg 71:407–12
2. Armitage NC, Perkins AC, Pimm MV, Wastie ML, Baldwin RW, Hardcastle JD (1985) Imaging of primary and metastatic colorectal cancer using an 111In- labelled antitumour monoclonal antibody (791T/36). Nucl Med Commun 6:623–631
3. Bjorge L, Hakulinen J, Vintermyr OK, Jarva H, Jensen TS, Iversen OE, Meri S, (2005). Ascitic complement system in ovarian cancer. Br J Cancer 92:895–905
4. Bjorge L, Hakulinen J, Wahlstrom T, Matre R, Meri S (1997) Complement-regulatory proteins in ovarian malignancies. Int J Cancer 70:14–25
5. Carroll MC (1998) The role of complement and complement receptors in induction and regulation of immunity. Annu Rev Immunol 16:545–568
6. Cerny J, Stockinger H, Horejsi V (1996) Noncovalent associations of T lymphocyte surface proteins. Eur J Immunol 26:2335–2343
7. Cheung NK, Walter EI, Smith-Mensah WH, Ratnoff WD, Tykocinski ML, Medof ME (1988) Decay-accelerating factor protects human tumor cells from complement-mediated cytotoxicity in vitro. J Clin Invest 81:1122–1128
8. Crnogorac-Jurcevic T, Efthimiou E, Neilsen T, Loader J, Terris B, Stamp G, Baron A, Scarpa A, Lemoine NR (2002) Expression profilling of microdissected pancreatic adenocarcinomas. Oncogene 21:4587–4594
9. Davis LS, Patel SS, Atkinson JP, Lipsky PE (1988) Decay-accelerating factor functions as a signal transducing molecule for human T cells. J Immunol 141:2246–2252
10. Durrant LG, Chapman MA, Buckley DJ, Spendlove I, Robins RA, Armitage NC (2003) Enhanced expression of the complement regulatory protein CD55 predicts a poor prognosis in colorectal cancer patients. Cancer Immunol Immunother 52(10): 638–642
11. Eichler W, Aust G, Hamann D (1994) Characterization of an early activation-dependent antigen on lymphocytes defined by the monoclonal antibody BL-Ac(F2). Scand J Immunol 39:111–115
12. Embleton MJ, Gunn B, Byers VS, Baldwin RW (1981) Antitumour reactions of monoclonal antibody against a human osteogenic-sarcoma cell line. Br J Cancer 43:582–587
13. Farrands PA, Perkins AC, Pimm MV, Embleton MJ, Hardy JD, Baldwin RW, Hardcastle JD (1982) Radioimmunodetection of human colorectal cancers by an anti-tumour monoclonal antibody. Lancet 2:397–400
14. Fishelson Z, Donin N, Zell S, Schultz S, Kirschfink M (2003) Obstacles to cancer immunotherapy: expression of membrane complement regulatory proteins (mCRPs) in tumors. Mol Immunol 40:109–123
15. Fonsatti E, Altomonte M, Coral S, De Nardo C, Lamaj E, Sigalotti L, Natali PG, Maio M (2000) Emerging role of protectin (CD59) in humoral immunotherapy of solid malignancies. Clin Ter 151:187–93

16. Freedland SJ, Seligson DB, Liu AY Pantuck AJ, Paik SH, Horvath S, Wieder JA, Zisman A, Nguyen D, Tso CL, Palotie AV, Belldegrun AS (2003) Loss of CD10 (neutral endopeptidase) is a frequent and early event in human prostate cancer. Prostate 55:71–80
17. Gelderman KA, Tomlinson S, Ross G, Gorter A (2004) Complement function in mAB-mediated cancer immunotherapy. Trends Immunol 25:158–164
18. Gomez-Scotto E, Seigneur M, Renard M, Houbouyan-Reveillard LL, Boisseau MR (2000) [Interest in variations in soluble ICAM-1 plasma levels. From physiology to clinical applications]. J Mal Vasc 25:156–165
19. Grossman WJ, Verbsky JW, Tollefsen BL, Kemper C, Atkinson JP, Ley TJ (2004) Differential expression of granzymes A and B in human cytotoxic lymphocyte subsets and T regulatory cells. Blood 104:2840–2848
20. Hakulinen J, Junnikkala S, Sorsa T, Meri S (2004) Complement inhibitor membrane cofactor protein (MCP; CD46) is constitutively shed from cancer cell membranes in vesicles and converted by a metalloproteinase to a functionally active soluble form. Eur J Immunol 34:2620–2629
21. Hamann J, Stortelers C, Kiss-Toth E, Vogel B, Eichler W, van Lier RA (1998) Characterization of the CD55 (DAF)-binding site on the seven-span transmembrane receptor CD97. Eur J Immunol 28:1701–1707
22. Hamann J, Vogel B, van Schijndel GM, van Lier RA (1996) The seven-span transmembrane receptor CD97 has a cellular ligand (CD55, DAF). J Exp Med 184:1185–1189
23. Hanna SM, Spiller OB, Linton S, Mead R, Morgan BP (2002) Rat T cells express neither CD55 nor CD59 and are dependent on Cry for protection from homologous complement. Eur J Immunol 32:502–509
24. Harris C, Lublin D, Morgan B (2002) Efficient generation of monoclonal antibodies for specific protein domains using recombinant immunoglobulin fusion proteins: pitfalls and solutions. J Immunol Methods 268:245–258
25. Harris CL, Abbott RJ, Smith RA, Morgan BP, Lea SM (2005) Molecular dissection of interactions between components of the alternative pathway of complement and decay accelerating factor (CD55). J Biol Chem 280:2569–2578
26. Harris CL, Hughes CE, Williams AS, Goodfellow I, Evans DJ,Caterson B, Morgan BP (2003) Generation of anti-complement 'prodrugs': cleavable reagents for specific delivery of complement regulators to disease sites. J Biol Chem 278(38):36068–36076
27. Hawkey CJ, Holmes CH, Smith PG, Austin EB, Baldwin RW (1986) Patterns of reactivity of the monoclonal antibody 791T/36 with different tumour metastases in the liver. Br J Cancer 54:871–875
28. Heeger PS, Lalli PN, Lin F, Valujskikh A, Liu J, Muqim N, Xu Y, Medof ME (2005) Decay-accelerating factor modulates induction of T cell immunity. J Exp Med 201:1523–1530
29. Hindmarsh EJ, Marks RM (1998) Decay-accelerating factor is a component of subendothelial extracellular matrix in vitro, and is augmented by activation of endothelial protein kinase C. Eur J Immunol 28:1052–62
30. Hiraoka S, Mizuno M, Nasu J, Okazaki H, Makidono C, Okada H, Terada R, Yamamoto K, Fujita T, Shiratori Y (2004) Enhanced expression of decay-accelerating factor, a complement-regulatory protein, in the specialized intestinal metaplasia of Barrett's esophagus. J Lab Clin Med 143:201–206
31. Hourcade D, Holers VM, Atkinson JP (1989) The regulators of complement activation (RCA) gene cluster. Adv Immunol 45:381–416
32. Inaba T, Mizuno M, Ohya S, Kawada M, Uesu T, Nasu J, Takeuchi K, Nakagawa M, Okada H, Fujita T, Tsuji T (1998) Decay-accelerating factor (DAF) in stool specimens as a marker of disease activity in patients with ulcerative colitis (UC). Clin Exp Immunol 112:237–41
33. Inoue T, Yamakawa M, Takahashi T (2002) Expression of complement regulating factors in gastric cancer cells. Mol Pathol 55:193–199
34. Jaspars LH, Vos W, Aust G, Van Lier RA, Hamann J (2001) Tissue distribution of the human CD97 EGF-TM7 receptor. Tissue Antigens 57:325–331
35. Jones EA, English A, Henshaw K, Kinsey SE, Markham AF, Emery P, McGonagle D (2004) Enumeration and phenotypic characterization of synovial fluid multipotential mesenchymal progenitor cells in inflammatory and degenerative arthritis. Arthritis Rheum 50:817–27
36. Jurianz K, Ziegler S, Donin N, Reiter Y, Fishelson Z, Kirschfink M (2001) K562 erythroleukemic cells are equipped with multiple mechanisms of resistance to lysis by complement. Int J Cancer 93:848–854
37. Kawada M, Mizuno M, Nasu J, Uesu T, Okazaki H, Okada H, Shimomura H, Yamamoto K, Tsuji T, Fujita T, Shiratori Y (2003) Release of decay-accelerating factor into stools of patients with colorectal cancer by means of cleavage at the site of glycosylphosphatidylinositol anchor. J Lab Clin Med 142:306–312
38. Kemper C, Verbsky JW, Price JD, Atkinson JP (2005) T-Cell Stimulation and Regulation: With Complements from CD46. Immunol Res 32:31–44
39. Kim JD, Choi BK, Bae JS, Lee UH, Han IS, Lee HW, Youn BS, Vinay DS, Kwon BS (2003) Cloning and characterization of GITR ligand. Genes Immun 4:564–569
40. Kim YJ, Mantel PL, June CH, Kim SH, Kwon BS (1999) 4–1BB costimulation promotes human T cell adhesion to fibronectin. Cell Immunol 192:13–23
41. Kohno H, Mizuno M, Nasu J, Makidono C, Hiraoka S, Inaba T, Yamamoto K, Okada H, Fujita T, Shiratori Y (2005) Stool decay-accelerating factor as a marker for monitoring the disease activity during leukocyte apheresis therapy in patients with refractory ulcerative colitis. J Gastroenterol Hepatol 20:73–78
42. Koretz K, Bruderlein S, Henne C, Moller P (1992) Decay-accelerating factor (DAF, CD55) in normal colorectal mucosa, adenomas and carcinomas. Br J Cancer 66:810–814
43. Law SK, Fearon DT, Levine RP (1979) Action of the C3b-inactivator on the cell-bound C3b. J Immunol 122:759–765
44. Leemans JC, te Velt AA, Florquin S, Bennink RJ, de Bruin K, van Lier RAW, van der Poll T, Hamann J (2004) The epidermal growth factor-seven transmembrane (EGF-TM7) receptor CD97 is required for neutrophil migration and host defense. J. Immunol. 172:1125–31
45. Li L, Spendlove I, Morgan J, Durrant LG (2001). CD55 is overexpressed in the tumour environment. Br J Cancer 84:80–86
46. Lin HH, Stacey M, Saxby C, Knott V, Chaudhry Y, Evans D, Gordon S, McKnight AJ, Handford P, Lea S (2001) Molecular analysis of EGF-SCR domain mediated protein-protein interactions-dissection of the CD97-CD55 complex. J Biol Chem 276(26):24160–24169
47. Liu J, Miwa T, Hilliard B, Chen Y, Lambris JD, Wells AD, Song WC (2005) The complement inhibitory protein DAF (CD55) suppresses T cell immunity in vivo. J Exp Med 201:567–577
48. Madjd Z, Durrant LG, Bradley R, Spendlove I, Ellis IO, Pinder SE (2004) Loss of CD55 is associated with aggressive breast tumors. Clin Cancer Res 10:2797–2803
49. Madjd Z, Durrant LG, Pinder SE, Ellis IO, Ronan J, Lewis S, Rushmere NK, Spendlove I (2005) Do poor-prognosis breast tumours express membrane cofactor proteins (CD46)? Cancer Immunol Immunother 54:149–156
50. Madjd Z, Pinder SE, Paish C, Ellis IO, Carmichael J, Durrant LG (2003) Loss of CD59 expression in breast tumours correlates with poor survival. J Pathol 200:633–9
51. Maenpaa A, Junnikkala S, Hakulinen J, Timonen T, Meri S (1996) Expression of complement membrane regulators membrane cofactor protein (CD46), decay accelerating factor (CD55), and protectin (CD59) in human malignant gliomas. Am J Pathol 148:1139–52
52. Marcenaro E, Augugliaro R, Falco M, Castriconi R, Parolini S, Sivori S, Romeo E, Millo R, Moretta L, Bottino C, Moretta A (2003) CD59 is physically and functionally associated with natural cytotoxicity receptors and activates human NK cell-mediated cytotoxicity. Eur J Immunol 33:3367–3376

53. Medof ME, Iida K, Mold C, Nussenzweig V (1982) Unique role of the complement receptor CR1 in the degradation of C3b associated with immune complexes. J Exp Med 156:1739–1754
54. Medof ME, Walter EI, Rutgers JL, Knowles DM, Nussenzweig V (1987) Identification of the complement decay-accelerating factor (DAF) on epithelium and glandular cells and in body fluids. J Exp Med 165:848–864
55. Miwa T, Maldonado MA, Zhou L, Sun X, Luo HY, Cai D, Werth VP, Madaio MP, Eisenberg RA, Song WC (2002) Deletion of decay-accelerating factor (CD55) exacerbates autoimmune disease development in MRL/lpr mice. Am J Pathol 161:1077–1086
56. Miwa T, Zhou L, Hilliard B, Molina H, Song WC (2002) Crry, but not CD59 and DAF, is indispensable for murine erythrocyte protection in vivo from spontaneous complement attack. Blood 99:3707–3716
57. 57.Morgan BP, Berg CW, Harris CL (2005) "Homologous restriction" in complement lysis: roles of membrane complement regulators. Xenotransplantation 12:258–265
58. Morgan BP, Campbell AK (1985) The recovery of human polymorphonuclear leucocytes from sublytic complement attack is mediated by changes in intracellular free calcium. Biochem J 231:205–208
59. Morgan J, Spendlove I, Durrant LG (2002) The role of CD55 in protecting the tumour environment from complement attack. Tissue Antigens 60:213–223
60. Muller-Eberhard HJ (1986) The membrane attack complex of complement. Annu Rev Immunol 4:503–528
61. Mustafa T, Klonisch T, Hombach-Klonisch S, Kehlen A, Schmutzler C, Koehrle J, Gimm O, Dralle H, Hoang-Vu C (2004) Expression of CD97 and CD55 in human medullary thyroid carcinomas. Int J Oncol 24:285–294
62. Perez OD, Mitchell D, Jager GC, South S, Murriel C, McBride J, Herzenberg LA, Kinoshita S, Nolan GP (2003) Leucocyte Functional antigen 1 lowers T cell activation thresholds and signalling through cytoadhesin-1 and Jun-activating binding protein 1. Nature Immunol 4:1083–1091
63. Perkins AC, Pimm MV, Gie C, Marksman RA, Symonds EM, Baldwin RW (1989) Intraperitoneal 131I- and 111In-791T/36 monoclonal antibody in recurrent ovarian cancer: imaging and biodistribution. Nucl Med Commun 10:577–584
64. Pimm MV, Embleton MJ, Perkins AC, Price MR, Robins RA, Robinson GR, Baldwin RW (1982) In vivo localization of anti-osteogenic sarcoma 791T monoclonal antibody in osteogenic sarcoma xenografts. Int J Cancer 30:75–85
65. Pimm MV, Perkins AC, Armitage NC, Baldwin RW (1985) Localization of anti-osteogenic sarcoma monoclonal antibody 791T/36 in a primary human osteogenic sarcoma and its subsequent xenograft in immunodeprived mice. Cancer Immunol Immunother 19:18–21
66. Pizzo P, Giurisato E, Tassi M, Benedetti A, Pozzan T, Viola A (2002) Lipid rafts and T cell receptor signaling: a critical re-evaluation. Eur J Immunol 32:3082–3091
67. Price MR, Campbell DG, Baldwin RW (1983) Identification of an anti-human osteogenic sarcoma monoclonal-antibody-defined antigen on mitogen-stimulated peripheral blood mononuclear cells. Scand J Immunol 18:411–420
68. Ramage JM, Metheringham R, Conn A, Spendlove I, Moss RS, Patton DT, Murray JC, Rees RC, Durrant LG (2004) Identification of an HLA-A*0201 cytotoxic T lymphocyte epitope specific to the endothelial antigen Tie-2. Int J Cancer 110:245–50
69. Roberts AI, Lee L, Schwarz E, Groh V, Spies T, Ebert EC, Jabri B (2001) NKG2D receptors induced by IL-15 costimulate CD28-negative effector CTL in the tissue microenvironment. J Immunol 167:5527–5530
70. Russell S (2004) CD46: a complement regulator and pathogen receptor that mediates links between innate and acquired immune function. Tissue Antigens 64:111–118
71. Salih HR, Antropius H, Gieseke F, Lutz SZ, Kanz L, Rammensee HG, Steinle A (2003) Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. Blood 102:1389–1396
72. Schmitt CA, Schwaeble W, Wittig BM, Meyer zum Buschenfelde KH, Dippold WG (1999) Expression and regulation by interferon-gamma of the membrane-bound complement regulators CD46 (MCP), CD55 (DAF) and CD59 in gastrointestinal tumours. Eur J Cancer 35:117–24
73. Shafren DR, Au GG, Nguyen T, Newcombe NG, Haley ES, Beagley L, Johansson ES, Hersey P, Barry RD (2004) Systemic therapy of malignant human melanoma tumors by a common cold-producing enterovirus, coxsackievirus a21. Clin Cancer Res 10:53–60
74. Shenoy-Scaria AM, Kwong J, Fujita T, Olszowy MW, Shaw AS, Lublin DM (1992) Signal transduction through decay-accelerating factor. Interaction of glycosyl-phosphatidylinositol anchor and protein tyrosine kinases p56lck and p59fyn 1. J Immunol 149:3535–3541
75. Shimo K, Mizuno M, Nasu J, Hiraoka S, Makidono C, Okazaki H, Yamamoto K, Okada H, Fujita T, Shiratori Y (2004) Complement regulatory proteins in normal human esophagus and esophageal squamous cell carcinoma. J Gastroenterol Hepatol 19:643–647
76. Sier CF, Gelderman KA, Prins FA, Gorter A (2004) Beta-glucan enhanced killing of renal cell carcinoma micrometastases by monoclonal antibody G250 directed complement activation. Int J Cancer 109:900–908
77. Simpson KL, Jones A, Norman S, Holmes CH (1997). Expression of the complement regulatory proteins decay accelerating factor (DAF, CD55), membrane cofactor protein (MCP, CD46) and CD59 in the normal human uterine cervix and in premalignant and malignant cervical disease. Am J Pathol 151:1455–1467
78. Spendlove I, Li L, Carmichael J, Durrant LG (1999) Decay accelerating factor (CD55): A target for cancer vaccines? Cancer Res 59:2282–2286
79. Symonds EM, Perkins AC, Pimm MV, Baldwin RW, Hardy JG, Williams DA (1985) Clinical implications for immunoscintigraphy in patients with ovarian malignancy: a preliminary study using monoclonal antibody 791T/36. Br J Obstet Gynaecol 92:270–276
80. Varsano S, Rashkovsky L, Shapiro H, Ophir D, Mark-Bentankur T (1998) Human lung cancer cell lines express cell membrane complement inhibitory proteins and are extremely resistant to complement-mediated lysis; a comparison with normal human respiratory epithelium in vitro, and an insight into mechanism(s) of resistance. Clinical and Experimental Immunology 113:173–82
81. Walport MJ (2001) Complement. First of two parts. N Engl J Med 344:1058–1066
82. Walport MJ (2001) Complement. Second of two parts. N Engl J Med 344:1140–1144
83. Watson NF, Durrant LG, Madjd Z, Ellis IO, Scholefield JH, Spendlove I (2005) Expression of the membrane complement regulatory protein CD59 (protectin) is associated with reduced survival in colorectal cancer patients. Cancer Immunol Immunother 1–8
84. Williams MR, Perkins AC, Campbell FC, Pimm MV, Hardy JG, Wastie ML, Blamey RW, Baldwin RW (1984) The use of monoclonal antibody 791T/36 in the immunoscintigraphy of primary and metastatic carcinoma of the breast. Clin Oncol 10:375–81
85. Zaffran Y, Destaing O, Roux A, Ory S, Nheu T, Jurdic P, Rabourdin-Combe C, Astier AL (2001) CD46/CD3 costimulation induces morphological changes of human T cells and activation of Vav, Rac, and extracellular signal-regulated kinase mitogen-activated protein kinase. J Immunol 167:6780–6785