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Original Paper

Expression and Regulation by Interferon-γ of the Membrane-bound Complement Regulators CD46 (MCP), CD55 (DAF) and CD59 in Gastrointestinal Tumours

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The membrane-bound complement inhibitors CD46 (membrane cofactor protein), CD55 (decay-accelerating factor) and CD59 (protectin) protect tumour cells against lysis by activated complement. In this study, a total of 14 (3 gastric, 3 colonic and 8 pancreatic) gastrointestinal tumour cell lines were examined for the expression of CD46, CD55 and CD59 with respect to the regulatory efficacy of interferon-γ (IFN-γ). The effects of IFN-γ on mRNA and protein expression levels of CD46, CD55 and CD59 were evaluated by Northern blot hybridisation, RT-PCR, flow cytometry and immunostaining. In unstimulated cell lines, CD46 and CD59 transcripts were expressed at comparable levels, whereas the basal expression of CD55 mRNA was heterogeneous. The complement inhibitor proteins were detected in all cell lines using specific antibodies. Additional immunohistochemical stainings of gastrointestinal tissue specimens supported these findings. IFN-γ evoked a weak induction of certain transcripts in a subset of the cell lines. Upregulation of protein expression was only observed in HT29 cells for CD55 and CD59 and was accompanied by a marked increase of the corresponding transcripts. We conclude that membrane-bound complement inhibitors are broadly expressed in gastrointestinal tumour cells and vary in their susceptibility to IFN-γ. Thus, they may be involved in tumour escape mechanisms in gastric, pancreatic and colorectal cancer. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: CD46 (MCP), CD55 (DAF), CD59 (protectin), colon cancer, complement regulators, gastric cancer, immunotherapy, interferon-γ, pancreatic cancer
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INTRODUCTION

COMPLEMENT-MEDIATED CYTOTOXICITY is involved in the immunological surveillance of tumour cells [1–3]. Complement can be activated on autologous tumour cells through various mechanisms, including deposits of immune complexes [4], spontaneous decay of C3 [5], altered cell-surfaces [6] and tissue injury due to necrosis [7], invasion or metastasis [2]. However, tumour cells also show a restricted susceptibility to final cytolysis [5, 7]. The resistance of tumour cells to cytolysis is strongly mediated by the expression of membrane-bound complement regulators [3, 7–13]. Several membrane-bound complement inhibitors have been identified: CD46 (membrane cofactor protein, MCP) [14], CD55 (decay-accelerating factor, DAF) [15] and CD59 (protectin) [9]. However, the expression of these molecules and their role in gastrointestinal cancer entities remain poorly understood.

The complement system is a cytolytic humoral defence mechanism against a variety of pathogens and abnormal cells [16]. It can be activated by two cascades of interacting plasma proteins. The classical pathway is triggered by antibody–antigen complexes and the alternative pathway is triggered by altered surfaces of foreign or tumour cells [1, 17]. The crucial step in either pathway is the escalating formation of C3 convertases after cleavage of C3 or C4 [18]. Both pathways lead to the assembly of C5b, C6, C7, C8 and C9

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into the cytolytic pore-forming membrane attack complex [19]. Cellular deposits of activated complement components could also affect undesired targets like normal host cells. Cells are protected from damage by autologous complement activation through the activity of membrane-bound complement regulatory proteins [20]. CD46 is a cofactor for factor I-mediated cleavage of C4b and C3b, thus preventing the formation of C3 convertases [21]. CD55 blocks the assembly and accelerates the decay of C3 convertases [15]. CD59 acts at the final stages of membrane attack complex formation by preventing the binding of C9 to C5b-8 [22]. By interfering with the complement cascade at different stages, each of these inhibitors has been shown to protect cells from subsequent cytolysis [23]. Coexpression of the molecules enhances the resistance against complement-mediated lysis [24]. All these factors are strictly intrinsic membrane inhibitors, acting only on expressing cells [9, 14, 15].

Knowledge of expression and regulation of complement inhibitory proteins is important for understanding complement-mediated surveillance of tumour cells. With respect to the gastrointestinal tract, several studies focusing on colorectal tissues have been published with controversial results in terms of the inhibitor expression in malignant tissues [25–28]. Comprehensive studies of the complement regulator expression in the pancreas are still lacking; only a few data of isolated cell lines or normal tissues have been published [11, 29]. To our knowledge, studies describing the complement regulator expression in gastric cells or tissues are not yet available. The possible role of complement inhibitors in cancer escape mechanisms and published data indicating an upregulation of complement regulatory proteins in cancer [25, 27] and chronic inflammatory diseases [30] drew our interest to the expression and inducibility of these proteins in gastrointestinal tumour cell lines. The aim of the present study was to characterise the basic expression of CD46, CD55 and CD59 at both the RNA and protein level in colon, gastric and pancreatic cancer cells and to evaluate their inducibility by the pro-inflammatory cytokine interferon-γ (IFN-γ).

MATERIALS AND METHODS

Cell lines and culture

Tumour cell lines were obtained from the American Type Culture Collection or contributed from different laboratories as described [31]. The present study includes the gastric cancer cell lines MKN-45, Mz-GC-3 (supplied by M. Heike, Mainz, Germany) and Mz-Sto-1, the colon cancer cell lines CaCo-2, HT29 and T-84 and the pancreatic cancer cell lines 818-4, ASPC-1, Capan-1, Capan-2, PaCa-2, PaCa-3, PaCa-44 and the pancreatic insulinoma cell line QGP-1.

All cell lines were maintained in CMRL-1066 medium, supplemented with 10% fetal calf serum (FCS, Biochrom, Berlin, Germany), 1% L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin, at 37°C in a humid atmosphere of 5% CO_2. Cell suspensions were obtained by incubating the cell cultures with 0.05% ethylen-diamine-tetra-acetate (EDTA) in phosphate-buffered saline (PBS). Cell viability was determined by the trypan blue exclusion technique with a viability always more than 95%.

Recombinant human IFN-γ was kindly provided by the Dr Rentschler Co. (Laupheim, Germany). For all experiments using IFN-γ, IFN-γ-containing medium (100 units/ml) was added when the cells had reached approximately 50% confluence. Controls received freshly prepared medium without IFN-γ. The cultures were incubated under the conditions described above for 48 h.

RNA extraction and Northern blotting

Total cellular RNA was extracted according to the guanidinium thiocyanate–phenol–chloroform single step method [32]. Total RNA (15 μg) was resolved on a 1% formaldehyde-agarose gel and transferred to a positively charged Nylon membrane (Hybond-N^+, Amersham, Braunschweig, Germany) by capillary blotting. The blot was fixed by oven baking at 80°C for 2 h.

CD46 transcripts were detected by a 1546 bp cDNA probe, corresponding to the full-length cDNA clone 'MCP-9' as described by Liszewski and colleagues [14]. The CD55 probe was a 2101 bp full-length cDNA as published by Lublin and Atkinson [33]. The 1154 bp cDNA probe 'YTH53.1/1' including the full coding sequence of CD59 was a kind gift from Dr H. Waldmann (University of Cambridge, Cambridge, U.K.) [9]. The blots were prehybridised at 65°C for 2 h in a solution containing 1% bovine serum albumin, 1 mM EDTA, 250 mM Na_2HPO_4 and 7% SDS and hybridised in the same solution supplemented with the ^{32}P-α labelled probe (activity > 2.5 Mio. cpm/ml) at 65°C for 12 h. After washing with a solution containing 1 mM EDTA, 20 mM Na_2HPO_4 and 1% SDS at 65°C, the blots were exposed to X-ray films at − 80°C. As a RNA loading control and as an internal standard not affected by IFN-γ, all Northern blots were normalised against the ^{32}P-γ labelled β-actin antisense oligonucleotide 5′-GTG GAT GCC ACA GGA CTC C-3′ [34] as described [35].

Reverse transcriptase polymerase chain reaction (RT-PCR)

Aliquots of 1 μg of total RNA were used for reverse transcription to cDNA. cDNA was prepared in a 20 μl reaction volume using the GeneAmp® RNA PCR kit (Perkin-Elmer Roche Molecular System Inc., Branchburg, New Jersey, U.S.A.) according to the methods given by the manufacturer. First strand cDNA synthesis was performed using specific reverse primers (Table 1). The primers were selected from the published cDNA sequence data of CD55 [33], CD59 [9] and β-actin [34]. The CD55 primers (Table 1) span several

Table 1. *Primer sequences used to detect CD55, CD59 and β-actin by RT-PCR*

	Sequence	Product length (bp)
CD55 forward primer	5′-GTACTGTGAATAATGATGAAGGAG-3′	
CD55 reverse primer	5′-TCTTAACTCTTCTTTGGCTAAGTC-3′	364/482
CD59 forward primer	5′-ACAATGGGAATCCAAGGAGGG-3′	
CD59 reverse primer	5′-CTTAGGGATGAAGGCTCCAGG-3′	391
β-actin forward primer	5′-CCTTCCTGGGCATGGAGTCCT-3′	
β-actin reverse primer	5′-GGAGCAATGATCTTGATCTT-3′	202

introns to prevent undiscerned genomic amplification and generate a 364 bp PCR fragment of a region nonhomologous to any other related complement factor. The CD59 primers (Table 1), also spanning two introns of the genomic sequence, generate a 391 bp fragment. Each PCR procedure was performed simultaneously with the β-actin primers as listed in Table 1 which give rise to a 202 bp fragment as control reaction. An aliquot of 2 µl of cDNA product was used for a PCR in a 50 µl reaction volume. Typical reaction conditions included initial denaturation at 95°C for 3 min followed by 35 cycles of 1 min at 95°C, 1 min annealing at 60°C and 2 min extension at 72°C and a final extension step at 72°C for 10 min. The annealing temperature was optimised for each primer pair. Following amplification, PCR products were resolved on 2% agarose gels. DNA fragments were visualised under ultraviolet (UV) light by staining with ethidium bromide.

Southern blot hybridisation

Southern blotting was performed according to a procedure described by Sambrook and colleagues [35]. The membranes (Hybond-N^+, Amersham) were hybridised against the specific probes in the conditions as described above for the Northern blotting procedure.

Flow cytometric analysis

For flow cytometry, cells were harvested, washed and resuspended in PBS. The following monoclonal antibodies (MAb) were used: J4-48 (IgG_1-isotype, murine) directed against CD46 (Serotec, Wiesbaden, Germany) [36]; BRIC 110 (IgG_1-isotype, murine) directed against CD55 (Serotec) [37]; YTH53.1 (IgG_{2b}-isotype, rat) directed against CD59 (Serotec) [9]; W6/32 (IgG_{2a}-isotype, murine) directed against the shared HLA-A/B/C-determinant (Dako, Hamburg, Germany) and irrelevant murine IgG_1- and IgG_{2b}-isotypes as negative controls.

5×10^5 cells/test were incubated with 100 µl aliquots of the primary MAbs (5–50 µg/ml) at 4°C for 30 min. The cells were then washed three times in PBS and incubated with the fluorescein-isothiocyanate-conjugated goat-anti-mouse (Coulter Immunology, Krefeld, Germany) or goat-anti-rat (Dianova, Hamburg, Germany) antibody at an appropriate dilution at 4°C for 30 min. The cells were subsequently washed in PBS and fixed in PBS containing 0.5% paraformaldehyde prior to analysis using a Becton-Dickinson FACScan (Heidelberg, Germany).

Immunostaining

In order to evaluate the immunohistochemical staining patterns of the MAbs used for flow cytometry, a series of surgically resected normal and malignant gastrointestinal tissue specimens from stomach, pancreas and colon was quick frozen immediately after removal. 7-µm-thick cryostat sections were stained shortly after mounting on glass slides. The slides were incubated with each of the primary MAbs as used for flow cytometry at an appropriate dilution for 45 min at room temperature in a humidified chamber. After washing twice with PBS, incubation with the horseradish peroxidase conjugated and pre-absorbed rabbit-anti-mouse antibody P260 (Dako) or rabbit-antirat antibody P162 (Dako) was carried out at 20-fold dilution in PBS including 35% immunoglobulin-free FCS (Gibco BRL, Berlin, Germany) for 45 min at room temperature. Specifically bound antibody was then visualised as described [38] by peroxidase-catalysed substrate conversion of 3-amino-9-ethylcarbazole with 0.03% H_2O_2 and subsequent counter-staining with haematoxylin. The cellular staining intensity of the chromogen was quantified by independent researchers with a scale scoring from +++ indicating very strong staining to +/− which indicates weak or no staining.

Data analysis

The intensities of the scanned Northern blot hybridisation signals were quantified by the image processing and analysis software NIH Image, version 1.49, from the National Institute of Health (U.S.A.). The statistical significance of differences between means of paired samples was evaluated by Wilcoxon's signed rank test. A P value of < 0.05 was considered significant.

RESULTS

Expression of CD46, CD55 and CD59 transcripts

Total RNA from several gastrointestinal tumour cell lines was analysed by Northern blot hybridisation to determine the relative abundance of CD46, CD55 and CD59 transcripts (Figure 1, lanes 'ut'). The total RNA was also hybridised to a β-actin probe detecting the β-actin transcript of 2.1 kb as a control for RNA loading (Figure 1d). After determination of the hybridisation signal intensities, all data have been transformed into normalised values relative to the corresponding β-actin intensities.

Despite multiple forms of CD46 transcripts generated by alternative splicing at the carboxy terminus [14], the length of all transcript species were approximately 3.2 kb and were indistinguishable from each other (Figure 1a). With the exception of the low level signals for the gastric carcinoma cell line Mz-GC-3 and the pancreatic cancer cell line 818-4, all cell lines showed a marked and relatively homogeneous RNA expression.

CD55 transcripts were detected (Figure 1b) in accordance with the previously described species of 1.5 and 2.2 kb due to the alternative use of different polyadenylation sites [39]. The relative intensities of the two forms varied slightly with a predominance of the 2.2 kb transcript. The CD55 mRNA expression was heterogeneous in every group of cell lines whereas a markedly low mean expression level was found in the colon group. In order to assess this expression profile, a RT-PCR generating a 364 bp fragment of CD55 was performed. A larger fragment (482 bp), corresponding to a partly unspliced and reading frame-shifted minor species leading to a secretory form of CD55, was not detected in any case. The RT-PCR confirmed the heterogeneous expression pattern of CD55 mRNA (Figure 2a).

Five discrete CD59 mRNA species of lengths 0.7, 1.3, 1.9, 2.1 and 5.8 kb generated by alternative polyadenylation have been previously reported [40]. The CD59 probe hybridised mainly to three distinct bands corresponding to the low expressed 0.7 kb species, the moderately abundant 1.3 kb species and the most abundant and comigrating 1.9/2.1 kb species (Figure 1c). The 5.8 kb species was detectable only in three pancreatic cell lines with a strong signal intensity for PaCa-44. The additional bands of PaCa-44 which do not agree with the length of the published species are not specific; these bands did not occur in a poly(A^+) RNA control hybridisation of PaCa-44 (data not shown). The distribution of CD59 transcripts was more homogeneous when compared with CD55, although the signals did not exhibit the same

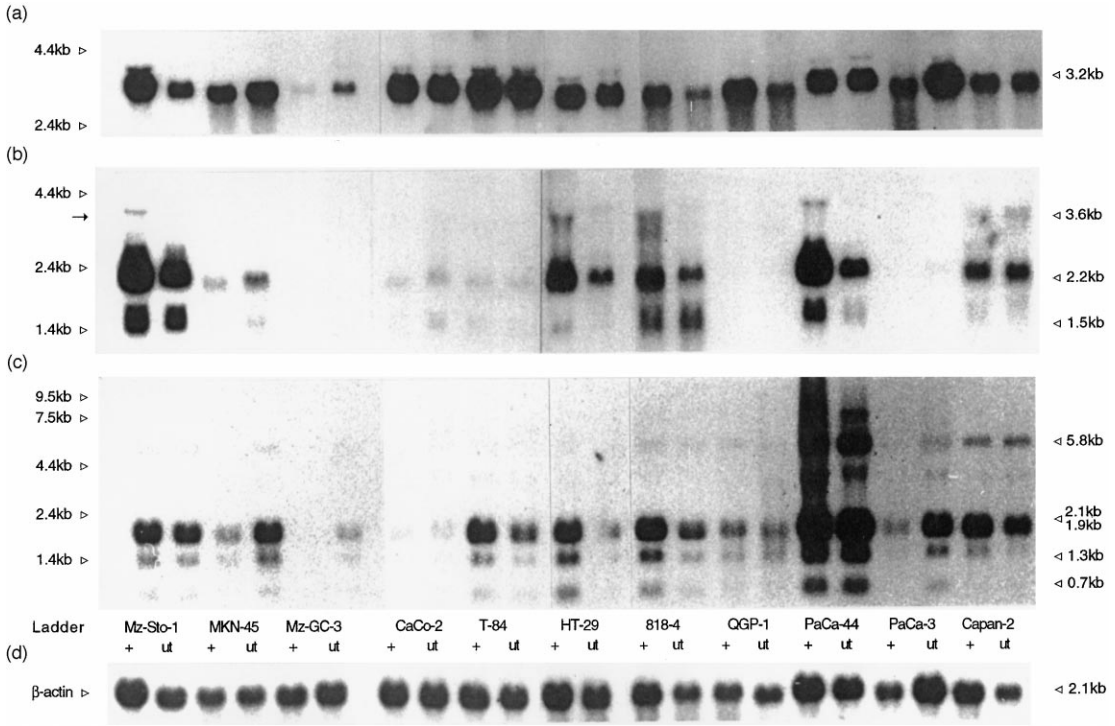

Figure 1. Northern blot analysis of membrane-bound complement inhibitors and the effect of IFN-γ on mRNA expression. Cells were incubated with IFN-γ (100 units/ml) for 48 h (+). Basal expression was evaluated in untreated (ut) conditions. CD46 (a); CD55 (b), a new 3.6 kb transcript species is indicated by an arrow; CD59 (c). All hybridisation signal intensities were normalised to the corresponding band intensities of the β-actin control (d).

uniformity as those of CD46. While gastric and pancreatic cell lines expressed CD59 at high levels, the mean expression level of the colon cell lines was very low, similar to the results of the CD55 Northern blot analysis. The RT-PCR analysis, generating one fragment of 391 bp independently of different polyadenylation sites, confirmed the ubiquitous expression pattern of CD59 (Figure 2b).

Protein expression of CD46, CD55 and CD59

The basal protein expression of the membrane-bound complement inhibitors was examined by flow cytometry using MAbs against CD46, CD55 and CD59 (Figure 3). As defined by isotype-matched control antibodies all data measured by flow cytometry represented positively stained cell preparations for all complement inhibitors. In every sample, only one peak of fluorescence intensity was detected corresponding to uniform expression intensities of each cell line. The highest intensities of fluorescence were measured in the positive control group with the MAb W6/32 against the shared HLA-A/B/C determinant. Consistent with the RNA analysis, the detection of CD55 using MAb BRIC 110 revealed the lowest mean fluorescence intensity. Furthermore, the deviation of the fluorescence intensites between the cell lines tested was the highest for CD55. Especially the colon cell lines showed, on average, a relatively low fluorescence level of CD59 and a very low level of CD55. These findings are consistent with the results from CD55 and CD59 in the Northern blot analysis. The relatively uniform and abundant expression of the CD46 transcripts is reflected by high fluorescence intensities.

The additional immunohistochemical analysis in a series of colonic, gastric and pancreatic tissue specimens was performed to characterise the *in situ* staining patterns of the

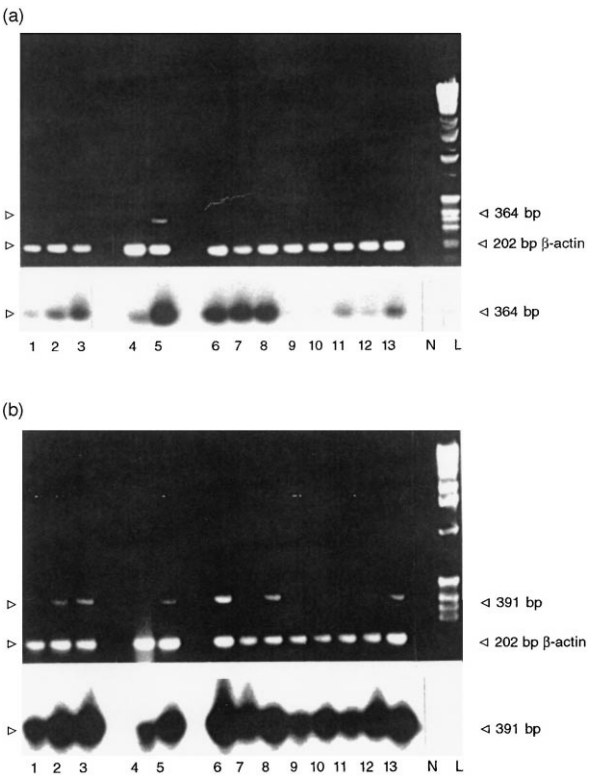

Figure 2. RT-PCR and corresponding Southern blot hybridisation of CD55 generating a 364 bp fragment (a) and CD59 generating a 391 bp fragment (b). Coamplification of a 202 bp β-actin fragment as internal control. Cell lines: Mz-Sto-1 (lane 1), MKN-45 (2), Mz-GC-3 (3), T-84 (4), HT29 (5), 818-4 (6), QGP-1 (7), PaCa-44 (8), PaCa-3 (9), PaCa-2 (10), Capan-2 (11), Capan-1 (12) and ASPC-1 (13).

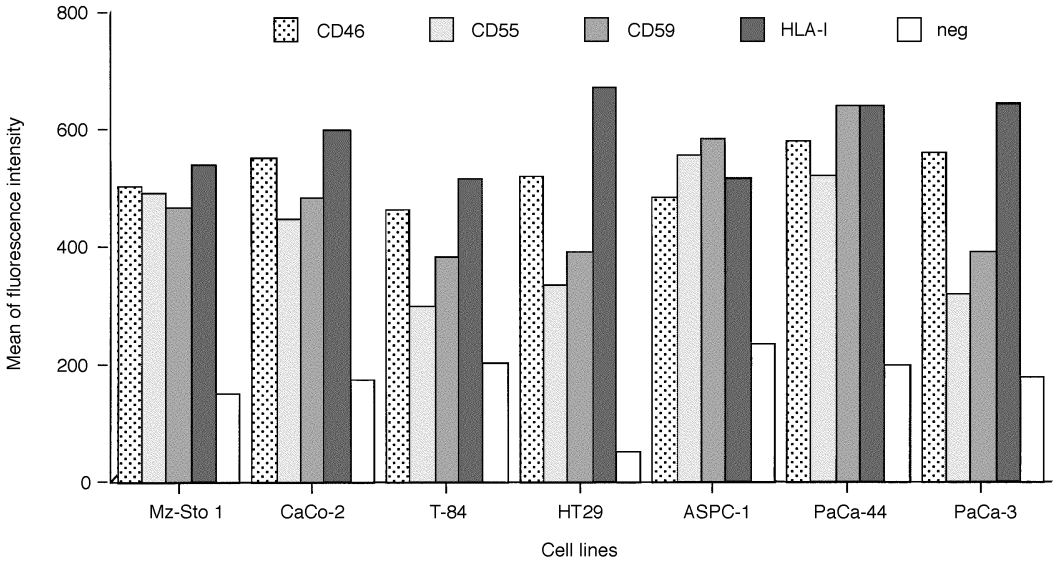

Figure 3. Flow cytometric analysis of the basic CD46, CD55 and CD59 protein expression levels in gastrointestinal cell lines. The corresponding levels of the isotype-matched controls were markedly lower (data not shown). HLA class I expression level as positive control. Irrelevant isotype as negative control (neg).

primary antibodies used in flow cytometric analysis. In gastric tissue samples from 5 patients, the normal mucosa was homogeneously positive for the CD46 and CD59 protein expression in all cases, whereas the staining pattern of CD55 was less intense and heterogeneous. The gastric cancer samples revealed a strong staining intensity for CD46 (Figure 4a) and CD59, but no detectable CD55 in malignant areas. Pancreatic tissue samples of 4 patients were evaluated by immunohistochemistry. The acinar and exocrine duct cells from normal pancreatic tissue expressed CD46 and CD59 predominantly in the cytoplasmic compartment, whereas the islet cells were negative. A heterogeneous and weak CD55 protein expression was only found in neoplastic samples. Immunoreactivity in pancreatic cancer samples against CD46 was strongly positive. CD59 (Figure 4b) was positive in the majority of cancer cells. Colorectal tissue specimens of 9 patients revealed a strong and homogeneous expression of CD46 throughout the normal mucosa with a similar staining pattern in the tumorous areas of nearly all colon cancer samples (Figure 4c, e and g). CD59 was expressed at different levels and predominantly on the luminal cell surface of the normal colonic mucosa. In the tumour samples tested, CD59 was positive in moderately or well differentiated areas, but remained partially negative in poorly differentiated areas. The expression of CD55 was found mostly positive in the normal colonic mucosa with a predominance on the luminal cell surface of the upper part of the crypts (Figure 4d). The malignant areas of the colonic cancer samples evaluated in this study were entirely negative for CD55 (Figure 4f and h). Stromal structures of all tissues tested were slightly positive for CD46 and broadly positive for CD55 and CD59. Taken together, these immunohistochemical data reveal the expression levels and patterns of CD46, CD55 and CD59 in primary clinical material and they strongly support our observations in tumour cell lines.

Influence of IFN-γ on transcript and protein expression levels

To determine the effect of IFN-γ on the expression of complement inhibitors, cell lines were incubated for 48 h in the presence or absence of IFN-γ (100 units/ml). In order to validate the efficacy of IFN-γ, a flow cytometric analysis with MAb W6/32 against the shared HLA-A/B/C determinant was performed. According to the data of Carrel and colleagues [41], IFN-γ significantly ($P < 0.05$) increased the expression of HLA class I antigens.

As detected by Northern blot hybridisation, IFN-γ evoked a weak induction of CD46 mRNA in Mz-Sto-1, CaCo-2 and 818-4 cells (Figure 1a). Both the 1.5 and the 2.2 kb CD55 transcript species were markedly upregulated in HT29 and PaCa-44 cells. Surprisingly, a weak CD55 hybridisation signal nearby 3.6 kb was detected for the first time. This band occurred in Mz-Sto-1, HT29, 818-4 and PaCa-44 cells exclusively after stimulation with IFN-γ (Figure 1b). The length of this 3.6 kb transcript may be due to the use of a further alternative polyadenylation site or due to another unspliced form. The entire group of CD59 transcript species was enhanced in T-84 and 818-4 cells and showed a marked 7.5-fold increase in HT29 cells (Figure 1c). All induction ratios higher than 1.25 are listed in Table 2; relevant reductions (ratio < 0.75) due to IFN-γ could be detected in none of the samples analysed. Interestingly, together the colon cancer cell lines showed on average a 2.6-fold increase of CD55 transcript expression and a 3.3-fold increase of CD59 tran-

Table 2. Induction of RNA and protein expression by IFN-γ (ratio cut-off > 1.25)

	RNA expression ratio*		Protein expression ratio†	
CD46	Mz-Sto-1	1.5	No increase	
	CaCo-2	1.4		
	818-4	1.5		
CD55	HT29	3.4	HT29	1.5
	PaCa-44	3.2		
CD59			HT	1.3
	T-84	2.0		
	818-4	1.4		

*RNA expression quantified by Northern blot hybridisation signal scanning (see Materials and Methods). †Protein expression by flow cytometric as mean fluorescence intensity. Ratio = IFN-γ-treated/untreated expression level.

script expression. A mean 1.6-fold induction of CD55 mRNA was observed in the pancreas group.

Regarding the protein level (Table 2), CD46 expression was increased in none of the cell lines tested by flow cytometry. Corresponding to the highest transcript induction ratios, only HT29 cells showed a moderate induction of CD55 (1.5-fold) and CD59 (1.3-fold) protein expression. The upregulated CD59 protein expression on HT29 cells is in good agreement with previous data published by Bjørge and colleagues [42].

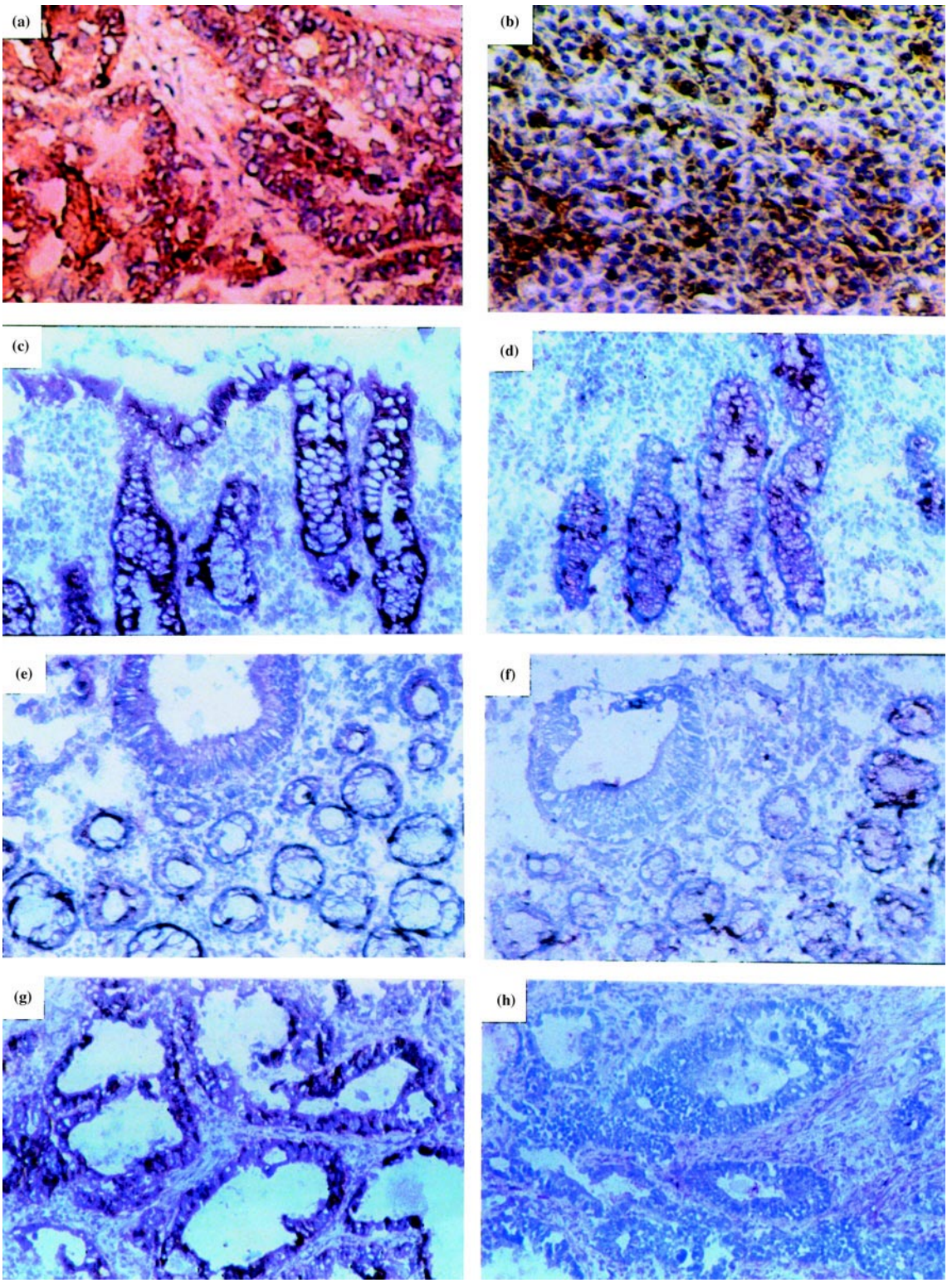

Figure 4. Immunohistochemistry of cryostat sections. Expression of CD46 (+++) in a gastric carcinoma (a); expression of CD59 (++) in a pancreatic carcinoma (b); expression of CD46 in normal colonic mucosa (c; +++), adenomatous region (e; +++) and carcinoma (g; +++) and of CD55 in the same normal colonic mucosa (d; +++), adenomatous region (f; +) and carcinoma (h; −). Original magnification: ×200.

DISCUSSION

Reports on the distribution of complement inhibitors in the gastrointestinal tract are limited. To our knowledge there are no published data on their expression in stomach and pancreas. Here we report a comprehensive study of 14 gastrointestinal tumour cell lines as well as fresh frozen tissue specimens. The goal was to evaluate the RNA and protein expression of the three membrane-bound complement inhibitors CD46, CD55 and CD59 by Northern blot hybridisation, RT-PCR, flow cytometry and immunostaining. The present study shows for the first time a synopsis of complement inhibitor RNA and protein expression and its regulation in cultured gastric and pancreatic cancer cells.

As reported by several immunohistochemical studies on colorectal tissue samples [25–28], CD46 was broadly expressed in the normal mucosa, whereas CD55 and CD59 were detected inconsistently on normal colonocytes. In colorectal cancer, CD46 expression remained unchanged, whilst CD55 was expressed heterogeneously with an upregulation in a subset of carcinomas and CD59 could be detected in most cases. The immunohistochemical results of our study confirm these findings. Interestingly, the staining patterns of the complement inhibitor proteins in stomach and pancreas are similar to those of colorectal tissues. The present study reveals a strong immunoreactivity against CD46 and CD59 in normal and neoplastic gastric and pancreatic tissue, but a weak and only sporadic expression of CD55 in these tissues.

At the RNA level, we demonstrated a homogeneous expression of CD46 and CD59 and a heterogeneous expression of CD55 in unstimulated cell lines from stomach, colon and pancreas. The three inhibitor proteins were detected in all cell lines by flow cytometry. These results are supported by additional immunohistochemical findings. The protein expression intensities of CD46, CD55 and CD59 in colon cancer tissue specimens are in good agreement with the studies published by Inoue and colleagues and Koretz and associates [25–27] and confirm our results generated by flow cytometry. With regard to the possible role of CD55 as a marker for colorectal cancer, we could not observe an enhanced staining intensity for CD55 in colon cancer tissue samples. Although CD55 seems to be upregulated in colon cancer, Koretz and colleagues reported in the same study, that 31/88 colorectal carcinomas were completely negative and 52/88 only focally positive for CD55 protein expression using MAb BRIC 110 [25]. It is not clear whether the presence of increased stool CD55 levels in colorectal cancer patients [43] derives from faecal occult blood or from colon cancer cells. Also, release of CD55 into the lumen may result from shedding of the membrane-bound CD55 from the cell surface by proteolytic cleavage or, alternatively, may be due to a secreted form of CD55. It is known that a partly unspliced minor transcript species of CD55 encodes for a secreted form of CD55 [39]. Our RT-PCR data contradict the presence of a secreted form in the colon cancer cells tested and hence suggest that luminal CD55 results from enzymatic cleavage or faecal blood cells. Thus, the origin of increased stool CD55 in colorectal cancer patients remains to be elucidated.

Tumour cells are protected against complement-mediated cytotoxicity by membrane-bound complement inhibitors [3, 7–13]. The potential mechanisms of complement inhibitor induction in malignant tissues are still unclear. An attractive hypothesis discusses the cytokine release of tumour-infiltrating mononuclear cells. Activated macrophages and T-lymphocytes in colonic adenocarcinomas secrete IFN-γ [44]. In colon cancer cells, IFN-γ has been shown to induce the expression of the proactivating complement components C3, C4 and factor B [45]. Besides its endogenous role, IFN-γ is a well established anticancer drug in the treatment of solid tumours. Therefore, the present study was intended to analyse whether IFN-γ stimulates the expression of membrane-bound complement inhibitors on gastrointestinal tumour cells.

The susceptibility of complement regulators to IFN-γ has been investigated in a few studies focused on single cell lines. Although there were different technical conditions compared with our study, Andoh and colleagues [13] reported no effect of IFN-γ on CD55 mRNA expression in HT29 cells as did Bjørge and associates [46], who did not observe any change of the CD55 protein level on these cells. Bjørge and associates reported increased protein expression levels of CD59 on HT29 cells after incubation with IFN-γ [42]. Moreover, inducibility of CD46 and CD55 proteins by treatment with IFN-γ was published for non-neoplastic thyroid cells [12] and oligodendrocytes [47].

In the present study, regulatory effects of IFN-γ on RNA and protein expression of the membrane-bound complement inhibitors were evaluated on gastric, colon and pancreatic cancer cell lines. IFN-γ evoked a weak induction of several inhibitor mRNAs in a subset of the cell lines. Upregulated protein expression was only observed in HT29 cells for CD55 and CD59 and was accompanied by a marked increase of the corresponding transcripts. Taken together, the cell lines tested in this study displayed differential responses to IFN-γ, whereas the colon cancer cell lines tended to be susceptible to IFN-γ. In conclusion, IFN-γ might promote tumour escape in subgroups of gastrointestinal cancer entities.

The results of the present study highlight the importance of the complement inhibitors for colon, gastric and pancreatic carcinoma. Immunotherapy of patients using antibodies against tumour associated antigens has to consider the putative role of complement inhibitors in anticancer treatment escape. Further studies are required to correlate clinical data with the expression of complement inhibitors in tumour tissues. Interference of complement inhibitors with the clinical success of immunotherapies may lead to cotreatment strategies against the inhibitors themselves.

1. Niculescu F, Rus HG, Retegan M, Vlaicu R. Persistent complement activation on tumor cells in breast cancer. *Am J Pathol* 1992, **140**, 1039–1043.
2. Yamakawa M, Yamada K, Tsuge T, *et al.* Protection of thyroid cancer cells by complement-regulatory factors. *Cancer* 1994, **73**, 2808–2817.
3. Bjørge L, Vedeler CA, Ulvestad E, Matre R. Expression and function of CD59 on colonic adenocarcinoma cells. *Eur J Immunol* 1994, **24**, 1597–1603.
4. Guidi L, Baroni R, Bartolini C, *et al.* Immune complexes in solid tumours precipitable by 3.5% polyethylene glycol: analysis of some nonspecific components. *Diagn Clin Immunol* 1988, **5**, 284–288.
5. Panneerselvam M, Welt S, Old LJ, Vogel C-W. A molecular mechanism of complement resistance of human melanoma cells. *J Immunol* 1986, **136**, 2534–2541.
6. Atkinson JP, Farries T. Separation of self from non-self in the complement system. *Immunol Today* 1987, **8**, 212–215.
7. Hakulinen J, Meri S. Expression and function of the complement membrane attack complex inhibitor protectin (CD59) on human breast cancer cells. *Lab Invest* 1994, **71**, 820–827.

8. Cheung N-KV, Walter EI, Smith-Mensah WH, Ratnoff WD, Tykocinski ML, Medof ME. Decay-accelerating factor protects human tumor cells from complement-mediated cytotoxicity *in vitro*. *J Clin Invest* 1988, **81**, 1122–1128.
9. Davies A, Simmons DL, Hale G, *et al.* CD59, an LY-6-like protein expressed in human lymphoid cells, regulates the action of the complement membrane attack complex on homologous cells. *J Exp Med* 1989, **170**, 637–654.
10. Seya T, Hara T, Matsumoto M, Sugita Y, Akedo H. Complement mediated tumor cell damage induced by antibodies against membrane cofactor protein (MCP, CD46). *J Exp Med* 1990, **172**, 1673–1680.
11. Kumar S, Vinci JM, Pytel BA, Baglioni C. Expression of messenger RNAs for complement inhibitors in human tissues and tumors. *Cancer Res* 1993, **53**, 348–353.
12. Tandon N, Yan SL, Morgan BP, Weetman AP. Expression and function of multiple regulators of complement activation in autoimmune thyroid disease. *Immunology* 1994, **81**, 643–647.
13. Andoh A, Fujiyama Y, Sumiyoshi K-I, Sakumoto H, Bamba T. Interleukin 4 acts as an inducer of decay-accelerating factor gene expression in human intestinal epithelial cells. *Gastroenterology* 1996, **111**, 911–918.
14. Liszewski MK, Post TW, Atkinson JP. Membrane cofactor protein (MCP or CD46): newest member of the regulators of complement activation gene cluster. *Annu Rev Immunol* 1991, **9**, 431–455.
15. Nicholson-Weller A, Wang C. Structure and function of decay accelerating factor CD55. *J Lab Clin Med* 1994, **123**, 485–491.
16. Morgan BP. Physiology and pathophysiology of complement: progress and trends. *Crit Rev Clin Lab Sci* 1995, **32**, 265–298.
17. Lachmann PJ, Hughes-Jones NC. Initiation of complement activation. In Müller-Eberhardt H, Miescher PA, eds. *Complement*. Berlin, Springer-Verlag, 1985, 147–166.
18. Reid KBM, Bentley DR, Campbell RD, *et al.* Complement system proteins which interact with C3b or C4b. A superfamily of structurally related proteins. *Immunol Today* 1986, **7**, 230–234.
19. Esser AF. Big MAC attack: complement proteins cause leaky patches. *Immunol Today* 1991, **12**, 316–318.
20. Morgan BP. Complement regulatory molecules: application to therapy and transplantation. *Immunol Today* 1995, **16**, 257–259.
21. Seya T, Atkinson JP. Functional properties of membrane cofactor protein of complement. *Biochem J* 1989, **264**, 581–588.
22. Meri S, Morgan BP, Davies A, Daniels RH, Olavesen MG, Waldmann H. Human protectin (CD59), an 18000–20000 MW complement lysis restricting factor, inhibits C5b-8 catalysed insertion of C9 into lipid bilayers. *Immunology* 1990, **71**, 1–9.
23. Oglesby TJ, White D, Liszewski K, Wright L, Van den Bogarde J, Atkinson JP. Protection of mammalian cells from complement-mediated lysis by transfection of human membrane cofactor protein and decay-accelerating factor. *Trans Assoc Am Phys* 1991, **104**, 164–172.
24. Brooimans RA, Van Wieringen PA, Van Es LA, Daha MR. Relative roles of decay-accelerating factor, membrane cofactor protein and CD59 in the protection of human endothelial cells against complement-mediated lysis. *Eur J Immunol* 1992, **22**, 3135–3140.
25. Koretz K, Brüderlein S, Henne C, Möller P. Decay-accelerating factor (DAF, CD55) in normal colorectal mucosa, adenomas and carcinomas. *Br J Cancer* 1992, **66**, 810–814.
26. Koretz K, Brüderlein S, Henne C, Möller P. Expression of CD59, a complement regulator protein and second ligand of the CD2 molecule, and CD46 in normal and neoplastic colorectal epithelium. *Br J Cancer* 1993, **68**, 926–931.
27. Inoue H, Mizuno M, Uesu T, Ueki T, Tsuji T. Distribution of complement regulatory proteins, decay-accelerating factor, CD59/homologous restriction factor 20 and membrane cofactor protein in human colorectal adenoma and cancer. *Acta Med Okayama* 1994, **48**, 271–277.
28. Niehans GA, Cherwitz DL, Staley NA, Knapp DJ, Dalmasso AP. Human carcinomas variably express the complement inhibitory proteins CD46 (membrane cofactor protein), CD55 (decay-accelerating factor) and CD59 (protectin). *Am J Pathol* 1996, **149**, 129–142.
29. Meri S, Waldmann H, Lachman PJ. Distribution of protectin (CD59), a complement membrane attack inhibitor, in normal human tissues. *Lab Invest* 1991, **65**, 532–537.
30. Uesu T, Mizuno M, Inoue H, Tomoda J, Tsuji T. Enhanced expression of decay accelerating factor and CD59/homologous restriction factor 20 on the colonic epithelium of ulcerative colitis. *Lab Invest* 1995, **72**, 587–591.
31. Dippold WG, Meyer zum Böschenfelde K-H. Proliferation of gastrointestinal carcinoma cells by T-lymphocyte factors interleukin-3 and granulocyte-macrophage-colony-stimulating factor. *Immunol Res* 1991, **10**, 258–260.
32. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform-extraction. *Anal Biochem* 1987, **162**, 156–159.
33. Lublin DM, Atkinson JP. Decay-accelerating factor: biochemistry, molecular biology and function. *Ann Rev Immunol* 1989, **7**, 35–58.
34. Ponte P, Sun-Yu N, Engel J, Gunning P, Kedes L. Evolutionary conservation in the untranslated regions of actin mRNAs: DNA sequence of a human beta-actin cDNA. *Nucleic Acids Res* 1984, **12**, 1687–1696.
35. Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory Press, 1989.
36. Pesando JM, Abed M, Hoffman P. Altered expression of surface antigens with appearance of HLA class II molecules on a malignant human B cell line. *Human Immunol* 1987, **19**, 235–243.
37. Hadam MR. Cluster report: CD55. In Knapp W, Dörken B, Gilks WR, *et al.*, eds. *Leukozyte Typing IV. White Cell Differentiation Antigens*. London, Oxford University Press, 1989, 694–697.
38. Dippold WG, Dienes HR, Knuth A, Meyer zum Büschenfelde K-H. Immunohistochemical localization of ganglioside GD3 in human malignant melanoma, epithelial tumors and normal tissues. *Cancer Res* 1985, **45**, 3699–3705.
39. Caras IW, Davitz MA, Rhee L, Weddell G, Martin Jr DW, Nussenzweig V. Cloning of decay-accelerating factor suggests novel use of splicing to generate two proteins. *Nature* 1987, **325**, 545–548.
40. Tone M, Walsh LA, Waldmann H. Gene structure of human CD59 and demonstration that discrete mRNAs are generated by alternative polyadenylation. *J Mol Biol* 1992, **227**, 971–976.
41. Carrel S, Schmidt-Kessen A, Giuffre L. Recombinant interferon-γ can induce the expression of HLA-DR and -DC on DR-negative melanoma cells and enhance the expression of HLA-ABC and tumor-associated antigens. *Eur J Immunol* 1985, **15**, 118–123.
42. Bjørge L, Skeie Jensen T, Ulvestad E, Vedeler CA, Matre A. The influence of tumour necrosis factor-α, interleukin-1β and interferon-γ on the expression and function of the complement regulatory protein CD59 on the human colonic adenocarcinoma cell line HT29. *Scand J Immunol* 1995, **41**, 350–356.
43. Mizuno M, Nakagawa M, Uesu T, *et al.* Detection of decay-accelerating factor in stool specimens of patients with colorectal cancer. *Gastroenterology* 1995, **109**, 826–831.
44. Van Ravenswaay-Claasen HH, Kluin PM, Fleuren GJ. Tumor infiltrating cells in human cancer. On the possible role of $CD16^+$ macrophages in antitumor cytotoxicity. *Lab Invest* 1992, **67**, 166–174.
45. Andoh A, Fujiyama Y, Bamba T, Hosoda S. Differential cytokine regulation of complement C3, C4 and factor B synthesis in human intestinal epithelial cell line CaCo-2. *J Immunol* 1993, **151**, 4239–4247.
46. Bjørge L, Skeie Jensen T, Matre R. Characterization of the complement-regulatory proteins decay-accelerating factor (DAF, CD55) and membrane cofactor protein (MCP, CD46) on a human colonic adenocarcinoma cell line. *Cancer Immunol Immunother* 1996, **42**, 185–192.
47. Gasque P, Morgan BP. Complement regulatory protein expression by a human oligodendrocyte cell line: cytokine regulation and comparison with astrocytes. *Immunology* 1996, **89**, 338–347.