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Review

Detection of cytotoxic CD13-specific autoantibodies in sera from patients with ulcerative colitis and Crohn's disease

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Abstract

Recent evidence suggests an association between inflammatory bowel disease (IBD) and human cytomegalovirus (HCMV) infection, but the exact pathogenic role of HCMV in this disease remains unclear. HCMV infection has for a long time been known to be associated with various autoimmune manifestations and the formation of autoantibodies. Previous studies from our group have shown that HCMV is associated with a human protein, CD13 (aminopeptidase N) and that autoantibodies against this protein are frequently found in HCMV infected bone marrow transplant patients with chronic graft versus host disease. We have recently observed that 90% of IBD patients have an active HCMV infection. In this study, we examined the presence and cytotoxicity of CD13-specific autoantibodies in sera obtained from 28 patients with ulcerative colitis and 26 patients with Crohn's disease, and in sera obtained from healthy blood donors by using flow cytometric assays against mouse cells transfected with human CD13 or a microcytotoxicity assay against different CD13 positive human cells. Cytotoxic CD13-specific autoantibodies were identified in 66% of the sera obtained from HCMV-IgG positive patients with ulcerative colitis and in 58% of the sera obtained from HCMV-IgG positive patients with Crohn's disease, but not in control individuals. These cytotoxic autoantibodies may interfere with biological cell functions and could thereby contribute to the chronic inflammation in patients with IBD.

Keywords: Inflammatory bowel disease; Cytomegalovirus; Autoantibody; CD13

1. Introduction

Human cytomegalovirus (HCMV) belongs to the Herpesviridae family and is highly prevalent in the population all over the world [1]. Like other herpesviruses, HCMV establishes latency, and the virus frequently reactivates in immunosuppressed patients such as transplant patients and HIV infected patients. Reinfection with a new genetically distinct HCMV strain that has been observed in transplant recipients, suggest that a previously established HCMV infection in a patient does not protect against re-infection [2]. In immunocompromised hosts, primary or reactivated HCMV infection can result in a variety of clinical manifestations, and sometimes the virus can cause life-threatening HCMV disease. HCMV disease of the gastrointestinal (GI) tract is a major cause of morbidity and mortality in immunocompromised patients. HCMV GI disease is often suspected in AIDS patients with GI symptoms [3]. During the last four decades, increasing evidence also suggests that HCMV may be prevalent in patients with inflammatory bowel disease (IBD), but the exact pathogenic role of HCMV in disease development remains uncertain [4–12]. We have previously provided evidence for a high prevalence of HCMV-DNA and HCMV antigens in intestinal tissue sections obtained from patients with IBD, which suggest a common presence of an active HCMV infection in these patients.

We have also previously shown that HCMV can be reactivated from latency in macrophages by an involvement of inflammatory cytokines [13,14], suggesting that viral reactivation occurs in inflammatory tissues. An increased number of intestinal macrophages can often be observed in active IBD,

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and they are especially prominent in granulomas that are often observed in the bowel of patients with Crohn's disease [15].

There is a general agreement that autoimmune diseases have a multifactorial etiology that is dependent on both genetic and environmental factors. Among the latter, viruses may play an important role since they can induce autoimmunity through several mechanisms, such as exposure of normally "hidden" epitopes, presentation of cross-reactive peptides, through upregulation of key molecules on antigen presenting cells, by molecular mimicry mechanism, and presentation of host cell-derived proteins in the viral envelope [16–20]. HCMV infection has been suggested to be associated with various autoimmune manifestations such as hemolytic anemia, granulocytopenia, the autoimmune like disease chronic graft versus host disease (GVHD) in bone marrow transplant patients (BMT) and the production of a variety of autoantibodies. Our group has previously shown that the cellular molecule CD13 becomes associated with HCMV particles during viral particle maturation in infected cells and that the HCMV-associated autoantigen CD13 appears to become immunogenic during HCMV infection [16–18]. In support of this hypothesis, we have previously found CD13-specific autoantibodies in bone marrow transplant (BMT) patients with an active HCMV infection [17]. These antibodies may bind to cells that are CD13 positive in the patients tissues, such as endothelial cells, smooth muscle cells, granulocytes and macrophages and may exert cytotoxic effects and may also affect important cellular functions.

A variety of immune abnormalities have been described in the intestine of IBD patients and antibodies against several different cell types such as neutrophil granulocytes, endothelial, epithelial, inflammatory, and smooth muscle cells have been reported in patients with both ulcerative colitis (UC) and Crohn's disease (CD) [21–25]. Such antibodies have been suggested to play a pathogenic role in IBD. Therefore, we examined the prevalence and the cytotoxic effect of autoantibodies directed against CD13, in sera obtained from patients with UC and CD.

2. Materials and methods

2.1. Patient samples

Sera were collected from 28 patients with active UC (13 sera of these came from patients with UC, that we have previously examined for the presence of HCMV [26]), and 26 patients with active CD (10 sera of these came from patients with CD, that we have previously examined for the presence of HCMV [26]). Colonoscopy, clinical, radiologic, and histologic criteria were used to establish the diagnosis in each case (Table 1). Twenty of 28 of patients with severe UC and 16/26 of patients with severe CD did not respond to conventional medical treatment and underwent surgery. Sera obtained from 20 healthy blood donors served as controls.

For detection of the presence of IgG antibodies in the intestinal tissue, paraffin-embedded intestinal tissue sections were obtained from 13 patients with UC, 10 patients with CD, and 10 samples from uninflamed intestinal tissues from the tumor-free resection end of the bowel of patients with intestinal tumor were used as negative controls (that we have previously examined for the presence of HCMV [26]).

2.2. Cells

THP-1 cells (acute monocytic leukemia cell line, American Type Culture Collection (Rockville, MD, USA), were maintained in bicarbonate-free RPMI1640 medium supplemented with 25 mM HEPES [4-(2hydroxyethyl)-1piperazine ethanesulfonic acid], 10% heat inactivated fetal calf serum, L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml) (all from GIBCO BRL, Grand Island, NY, USA). The NIH-3T3 transfected with and expressing human aminopeptidase N (hAPN-3T3, at the cell surface) were kindly provided by A.T. Look, St Jude Children's Research Hospital, Memphis, TN, USA. These cells were maintained in bicarbonate-free minimal essential medium with Hanks' salts (Gibco BRL) including supplements with the addition of 800 μg of G418 (Geneticin; Gibco BRL) per ml. Human pulmonary arterial endothelial cells (HPAEC, Clonetics) were cultured in 37 °C and 5% CO_2 in EGM-2 medium (Clonetics, CA, USA) containing growth factors according to the manufacturers instructions. CaCo-2 cells were obtained from the American Type Culture Collection and cultured at 37 °C in an atmosphere of 5% CO_2 in complete medium (Dulbecco's modified essential medium [Gibco BRL] containing 20% fetal bovine serum and supplemented with 4 mM L-glutamine, penicillin [100 U/ml] and streptomycin [100 μg/ml]).

Neutrophil leukocytes were isolated from human peripheral blood from healthy donors according to Böyum et al. [27]. Briefly, 25 ml of heparinized whole blood was layered on a gradient consisting of 20 ml Polymorphoprep™ overlayered by 5 ml Lymphoprep™, and centrifuged for 40 min at 480 × *g* at room temperature. The band rich in polymorphonuclear cells was collected and contaminating erythrocytes were removed by hypotonic lysis. The cells were washed twice in Krebs-Ringer phosphate buffer without Mg^{2+} and Ca^{2+} and resuspended in KRG.

2.3. Antibodies

Monoclonal antibodies against CD13 (WM 15, Dakopatts, Glostrup, Denmark) were used as a positive control in flow cytometry (FACS). The following mouse monoclonal antibodies were used for the blocking assays: anti-CD33 (Becton Dickinson, Stockholm, Sweden), anti-CD34 (Dakopatts), anti-CD13 (MY7, Beckman Coulter, CA, USA) and WM15 (Dakopatts).

2.4. Binding of human sera to mouse cells expressing human CD13

For flow cytometric investigations, mouse NIH-3T3 cells or CD13 transfected mouse cells (hAPN-3T3) were incubated with 50 μl of patient serum (which had been pre-absorbed to NIH-3T3 cells to remove natural antibodies), for 30 min at

Table 1
Main characteristics of the IBD patients enrolled in the study

Diagnosis	Patients (*n*)	Age range (y)	Sex		Corticosteroid treatment (%)	HCMV IgG (%)	Cytotoxic anti CD13-IgG pos/ severe inflammation (%)	Cytotoxic anti CD13-IgG pos/ HCMV-IgG pos (%)	Cytotoxic anti CD13-IgG pos/ HCMV-IgG neg (%)
			M	F					
Ulcerative colitis (UC)	28	18–78	15	13	20 (71)	24 (86)	17/20 (85)	16/24 (66)	1/4 (25)
Crohn's disease (CD)	26	31–72	10	16	16 (61)	19 (73)	14/16 (87)	11/19 (58)	3/7 (43)
Healthy blood donors	20	–	–	–	–	15 (75)	–	0/15 (0)	0/5 (0)

Serological status and details about patients from whom sera were examined. By using ELISA, IgG antibodies specific against HCMV in sera were detected in 86% of patients with UC, in 73% of patients with CD and in 75% of healthy blood donors. Autoantibodies against CD13 in sera were examined by using CD13-transfected mouse cells (hAPN-3T3) and analyzed by FACS. Cytotoxic anti CD13 autoantibodies which could be blocked by using monoclonal antibodies against CD13 were detected in 66% and 58% of HCMV-IgG positive patients with UC and CD, respectively. Cytotoxic anti CD13 antibodies could be detected in 85% of patients with severe UC and in 87% of patients with severe CD.

room temperature (RT). The cells were washed with PBS and incubated with fluoresceinated (FITC) goat anti-human IgG (Dakopatts) antibodies separately for 25 min at 4 °C. The cells were washed twice, resuspended in PBS and analyzed by FACS.

2.5. Blocking of antibody binding to CD13 expressed on mouse cells by CD13-specific antibodies

Mouse NIH/3T3 or CD13 transfected mouse cells (hAPN-3T3) were incubated with $F(ab)_2$ fragments of mouse monoclonal antibodies CD13 (MY7, WM15), CD33 and CD34 for 1 h at RT. The cells were washed, incubated with sera (which had been pre-absorbed to NIH/3T3 cells to remove natural antibodies) followed by a FITC conjugated antibody and the cells were thereafter analyzed by flow cytometry. The criterion chosen for positive inhibition was a reduction by at least 10 channels in mean fluorescence compared to controls.

2.6. Microcytotoxic assay

All sera were screened for the presence of cytotoxic antibodies directed against THP-1 cells, fresh neutrophil granulocytes, CaCo-2 cells and HPAEC in triplicate. Cytotoxicity was detected by rabbit complement with the dye acridine orange (Sigma–Aldrich, Stockholm, Sweden) and ethidium bromide added. Sera were added to the cells and the results of the cytotoxicity assay were evaluated in an inverted fluorescence microscope. The dead cells versus alive cells were counted and expressed as a percentage. A blocking assay was performed by incubating THP-1 cells, fresh neutrophil granulocytes, HPAEC and CaCo-2 with $F(ab)_2$ fragments of mouse monoclonal antibodies CD13, for 1 h at RT. Anti-CD13 $F(ab)_2$ fragments (WM15 and MY7 [IgG1]) was obtained by treatment with pepsin (2 mg/ml IgG) for 24 h at 37 °C followed by dialyzation against an acetate buffer (pH 4.0) for 4 h at 4 °C; the reaction was stopped by adding 2 M Tris. The digested proteins were thereafter dialyzed against PBS over night and the $F(ab)_2$ fragments were separated from the cleaned Fc fragments by incubation with protein A for 1 h at 4 °C. $F(ab)_2$ fragments of CD33 and CD34-specific antibodies were obtained in the same way and served as controls. All sera were added to the cells and the results were quantified as described above.

2.7. Detection of IgG antibodies in sera from patients with UC and CD reactive against different cell types

To investigate the presence of IgG antibodies in sera from IBD patients, 50 μl of a tenfold dilution of each serum was incubated with unfixed fresh neutrophil granulocytes, CaCo-2, HPAEC and THP-1 for 30 min at 4 °C. After washing, bound antibodies were identified by incubation with FITC conjugated goat anti-human IgG antibodies (Dakopatts). The cells were spotted on slides by using a cytocentrifuge (Cytospin 3, Shandon Southern Products Ltd., Runcorn, UK) and fixed in cold Cytofix (Dakopatts) and examined in a fluorescence microscope.

To investigate whether antibodies in the patient sera reacted with CD13, we performed blocking experiments. The unfixed THP-1 cells, fresh neutrophil granulocytes, CaCo-2 cells and HPAEC were incubated with $F(ab)_2$ fragments of a mixture of two CD13-specific monoclonal antibodies (MY7, WM15) or anti-CD33 antibodies for 30 min at 4 °C. Thereafter, the cells were assayed for the binding of IgG in sera obtained from IBD patients as described above. FITC conjugated CD13-specific antibodies served as positive controls.

2.8. Serological screening for detection of HCMV-IgG

All sera from IBD patients were examined for detection of IgG against HCMV antigens by using ELISA as previously described by Sundqvist et al. [28]. Sera from HCMV positive and negative healthy blood donors were used as control sera.

2.9. Detection of IgG antibodies in paraffin-embedded intestinal tissue sections from patients with UC and CD

To investigate the presence of IgG antibodies in paraffin embedded intestinal tissues obtained from IBD patients, immunohistochemical staining was performed. Briefly, formalin fixed tissues embedded in paraffin, were sectioned, deparaffinized in xylene, treated with H_2O_2 in methanol, rehydrated through a series of graded alcohols, and washed in PBS.

Proteinase K treatment and non-specific blocking in 1% BSA preceded immunostaining. The specimens were incubated with horseradish peroxidase-conjugated goat anti human antibody (Dakopatts) for 30 min at RT. The presence of IgG immunoglobulin was detected using the diaminobenzidine (Dakopatts). The sections were counterstained with hematoxylin, and mounted in Aquamount (Dakopatts). Paraffin-embedded intestinal tissue sections from an uninflamed part of intestinal resection specimens obtained from patients with intestinal cancer, were used as controls.

2.10. Statistical analysis

Data are given as mean ± S.E.M. Student's *t*-test was used for statistical analysis. $*P < 0.05$ and $**P < 0.0005$ were considered as significant.

3. Results

3.1. Deposition of IgG antibodies in intestinal tissue sections obtained from IBD patients

Intestinal tissue sections obtained from patients with UC or CD were examined for the presence of IgG, and revealed staining of different cell types in the mucosa and in the deeper layer of intestine such as endothelial cells, inflammatory cells, epithelial cells, and smooth muscle cells that was not visible in the uninflamed intestinal sections that served as controls (Fig. 1).

3.2. Antibodies in sera obtained from patients with UC and CD recognize CD13 on different cell types

To investigate if sera obtained from IBD patients contained autoreactive antibodies against CD13, sera were tested for binding to THP-1, neutrophil granulocytes, CaCo-2, and HPAEC with or without blocking of CD13 (with the CD13 antibodies WM15 and MY7) and CD33. While blocking of CD13 on different cells almost completely blocked serum reactivity to the cells, CD33 antibodies did not significantly reduce this reactivity (Fig. 2).

3.3. Detection of HCMV-IgG and CD13-specific autoantibodies in sera obtained from patients with UC and CD

Expression of CD13 on NIH/3T3 and hAPN-3T3 was detected by using a monoclonal antibody against CD13 (WM15) (Fig. 3A). Each serum sample was pre-absorbed to mouse NIH-3T3 and tested for reactivity to CD13-transfected mouse cells (hAPN-3T3) and to control NIH/3T3 cells, and analyzed by flow cytometry. This method identified higher levels of reactive antibodies in sera obtained from IBD patients against mouse cells expressing CD13 as compared to sera from the control group (Fig. 3B). The CD13-specificity was determined by blocking of CD13 on mouse cells by using $F(ab)_2$ fragments of anti-CD13 monoclonal antibodies (WM15 and MY7) and anti-CD33 monoclonal antibodies were used as controls (Fig. 4A–C). By using ELISA, IgG antibodies specific for HCMV were detected in sera from 86% of patients with UC, from 73% of patients with CD and in 75% of healthy blood donors (Table 1). Cytotoxic autoantibodies against CD13 that were blocked by monoclonal antibodies against CD13 were detected in 16/24 (66%) and 11/19 (58%) of HCMV-IgG positive and in 1/4 (25%) and 3/7 (43%) of HCMV-IgG negative patients with UC and CD, respectively (Table 1).

Twenty of 28 (71%) of patients with severe UC and 16/26 (61%) of patients with severe CD did not respond to conventional medical treatment and underwent surgery. In this group of patients, cytotoxic anti-CD13 autoantibodies could be detected in 17/20 (85%) of patients with severe UC and in 14/16 (87%) of patients with severe CD. All these patients were positive for CMV-IgG except for one patient with UC and 3 patients with CD who were CMV-IgG negative (Table 1). However, in the non-severe group, lower levels of antibodies reactive against CD13 transfected mouse cells could be detected by using FACS. CD13 reactive sera were separated in two categories >50 (high) and <50 (low) in mean fluorescence intensity (MFI) in FACS analysis for CD13. All these sera were analyzed for cytotoxicity against THP-1 cells, neutrophil granulocytes, CaCo-2 cells and HPAEC (Fig. 5A–D). In eight sera obtained from UC patients with high MFI, CD13-specific $F(ab)'_2$ fragments of monoclonal antibodies in sera obtained from patients with UC blocked cytotoxicity by approximately 78% in THP-1, 50% in CaCo-2 cells, 42% in

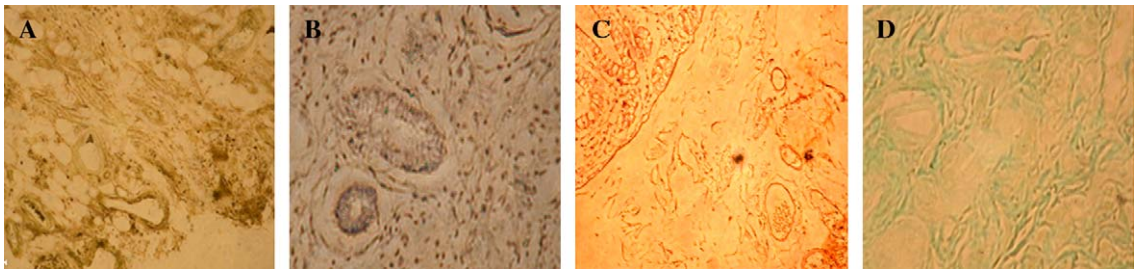

Fig. 1. Deposition of IgG antibodies in intestinal tissue sections from IBD patients. Intestinal tissue sections from patients with UC (A,C) or CD (B) examined for the presence of IgG by using immunohistochemistry and shows staining of different cell types in the mucosa and deeper layer of intestine such as endothelial, inflammatory, epithelial, and smooth muscle cells in tissue sections from IBD patients. Such antibodies were not detected in uninflamed intestinal tissue sections (D).

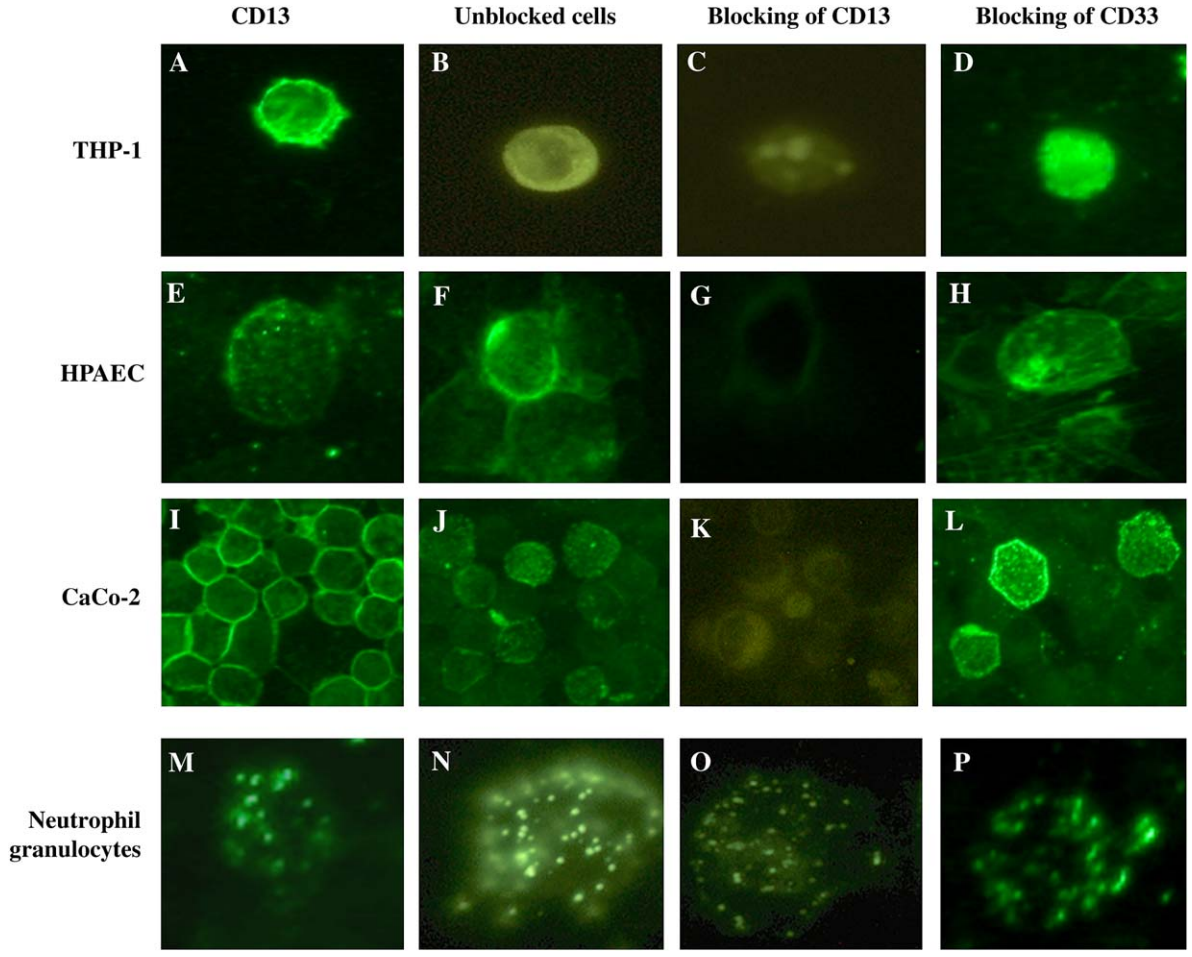

Fig. 2. Antibodies in sera recognize CD13 on the different cells types. To investigate if sera (with mean fluorescence intensity > 50 (high) in flow cytometry for CD13 antibodies) obtained from IBD patients contained autoreactive antibodies against CD13, sera were tested for binding to THP-1 cells, HPAEC, CaCo-2 cells and neutrophil granulocytes, before (B,F,J,N), and after blocking of CD13 (C,G,K,O) or after blocking of CD33 (D,H,L,P). Results from this experiment showed that antibodies in sera obtained from IBD patients bind to CD13 molecules on the cells. The expression of CD13 on different cell types were examined by FACS using monoclonal antibodies to CD13 (A,E,I,M).

HPAEC and 6% in neutrophil granulocytes. In nine sera obtained from patients with CD cytotoxicity was blocked by 78% in THP-1 cells, 43% in CaCo-2 cells, 21% in HPAEC and by 3% in neutrophil granulocytes as compared to blocking with monoclonal antibodies against CD33 or CD34 (Fig. 5A–D). Furthermore, in all sera obtained from IBD patients (UC: 20 patients, CD: 17 patients) with a low MFI, CD13-specific monoclonal antibodies blocked cytotoxicity in sera obtained from UC by approximately 58% in THP-1 cells, 35% in CaCo-2 cells, 6% in HPAEC and 20% in neutrophil granulocytes and in sera obtained from patients with CD by 68% in THP-1 cells, 5% in CaCo-2 cells, 31% in neutrophil granulocytes, but not for HPAEC. We observed that CD33 and CD34 antibodies also blocked cytotoxicity in some cell types (CaCo-2 cells, HPAEC) but not to the extent that was observed using CD13-specific antibodies (Fig. 5A–D). However, neither CD13, CD33 nor CD34 monoclonal antibodies blocked cytotoxicity to different cell types examined by using sera obtained from healthy blood donors (Fig. 5A–D). Furthermore, treatment of the different cell types with a cocktail of monoclonal anti-CD13 antibodies (MY7 and WM15) resulted in >90% cell lysis in the presence of complement.

4. Discussion

HCMV infection is a common virus infection worldwide, since the virus is infecting 60–100% of the world's population [29] and the virus persists indefinitely within the host after a primary infection. The current view is that peripheral blood monocytes constitute a major site of viral latency and that differentiation into tissue macrophages as a result of stimulation of cells with pro-inflammatory cytokines leads to HCMV reactivation and a productive infection [13,30–32]. Previous studies have demonstrated that patients with severe refractory IBD tested positive for HCMV IgG antibodies, and it seems plausible that the observed HCMV complications such as infection in the bowel are the results of HCMV reactivation rather than de novo infection in these patients [4,33].

A pathogenic association between IBD and HCMV has also been suggested by several investigators [4–12]. We have

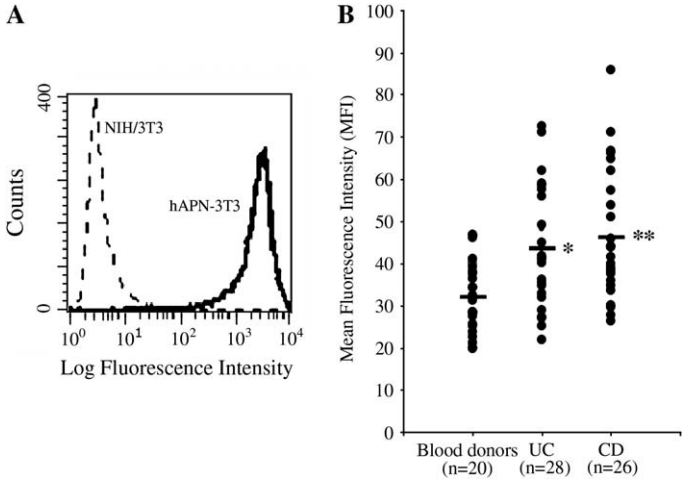

Fig. 3. Binding of human sera to mouse cells expressing human CD13. A monoclonal antibody against CD13 (WM15) was used for detection of CD13 on CD13-transfected mouse cells (hAPN-3T3) and NIH/3T3 cells (A). Each serum sample (pre-absorbed to NIH/3T3) obtained from patients with UC, CD and the control group was tested for binding to CD13-transfected mouse cells (hAPN-3T3) and to the control NIH/3T3 cells, and analyzed by flow cytometry. This method identified higher levels of CD13-specific antibodies in sera from patients with IBD as compared to the control group (B).

previously observed an active HCMV infection in 85% of patients with UC and in 100% of patients with CD [26], and Wakefield et al. [9] detected HCMV-DNA in intestinal tissue samples in 81% and 66% of patients with UC and CD, respectively, by using a nested PCR assay. Recently, Hommes et al. [34], by computer search (Medline, from 1966 to 2002; EMBASE, from 1980–2002) reported 33 case reports addressing the role of HCMV in IBD patients. The majority of these reports describe patients with severe attacks of UC, who had received corticosteroids for a prolonged time before HCMV was recognized. Toxic dilatation was present in 15% of patients, and colectomies were performed in 62% of patients. Mortality rates as high as 44% were observed in this group of patients, which illustrates the severity of disease in the reported cases. By computer search (Medline and EMBASE; from 2003 to 2006) we have identified 18 new case reports [35–52] demonstrating HCMV infection in patients with IBD, indicating an increased observed prevalence of HCMV infection among IBD patients. Several of these investigators have reported a beneficial role of antiviral treatment in IBD patients. However, the possible pathogenic role of HCMV in IBD patients is still unclear and needs further investigation.

HCMV infection has been implicated in the development of autoimmunity [1] and a variety of autoantibodies have been associated in both bone marrow and solid organ transplant recipients [16–17,53–55]. These observations pose the question whether HCMV induced autoimmunity may play a role in the pathogenesis of IBD. We have previously identified the CD13 molecule to be present on most cell types that are susceptible to HCMV infection in vitro, and have also provided evidence for CD13 to be a potential cellular receptor for the virus [56]. We have also previously described that this molecule is associated with HCMV particles and that CD13 appears to be immunogenic in bone marrow transplant patients (BMT) during clinical HCMV infection. We found that CD13-specific autoantibodies were identified in BMT patients with chronic graft-versus-host disease, but exclusively in patients who had experienced either HCMV disease or HCMV viremia, and these antibodies appeared at the time of HCMV detection [17].

In the present study, we detected higher levels CD13-specific autoantibodies with cytotoxic effects against different cell types, in sera obtained from 17/28 (61%) and in 14/26 (54%)

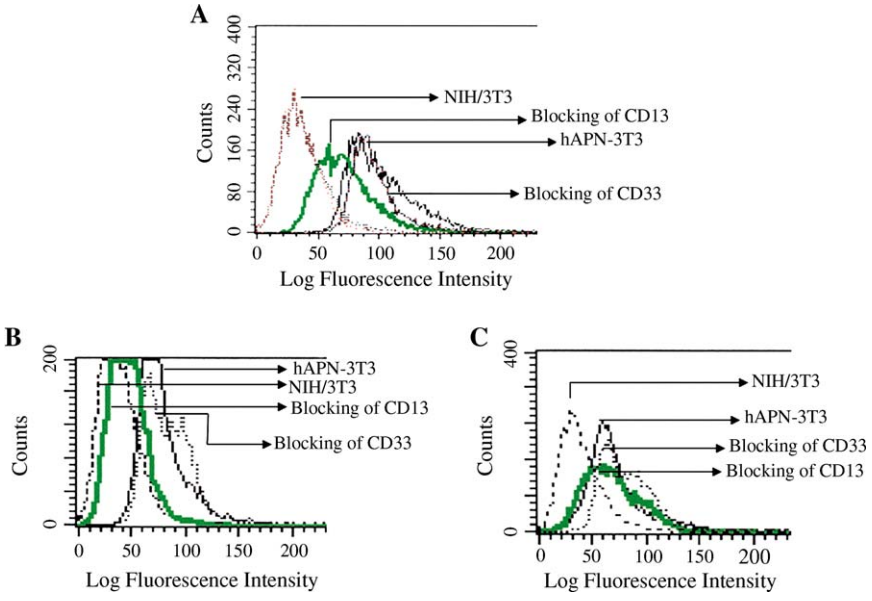

Fig. 4. Blocking of serum reacting to mouse cells expressing human CD13 by CD13-specific antibodies. The CD13-specificity was determined by blocking of CD13 expression on mouse cells by $F(ab)'_2$ fragments of anti-CD13 monoclonal antibodies (WM15, MY7) in a sera obtained from a patient with UC (MFI > 50) (A), in a sera obtained from a patient with CD (MFI < 50) (B) and in sera obtained from healthy blood donors (MFI < 50) (C). Anti-CD33 antibodies served as control antibodies.

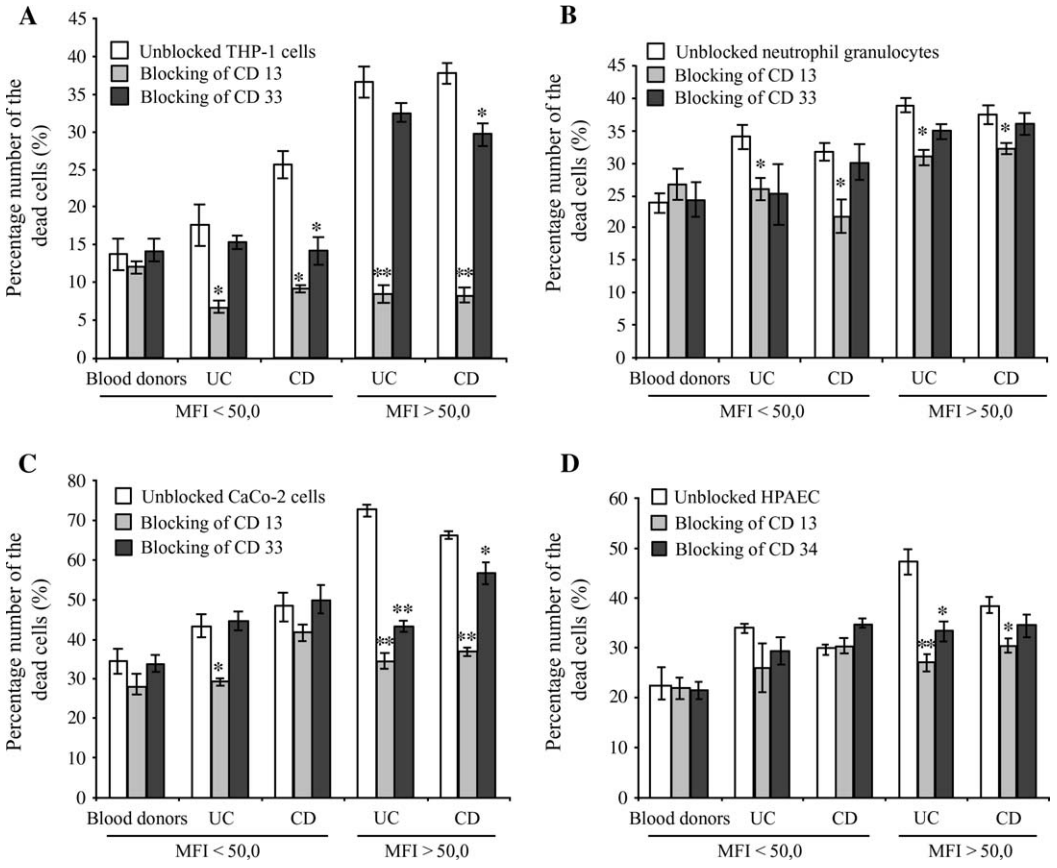

Fig. 5. Cytotoxicity of CD13 reactive sera obtained from IBD patients against different cell types. In eight sera obtained from UC patients with MFI > 50 (high), CD13-specific monoclonal antibodies in sera obtained from patients with UC blocked cytotoxicity by approximately 78% in THP-1 cells, 50% in CaCo-2 cells, 42% in HPAEC and 6% in neutrophil granulocytes and in nine sera obtained from patients with CD with MFI > 50 (high) cytotoxicity was blocked by 78% in THP-1 cells, 43% in CaCo-2 cells, 21% in HPAEC and by 3% in neutrophil granulocytes as compared to blocking with monoclonal antibodies against CD33 or CD34 (A–D). In all sera obtained from IBD patients (UC: 20 patients, CD: 17 patients) with MFI < 50 (low), CD13-specific monoclonal antibodies blocked cytotoxicity in sera obtained from UC by approximately 58% in THP-1 cells, 35% in CaCo-2 cells, 6% in HPAEC and 20% in neutrophil granulocytes and in sera obtained from patients with CD by 68% in THP-1 cells, 5% in CaCo-2 cells, 31% in neutrophil granulocytes, but not for HPAEC. In some cell types (CaCo-2 cells, HPAEC), CD33 and CD34 antibodies also blocked cytotoxicity but not to the extent that was observed using CD13-specific antibodies (A–D). Neither CD13, CD33 nor CD34 monoclonal antibodies blocked cytotoxicity to different cell types examined by using sera obtained from healthy blood donors (A–D).

of patients with UC and CD, respectively. Importantly, sera obtained from patients with UC and CD even with MFI < 50 also had cytotoxic effects on different cell types, but sera from healthy donors never exhibited cytotoxic effects. This finding indicates that antibodies with low reactivity against CD13 expressed on mouse cells or different types of cells (HPAEC, neutrophil granulocytes, CaCo-2 cells, THP-1 cells) in sera obtained from the control group most likely represent an unspecific reactivity and possibly with no pathogenic role.

In this study, cytotoxic CD13-specific autoantibodies could be detected in 16 out of 24 CMV-IgG positive patients with UC and in 11 out of 19 CMV-IgG positive patients with CD. Cytotoxic anti-CD13 autoantibodies could be detected in one out of four CMV-IgG negative patients with UC and in three out of seven CMV-IgG negative patients with CD.

Interestingly, cytotoxic CD13-specific autoantibodies could be detected in 85% of patients with severe UC and in 87% of patients with severe CD who underwent surgical treatment. The pathophysiological role of anti-CD13 antibodies is presently unknown, but they may play pathogenic role since these antibodies were commonly found in patients with severe inflammation.

We have suggested that CD13 autoantibody formation results from T-cell activation of clones with specificity for HCMV derived viral peptides on antigen presenting cells. This could result in activation of CD13 reactive non-tolerant B cells that have taken up CD13 containing HCMV particles, leading to processing and presentation of viral peptides in association with HLA class II molecules for stimulation of helper T cells, which results in production of CD13-specific antibodies. This principle of activation of a specific autoimmune response against selective virus-associated host proteins may be operative in IBD as well as in other autoimmune diseases such as type I diabetes, rheumatoid arthritis, multiple sclerosis, and vasculitis that may be associated with other viral infections.

The presence of CD13 autoantibodies in CMV negative patients is unknown but autoantibodies may also develop as a consequence of other viral infections. In support of this hypothesis, CD13 has been identified as the receptor for certain coronaviruses [57]. Interestingly, Yeager et al. [58]

reported that a novel coronavirus was identified as the causative agent of severe acute respiratory syndrome (SARS). They found that the hematological findings in SARS patients may be related to (1) a direct infection of blood cells and bone marrow stromal cells via CD13 or CD66a; and/or (2) an induction of autoantibodies and immune complexes that may result in damage to these cells. Unfortunately, the authors did not investigate occurrence of autoantibodies against CD13. Perhaps coronaviruses and other unknown viruses may be involved in the induction of CD13-specific autoantibodies in certain individuals. Unfortunately, there are no data regarding other viral infections in our patient groups.

The results from the cytotoxic assay show different cytotoxic effects of autoantibodies against CD13 on different cell types. We did not observe any differences in the levels of expression of CD13 on the different cell types (data not shown). These observations may instead be explained by other factors, e.g. different distribution of complement activating or complement inhibitory factors (e.g. CD55, CD59) on different cell types.

The CD13-specific autoantibodies may in different ways contribute to the pathophysiology of IBD when reacting with different cell types and perhaps, by stimulating different effector mechanisms in antibody-mediated tissue injury. For instance, such antibodies could through antibody dependent cellular cytotoxicity, phagocytosis of opsonized cells by complement mediated cell lysis, or by complement/Fc receptor mediated leukocyte recruitment, lead to enhanced inflammation that results in release of enzymes and reactive oxygen species, leading to increased tissue injury. Furthermore, the enzymatic activity of CD13 (aminopeptidase N) may also be of crucial importance for normal cell physiology. Aminopeptidases catalyze the cleavage of amino acids from the amino terminus of protein or peptide substrates. CD13 has been demonstrated to play a role in trimming of antigenic peptides for presentation by major histocompatibility complex (MHC-II) [59,60]. CD13 has also been suggested to modulate the cell cycle. Occupation of the active center of CD13 with artificial ligands such as monoclonal antibodies induces a prolongation of the cell cycle and a decrease in the rate of cell growth [61]. CD13-specific antibodies may also cause non-immunological damage of target cells, e.g. by inducing apoptosis. In addition, the CD13 molecule has been implied to mediate control of cellular differentiation [62]. We have recently found that HCMV particles that interact with the CD13 molecules on monocytes lead to an inhibition of macrophage differentiation [63]. Therefore, binding of CD13-specific antibodies to cells and structures expressing CD13 could perhaps also contribute both to tissue damage and chronic inflammation.

In conclusion, the number of reports describing HCMV (re)activation in IBD patients is increasing. We here provide evidence of a CD13-specific autoimmunity in IBD patients. Such autoantibodies have previously only been observed in HCMV-infected patients. These autoantibodies may have a pathogenic role in the intestine of IBD patients by a specific target attack of CD13 positive tissue structures and may also explain immunological phenomena not involving the bowel, e.g. manifestation in the eye, joint and skin that are observed in these patients. Further identification of the immunogens eliciting specific antibody responses could offer new clues as to the pathogenesis of IBD and may and provide new possibilities for development of specific therapies.

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