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Inflammation in common variable immunodeficiency is associated with a distinct CD8+ response to cytomegalovirus

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Abstract

Background—Common variable immunodeficiency is the most common primary immunodeficiency. A subset of patients suffers from debilitating inflammatory complications.

Objectives—We investigated the role of cytomegalovirus, and the T cell response targeted at this virus, in this inflammatory disease.

Methods—Phenotypic and functional assays were used to profile CMV specific T cells in common variable immunodeficiency patients with and without inflammatory complications. Highly sensitive immunohistochemistry was used to detect cytomegalovirus antigens at sites of inflammation.

Results—Cytomegalovirus was significantly associated with inflammatory disease, which occurred in 31/43 (72%) virus-exposed patients and 8/31 (26%) naïve patients ($p=0.0001$). Cytomegalovirus pp65-NLV epitope specific CD8+ T-cell frequencies were significantly elevated in inflammatory patients but these cells did not show evidence of exhaustion, with low levels of PD-1 and high T cell receptor avidity. Rather, they showed features consistent with high in vivo functionality and proliferative activity including reduced levels of the anti-inflammatory marker CD73 (1.67% NLV+ cells were CD73+ vs 42.01% in non-inflammatory patients $p=0.004$) and increased Ki-67 expression (37% vs 2% in non-inflammatory patients. $p<0.0001$). In vitro, the cytomegalovirus specific T cells showed high antigen specific proliferative potential compared to

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Authorship: S. Mahdi Marashi and Mohammad Raeiszadeh performed the bulk of the technical and analytical works equally. Afsar Rahbar and Cecilia Soderberg-Naucler performed the immunohistochemistry. Paul Klenerman provided the null tetramers. Sarita Workman collected the blood samples, developed the ethical framework for the study and counselled the patients. Most of the patients were under the care of Ronnie Chee. Richard Milne, Vincent Emery and David Webster designed experiments, supervised the work and wrote the paper.

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cells from non inflammatory patients. Using sensitive immunohistochemistry we detected for the first time viral antigen at the sites of inflammation, indicative of active viral replication.

Conclusions—Our data strongly support a direct role for cytomegalovirus and a hyper-reactive cytomegalovirus specific immune response, in the debilitating chronic inflammatory complications of common variable immunodeficiency.

Keywords

Cytomegalovirus; Common variable immunodeficiency; inflammation; CD8+ T cells

INTRODUCTION

Common Variable Immunodeficiency (CVID) is the commonest clinically relevant primary antibody deficiency. Patients usually present after their second decade of life with defective antibody production and increased susceptibility to infection.¹⁻³ Monogenic defects have been described in a minority of patients.⁴ Complications include recurrent and chronic infections, chronic inflammatory diseases, autoimmunity and lymphoid malignancies. The inflammatory phenotype in CVID is expressed as organ specific involvement that may lead to fibrosis and granuloma formation or as multisystem granulomatous disease. The organs most commonly affected are the lung, liver, spleen, skin, and gastrointestinal tract. Persistent viral infection(s) has been suggested as a cause of granulomatous disease in CVID.²

While CVID is a B-lymphocyte-related disease, reports have described alterations in the phenotype and function of T cells.⁵ CVID patients have not been considered at risk of serious viral infections, however, some develop persistent enteroviral infection.⁶ In addition, CVID patients infected with HCV by contaminated immunoglobulin batches have an aggressive clinical course of hepatitis.⁷

The betaherpesvirus human cytomegalovirus (CMV) infects between 60-90% of the population worldwide. After primary infection the virus establishes latency, with periodic reactivations which are controlled in most infected individuals despite the virus producing many immunomodulatory proteins.⁸⁻¹⁰ Although there is a considerable literature describing defective cellular immunity in CVID,⁵ we reported robust CD8+ T cell responses to CMV and Epstein Barr virus (EBV) in CVID patients with CMV responses being elevated compared to healthy controls.¹¹

Here, we investigated the function of CMV specific T-cells in CVID patients and addressed the hypothesis that a distinct CMV specific CD8+ T-cell phenotype coupled with CMV replication in affected organs contributes to the inflammation observed in a subset of CVID patients.

METHODS

Patients and control subjects

74 CVID patients (33 male, mean age 50 $\pm$ 15 years) attending the Royal Free Hospital diagnosed according to established criteria^{12,13} were recruited. All but one patient was receiving immunoglobulin therapy.

27 healthy subjects (9 male, mean age 40 $\pm$ 12 years) were recruited and their CMV status determined using a CD4+ T cell assay (see below) and a CMV specific IgG assay (VIDAS (BioMérieux, Marcy L-Etoile, France). Exposure to CMV in CVID patients was assessed by measuring IFN- γ production by CD4+ T cells stimulated with viral lysate. A value of $>0.05\%$ positive cells (two standard deviations above the mean for healthy CMV negatives)

was taken as evidence of CMV exposure and the CVID patients classified on this basis. (All healthy subjects also fulfilled this criterion.) EBV exposure was assessed by GLC pentamer staining (see below). An HLA-A*0201 positive subset of CMV exposed patients participated in a more detailed study of T cell function. Of these, 9 were “inflammatory” and 16 “non-inflammatory” (see below). None of these patients had TACI mutations. Due to limitations on the amount of blood that could be obtained, some subjects were not included in all of the assays presented in this paper.

All participants gave informed consent and the study was approved by the Local Ethics Committee (reference:04/Q0501/119).

Inflammatory disease definitions in CVID patients

Patients with any of the following features were categorised as “inflammatory”, the remainder as “non-inflammatory”.

1. Hepatitis without evidence of hepatitis B or C virus infection: only those with raised blood alkaline phosphatase (ALP) levels >200IU/L were included, although usually alanine (ALT) and aspartate transaminase (AST) levels were also moderately raised; most had had a liver biopsy showing mild/moderate peri-portal lymphocyte infiltration and cholestasis and evidence of nodular regenerative hyperplasia; granulomas were present in a few cases and some patients had early signs of cirrhosis and/or portal hypertension.
2. Splenomegaly defined as a clinically enlarged spleen on palpation and/or > 13cms in length on ultrasound. Splenomegaly was included as a marker of inflammation because large spleens surgically removed from CVID patients often show granulomatous infiltration¹⁴ and enlargement is also an early marker of portal hypertension secondary to liver inflammation. However, the splenomegaly associated with autoimmune blood dyscrasias in CVID may not show inflammatory changes so it is important to note that splenomegaly may overestimate the frequency of inflammatory disease in the patient groups.
3. Biopsy and clinical evidence of chronic enteropathy involving small and/or large bowel not explained by infection or gluten sensitivity.
4. Interstitial lung disease (ILD) not explained by infection. These patients had evidence of deteriorating CO gas transfer in the lungs and CT scan features of interstitial disease (‘ground glass’ appearance, micro-nodularity and fibrosis).

CD8+ T cell phenotyping

PBMC were purified by Ficoll-paque PLUS centrifugation.

1. Cytokine production and degranulation. PBMC were stimulated with a peptide pool encompassing the CMV ppUL83(pp65) protein (JPT, Germany). Cytokine production¹⁵ and CD107 expression¹⁶ were measured using published methods.
2. Class I HLA pentamers and phenotyping. 1×10^6 PBMC were stained with PE conjugated HLA-A*0201 pentamers (Proimmune) for the NLVPMVATV epitope (from CMV ppUL83(pp65) or the GLCTLVAML epitope (from the EBV BMLF1 protein) at 10µg/ml for 15 min at room temperature. Cells were washed with PBS (0.1% sodium azide) and stained with PerCP-Cy5.5 labelled anti-CD8 and, as appropriate, anti-PD-1 FITC, anti CD57 FITC (BD Biosciences), anti CD73 APC (eBiosciences), anti HLA DR APC (R&D systems). For Ki-67 staining, thawed cryopreserved cells were pentamer/CD8 stained as above, then fixed, permeabilised

(Fix & Perm, Invitrogen) and stained with anti-Ki-67-FITC (BD Biosciences). Isotype controls were from BD Biosciences.

Data were acquired on a FACScalibur (Becton Dickinson) and analyzed with FlowJo (Tree star LTD).

3. Differential avidity Class I HLA tetramers. NLV epitope tetramers with differential avidity were synthesised and refolded as described.¹⁷ The CD8 null tetramer has reduced avidity because of a mutated CD8 binding domain.¹⁸ 1×10^6 cells were stained with 4 μ g of PE-labeled tetramers in 0.5ml then washed and surface stained with anti-CD8 antibodies..

T cell proliferation assays

Cell proliferation was analysed using carboxyfluorescein diacetate succinimidyl ester (CFSE).¹⁹ Cells (10^7 /ml) were labelled with 2.5 μ M CFSE (Invitrogen) for 5 min at room temperature in the dark. Labelled cells were cultured in 96-well plates at a concentration of 1×10^5 cells/200 μ l in RPMI containing 10% FCS for 5 days in 37°C 5% CO₂.

CMV NLV peptide, EBV GLC peptide (ProImmune), CMV pp65 peptide pool, and PHA (Sigma) were used at 10 μ g/ml to stimulate the cells. After five days, cells were stained with pentamers and anti-CD8 antibody as above. ModFit LT 3.0 (Verity software) was used to quantify cycles of cell division.

Immunohistochemical detection of CMV in inflamed tissues

Formalin fixed paraffin embedded, colon biopsies from CVID patients with chronic diarrhoea and histological evidence of mild and moderate colitis were analysed for CMV early and late antigens by immunohistochemistry using a recently developed protocol with highly optimised tissue preparation and blocking^{20,21,30}. Control colon biopsies were healthy tissue removed during resection surgery for bowel cancer. Tissue sections (6 μ m) were deparaffinized, rehydrated and postfixed with 4% formalin. Sections were treated with pepsin (Biogenex,) at 37°C for 3 min then antigen retrieval solution (BioSite). Nonspecific peroxidase was blocked with 3% H₂O₂ (Sigma), Fc receptors were blocked with Fc-receptor blocker (Innovex), biotin and avidin were blocked with Biotin/Avidin Blocking Kit (DakoCytomation) and slides were treated with Background Buster (Innovex). Primary antibodies: mouse anti-CMV IEA, anti-CMV LA, anti-human α -actin (Chemicon, USA). Incubation with primary antibodies was overnight at 4°C. Antibodies were detected with biotinylated secondary antibody and streptavidin-conjugated horseradish peroxidase. Slides were developed with chromogen diaminobenzidine (Innovex) and counterstained with hematoxylin (Sigma). Images were captured at room temperature using a Leica Microsystems AB, Type DC 480 microscope and a Leica DFC320 camera. Adobe Photoshop was used for image acquisition, formatting and annotation.

Statistical analysis

Fisher's exact test was used to compare frequencies of clinical complications between the CMV exposed and naïve CVID patients. The Mann-Whitney test was used for comparing data sets that were non-normally distributed. For normally distributed data, mostly phenotypic analyses, Student's T test was deployed. P-values ≤ 0.05 were regarded as significant.

RESULTS

CMV infection is associated with inflammatory disease in CVID

We asked whether exposure to CMV was associated with the inflammatory complications characteristic of a subset of CVID patients. Using CD4⁺ T cell responses to CMV infected cell lysates¹¹ to identify CMV infected patients because serological confirmation of CMV exposure is impossible in immunoglobulin deficient individuals, we found a highly significant association between CMV exposure and inflammatory complications (Table E1). Thirty one of 43 (72%) CMV exposed patients had one or more features of chronic inflammation compared to 8 of 31 (26%) CMV naïve patients. The CD4/CD8 lymphocyte ratio was lower in CMV exposed patients and malignancy was more common in this group. In contrast there was no difference in the incidence of autoimmune disease between the CMV exposed and naïve CVID patients. Importantly, when the CMV exposed patients were stratified according to the presence or absence of inflammatory disease, there was no significant difference in the CMV specific CD4⁺ T-cell responses between the two groups so CD4 responses were not further investigated.

Functional integrity of CMV- specific CD8⁺ T-cells from CVID patients

To assess the functional potential of the CMV specific CD8⁺ T-cells, we used a pp65 peptide pool to analyse responses irrespective of Class I HLA haplotype. Mobilization of CD107a/b molecules and simultaneous production of IFN- γ and TNF- α by CD8⁺ T-cells after stimulation were determined in CMV exposed CVID patients and CMV IgG positive healthy controls. Figure E1A shows that in all samples, the majority of CMV specific CD8⁺ T-cells produced both cytokines, however, the frequency of double producer cells in peripheral blood of CVID patients, in particular those with inflammatory complications, was significantly higher than the frequency of these cells in healthy individuals (non-inflammatory: 1.09%, inflammatory: 5.05%; healthy: 0.67% (Figure E1B)). The double producer cells in both groups were capable of degranulation, as shown by mobilization of CD107a/b molecules to the cell surface (Figure E1C).

CMV specific CD8⁺ T-cell frequency is elevated in inflammatory CVID patients

To investigate more subtle aspects of the CD8⁺ T-cell response in inflammatory CVID patients we focused on the HLA-A*0201 positive subsets of our panel of CVID patients (n=25) and healthy controls (n=14) whose CD8⁺ T-cells would be expected to recognise the relevant restricted peptides in CMV pp65 (NLVPMVATV) and, for comparison, the EBV BMLF1 protein (GLCTLVAML). Enumeration of HLA class I pentamer-specific CD8⁺ T-cell frequencies showed a significantly increased frequency of CMV NLV-specific CD8⁺ T-cells in PBMC from CVID patients compared to healthy controls (CVID: mean frequency 2.6%, SD 0.49%; Healthy: mean frequency 0.66%, SD 0.2%. P=0.005 (Figure 1A)). There was no difference in the frequency of EBV GLC-specific CD8⁺ T-cells between the two groups (CVID: mean frequency 0.18%, SD 0.04%; Healthy: mean frequency 0.32%, SD 0.19%; P=0.35 (Figure 1A)). Within the CVID patient group, the frequency of CMV-specific cells was significantly higher than the frequency of EBV-specific cells with an average difference in frequency of 14.4-fold. This was much greater than the difference observed between the same responses to these two viral epitopes in healthy individuals (2.1-fold).

Nine of the 25 HLA-A*0201 positive CVID patients were suffering from inflammatory disease and these patients had a significantly higher frequency of CMV NLV-specific CD8⁺ T-cells compared to non-inflammatory CMV exposed CVID patients (mean frequency 4.8%, SD 0.77% versus 1.4%, SD 0.39%. P=0.0002 (Figure 1B)). Moreover, in the latter group of CVID patients, CMV NLV-specific CD8⁺ T-cell frequencies were not significantly

different from those in healthy CMV positive individuals (Figure 1B). CMV NLV-specific CD8⁺ cell frequencies were broadly consistent in individuals over the course of the study (Figure 1C). Frequencies of EBV GLC-specific CD8⁺ T-cells were similar in all groups (Figure 1D).

Since CMV NLV-specific CD8⁺ T-cell frequencies were elevated in the inflammatory patients, we next investigated the surface phenotype of these cells. We found no significant differences between the inflammatory and non-inflammatory groups in expression levels or expression frequencies of PD-1, a marker associated with T cell exhaustion, on CMV or EBV pentamer⁺ CD8⁺ T-cells. The frequency of PD1⁺ cells in the non-inflammatory group was elevated compared to the healthy controls for CMV and EBV pentamer⁺ cells, but, in general, all CD8⁺ T-cells expressed low levels of PD-1 (Figure 2A and B; Figure E2 A and B). However, inflammatory CVID patients showed significantly higher frequencies of Ki-67 (a marker of in vivo cycling) positive CD8⁺ T-cells than non-inflammatory patients or healthy controls (inflammatory patients: mean 2.4%, SD 0.37% vs. i) non-inflammatory patients 0.73%, SD=0.14%; P=0.0002; ii) Healthy controls: 0.32%, SD=0.03%, P=0.002) (Figure 2C)). This distinction was greatly enhanced when Ki-67 expression in CMV NLV pentamer⁺ CD8⁺ T-cells was analysed, with healthy controls and non-inflammatory patients showing less than 2% and inflammatory patients around 40% Ki-67 positive (Figure 2D; Figure E2C). This elevation was not seen with EBV GLC pentamer positive cells (data not shown). Frequencies of CD57⁺ CMV NLV pentamer⁺, but not EBV GLC pentamer⁺, CD8⁺ T cells were elevated in the inflammatory sub group (Figure E2D and E) as were HLA DR⁺ CD8⁺ T cells and HLA DR⁺ CMV NLV pentamer⁺ CD8⁺ T cells (Figure 2 E and F).

To further investigate the association between activated cycling CMV specific CD8⁺ T cells and inflammatory CVID, we measured expression of CD73, a marker of anti-inflammatory function. The two CVID patient groups and the healthy controls had similar frequencies of CD73⁺ CD8⁺ T cells (Figure 2G). However, we saw a striking distinction between the two CVID groups with CD73⁺ NLV pentamer⁺ CD8⁺ T-cell frequencies significantly lower in inflammatory patients than in non-inflammatory patients or in healthy controls (Inflammatory: mean 1.67%, SD=0.89; non inflammatory: mean 42.01%, SD=8.7%, P=0.004; Healthy: mean 19.89%, SD=7.94%, P=0.03) (Figure 2H). There was no such distinction on EBV GLC pentamer⁺ CD8⁺ T cells (data not shown).

Proliferative potential of CMV specific CD8⁺ T cells in CVID patients

In view of the high proportion of cycling CMV NLV specific CD8⁺ T-cells in the inflammatory CVID patients, we next investigated their proliferative potential. Initially, in a conventional assay using 5 days stimulation of CFSE labelled PBMC with the pp65 peptide pool in the presence of exogenous IL-15, we observed expansion of CMV specific T-cells in both groups of CVID patients (data not shown). We next assessed proliferation of CD8⁺ T-cells in response to peptide stimulation in the absence of exogenous cytokines or co-stimulators. PBMC from inflammatory (n=6) or non-inflammatory (n=8) CVID patients were stimulated with PHA, CMV NLV peptide, or EBV GLC peptide. NLV peptide driven CD8⁺ T-cell proliferation was observed in all samples from inflammatory CVID patients after 5 days stimulation with CMV NLV peptide (Figure 3A and B). This autonomous proliferation was not observed in samples from non-inflammatory CVID (Figure 3C) patients or healthy controls (data not shown). The extent of CD8⁺ T-cell proliferation was equivalent to 5 divisions consistent with a doubling time of approximately 24h (Figure 3B, insert). Similar results were obtained after stimulation with pp65 peptide pool (data not shown). No expansion was observed following stimulation with EBV GLC peptide (Figure 3 A-C). Combined stimulation of PBMC from inflammatory patients using different ratios of the CMV and EBV peptides showed that the majority (>95%) of the proliferated cells were

CMV specific and that, by extension, the EBV specific CD8+ T-cells did not proliferate even in an environment favouring proliferation of CMV specific cells (Figure 3 D and E).

Binding avidity of CMV specific CD8 T cells in CVID patients

The capacity of CMV NLV specific CD8+ T-cells from inflammatory patients to undergo autonomous proliferation in response to peptide stimulation may indicate an enhanced functional sensitivity.²² To assess this, we used CD8 binding site-mutated (null) and normal class I HLA tetramers refolded with the NLV peptide to estimate the avidity of the HLA class I-TCR interactions on CD8+ T-cell populations present in the different groups of CVID patients (Figure E3). In 4 of 5 inflammatory CVID patients studied (Figure E3 A and B), the normal and null CMV tetramers identified similar numbers of cognate CD8+ T-cells, indicating that these are mainly high avidity cells (ie CD8 independent). In contrast, in non-inflammatory CVID patients (Figure E3C and D) the cognate CD8+ T-cells were mostly CD8 dependent (ie did not bind the null tetramer).

Detection of CMV in inflamed tissues

Using sensitive immunohistochemistry, we analysed colon biopsies from two patients with histological features of mild/moderate colitis and granulomatous disease, classified as inflammatory CVID. All biopsies were positive for CMV immediate-early (IE) and late antigens, the latter indicative of active virus replication, with 40-70% of cells in any given field expressing viral IE antigen in their nuclei and 5-10% cells expressing viral late antigen. A variety of cells expressed viral antigens, including vascular endothelium, glandular epithelium, smooth muscle cells in the vessel wall and inflammatory cells, all known targets for CMV (Figure 4). We have seen a similar pattern of staining in a liver biopsy from a CVID patient with liver disease (data not shown). Three control colon biopsies from healthy bowel (Figure 4) as well as kidney biopsies from CMV negative renal transplant recipients (data not shown) were uniformly negative.

DISCUSSION

Inflammatory disease is a significant and persistent burden for many CVID patients. In this study we have demonstrated that: 1) inflammatory disease is significantly more prevalent in patients with evidence of CMV exposure; 2) CMV specific CD8+ T-cell frequencies are specifically elevated in inflammatory CVID patients; 3) CMV specific CD8+ T-cells from inflammatory CVID patients show evidence of substantial proliferation in vivo and respond rapidly to antigen in vitro; 4) CMV antigens can be directly detected in inflamed organ systems. Taken together these results strongly suggest that the combination of CMV replication in the organ and a unique CMV specific CD8+ T-cell response directly results in the inflammatory disease.

Until now there has been no rational explanation for the frequent occurrence of many of the inflammatory complications associated with CVID,^{14,23,24} or the persistent lymphocyte activation or oligoclonal expansions of activated CD8+ T-cells described in a subset of CVID patients.²⁵⁻²⁸ This has been compounded by the inability to provide evidence of pathogens in inflamed organs using conventional histology/immunohistochemistry despite gene array analysis of PBMCs suggesting that the T-cells in these CVID patients are responding to a persistent stimulus.²⁹ We have not detected evidence of CMV DNAemia using sensitive quantitative PCR approaches, but CMV associated gastrointestinal disease can occur in the absence of viraemia^{30,31} and, utilising an enhanced immunohistochemistry approach^{21,32} we provide the first direct evidence of actively replicating virus in the inflamed tissues. On the basis of the data presented here we conclude that CMV infection is a major factor accounting for the aforementioned observations. Although there were patients

with inflammation in the CMV negative group, much of this was attributable to splenomegaly. A role for other infectious agents in the inflammation or an unusually low CD4+ T cell response to CMV in these patients cannot be excluded and remain formal possibilities.

Since persistent CMV replication in inflamed tissues could lead to an environment in which CMV specific CD8+ T-cells are driven to immune exhaustion we assessed the phenotype and proliferative capacity of the CD8+ T-cells from patients with inflammation compared to CVID patients without inflammation. T-cell exhaustion, especially of high avidity cell populations, has been described in HIV, HCV and LCMV and in other settings.³³⁻³⁵ We showed that CMV NLV specific CD8+ T-cells in patients with inflammation were of high avidity, predominantly IFN- γ and TNF- α co-producers, and able to degranulate upon stimulation implying good functional capacity.^{36,37} The majority expressed HLA DR and CD57, but not PD1 a phenotype not consistent with an exhausted population but rather one that is able to proliferate and maintain full functionality. Frequencies of PD1 expression on CMV NLV specific CD8+ T cells in the non-inflammatory patients were significantly elevated, suggesting that these cells would be less able to proliferate and mediate inflammatory processes. To investigate this further we analysed Ki-67 expression and found it be very high on CMV specific T-cells (average of 37%) compared to non-inflammatory CVID patients and healthy controls implying a high proportion of the CMV T-cells were actively cycling. When PBMC were cultured with CMV peptides in the absence of exogenous cytokines such as IL-15 we observed that CMV CD8+ T-cells from CVID patients with inflammation proliferated with a doubling time of approximately 1 day whereas cells from the other groups showed no proliferation under these conditions. Interestingly, there was no proliferation in response to EBV peptides in parallel experiments indicating that this was a CMV specific effect. These data confirm the substantial proliferative capacity of the CD8+ T cells in CVID patients and underline the fact that the CD8+ T cell response is not exhausted. The mechanism behind the enhanced proliferative capacity in these experiments is unknown but could include differences in avidity, cytokine production (which could have an autocrine effect in vitro), cytokine responsiveness and response to endogenous co-stimulation or protection against cell death.

Further support for a role of CMV specific CD8+ T-cells in the inflammatory disease seen in CVID patients was our observation that the frequency of CD73+ CMV NLV pentamer+ CD8+ T-cells was significantly reduced in inflammatory patients. CD73 is an ecto-5-nucleotidase which acts in the purine salvage pathway to generate nucleosides such as adenosine, which has anti-inflammatory properties.^{38,39} Although relatively little is known of the functional implications of CD73 expression in CD8+ T cells, our data suggest that down regulation of CD73 may well impact on the capacity of these cells to mediate inflammatory processes. Indeed in a recent study, *Borrelia garinii* infection of CD73 -/- mice was associated with joint swelling and the authors suggested that low levels of adenosine may favour local inflammatory responses and persistent infection.⁴⁰

Although much of our data has been generated using pp65 A*0201 restricted pentamers, where possible we have also performed experiments using pp65 and IE1 peptide pools with multiple HLA specificities and seen comparable results.

Our data suggest that the inflammation observed in these CVID patients likely reflects the combination of intra-organ CMV infection and an aggressive and sustained CD8+ T-cell response. In this context, treatment of CVID patients with anti-TNF- α therapy (Infliximab) reduces inflammatory disease^{30,41} and inhibition of CMV replication with the antiviral drug ganciclovir has a similar effect.¹¹ Indeed, in preliminary studies we have observed that a marked reduction in CMV NLV pentamer+ CD8+ T-cell frequencies (4% of CD8+ T cells

pre-therapy to 0.3% post-therapy) parallels clinical improvement in an inflammatory CVID patient undergoing antiviral therapy. Thus, removal of the antigen or suppression of the inflammatory T-cell response can be effective in controlling inflammatory disease in this setting.

Conclusions

Our data provide a compelling evidence base in support of controlled clinical trials of treatment protocols targeting both sides of this virus-host interaction and emphasise the importance of considering CMV status both in patient management and in laboratory studies of CVID patients. In addition, we suggest that the CD8+ T-cell response to CMV in CVID, and in particular the differences we have observed between the two CVID patient groups in the response to CMV, but not EBV, provides a powerful and tractable model of immune regulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

CMV	Cytomegalovirus
CVID	Common Variable Immunodeficiency
EBV	Epstein Barr virus
TCR	T-cell receptor
CFSE	Carboxyfluorescein diacetate succinimidyl ester

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Clinical Implications

We describe an association between CMV and inflammatory CVID. Our results provide a rationale for investigations of antiviral or anti-inflammatory interventions for management of the inflammatory complications of CVID.

Capsule Summary

We describe a novel and distinct CMV-specific T cell phenotype associated with inflammation in CVID. We show that CMV is present at sites of inflammation, providing an evidence base for development of novel therapeutic strategies.

Figure 1.

(A) Percentage of CMV (NLV) or EBV (GLC) pentamer+ cells in CVID or healthy controls. (B, D) Data from (A) showing inflammatory and non inflammatory groups. (C) Frequency of CMV (NLV) pentamer + cells in serial samples from four patients (Pt). Lines in A, B, D indicate means Error bars in C: ± 1 SD.

Figure 2. Phenotyping of CMV NLV specific CD8+ T cells. (A,B) PD-1 (MFI); (C, D) Ki-67 (E,F) HLA DR (G, H) CD73. Bars indicate mean percentage values.

Figure 3.

Proliferation of CMV specific CD8+ T cells. (A) Representative FACS plots. Numbers indicate proliferating cells as percent of CD8+. (B, C) Analysis of inflammatory (n=6) and non inflammatory (n=8) samples. Insert in (B) shows generational analysis after NLV stimulation. (D) Mixed peptide stimulation. (E) Specificity (CMV black; EBV white) of expanded cells from (D). Data obtained 5 days after stimulation.

Figure 4.

Immunohistochemical detection of CMV antigens. Colon tissue from representative inflammatory CVID (A,B,E,F,I,J) or healthy control (C,D,G,H,K,L) subject was stained for CMV IE (A, C, E, G) or L (B,D,F,H) antigen; α -actin (I, K); or processed without primary antibody (J,L). E, F, G and H are enlargements of areas boxed in A, B, C and D respectively. Brown staining (diaminobenzidine chromogen) indicates presence of antigen. Original magnification 40x (A and B); 20x (C,D,I-L). Bars are 50 μ m.