Protection from cytomegalovirus viraemia following glycoprotein B vaccination is not dependent on neutralising antibodies

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Abstract (243)

Human cytomegalovirus (HCMV) is an important pathogen in transplant patients and in congenital infection. Previously, we demonstrated that vaccination with a recombinant viral glycoprotein B (gB)/MF59 adjuvant formulation before solid organ transplant reduced viral load parameters post-transplant. Reduced post-transplant viraemia was directly correlated with antibody titres against gB consistent with a humoral response against gB being important. Here we show that sera from the vaccinated seronegative patients displayed little evidence of a neutralising antibody response against cell-free HCMV in vitro. Additionally, sera from seronegative vaccine recipients had minimal effect on the replication of a strain of HCMV engineered to be cell-associated in a viral spread assay. Furthermore, although natural infection can induce antibody dependent cellular cytotoxicity (ADCC) responses, serological analysis of seronegative vaccinees again presented no evidence of a substantial ADCC-promoting antibody response being generated de novo. Finally, analyses for responses against major antigenic domains of gB following vaccination were variable and their pattern was distinct when compared to natural infection. Taken together, these data argue that the protective effect elicited by the gB vaccine is via a novel mechanism of action in seronegative vaccinees that cannot be explained by neutralisation or the induction of ADCC. More generally, these data, which are derived from a human challenge model that demonstrated that the gB vaccine is protective, highlight the need for more sophisticated analyses of new HCMV vaccines over and above the quantification of an ability to induce potent neutralising antibody responses in vitro.
Conventionally, vaccines are screened for induction of a neutralising antibody response in human volunteers before proceeding to late stage clinical trials. We present results from a human cytomegalovirus (HCMV) challenge study suggesting that this paradigm may not apply universally to all viruses. Instead viruses like HCMV, which establish lifelong infections and grow both cell-free and cell-associated, may be controlled independently of a potent neutralising antibody response. Our results suggest that more detailed laboratory studies are required to identify correlates of immune protection for such viruses and failure of a vaccine to induce a neutralising antibody response should not necessarily be considered as a key go-no-go decision point in the design of future vaccine studies.
Human cytomegalovirus (HCMV) causes substantial morbidity in multiple patient populations with impaired or immature immune responses (1, 2). The threat posed during organ transplantation or congenital infection led to HCMV vaccine development being categorised as the highest priority (3). Several vaccines against HCMV (from whole virus, DNA and viral subunits) have been studied in different patient cohorts establishing, in general, that a vaccination strategy targeted against HCMV is a viable option with major clinical implications (4-10).

One vaccine target is the viral glycoprotein B (gB) which has been shown to be partially protective in three phase 2 clinical trials when presented with MF59 adjuvant (6, 9, 11). The gB protein is an essential virion component required for viral entry (12, 13) and represents a major target of the humoral immune response, including neutralisation (14-16). Conventionally, neutralising antibody titres have been considered the benchmark by which vaccines are assessed as this represents a potent anti-viral mechanism. However, the humoral immune response is far more complex and can produce antibodies that can drive antibody dependent cell cytotoxicity (ADCC), that can bind to both the pathogen directly or to the target antigen expressed on the infected cell surface to recruit complement and promote pathogen or cell lysis, they can promote pathogen phagocytosis as well as modulate the downstream response of both the adaptive and innate immune responses (17). Here we report data showing limited evidence of a neutralising antibody response as a correlate of protection for the gB vaccine in a phase 2 study where transplant
patients were challenged with wild type HCMV (9). An inability to detect evidence of neutralisation of clinical Merlin was consistent with previous data demonstrating no effect of antibody plus complement against the laboratory strain Towne in a classical plaque assay (9). Furthermore, we provide evidence that the humoral response against gB induced in seronegatives by vaccination displays a distinct biological spectrum compared to that observed in naturally infected seropositives.

Results

Sera from seronegative vaccinated patients do not neutralise HCMV infection in single round infection assays

Note that throughout this paper the term seronegative refers to patients who were seronegative before being given gB/MF59 vaccine or placebo. We tested for evidence of neutralisation of HCMV infection using a high throughput assay that measured the establishment of a lytic infection by enumeration of immediate-early (IE) positive cells (Fig. 1). Two anti-gB monoclonal antibodies (ITC88; an anti-AD-2 antibody demonstrated to prevent gB fusion post binding (23) and 2F12; a commercial monoclonal antibody against an unspecified region of gB) inhibited HCMV infection in a concentration-dependent manner (Fig. 1a). Next we tested a panel of sera from our vaccine study under the same conditions. Prior to vaccination, sera from seronegative individuals had no impact on HCMV infection. Importantly, no evidence of activity against HCMV was observed in this assay when seronegative sera post vaccination was assessed (Fig. 1b; Fig. S1a-d) even when exogenous complement was added to the sera prior to infection (Fig. S2). In contrast, sera from seropositive patients had inherent neutralising activity against HCMV prior to
transplantation (Fig. 1b; Fig. S1e-h) but no evidence of increased neutralising capacity was observed post vaccination (Fig. 1b; Fig. S1e-h).

To address the possibility that the sera from seronegative vaccinees contained antibodies capable of inducing abortive/quiescent infections, as proposed for varicella infection (18), a parallel analysis was performed that measured pp28 (a viral late gene) positivity (Fig. 1c,d). Unsurprisingly, pp28 positive cells were rare in the ITC88 control (Fig. 1c) given that IE positive cells were rarely seen (Fig. 1b). In contrast, and consistent with the IE data, no effect on pp28 positivity was observed using the sera from vaccinated seronegative transplant recipients (Fig. 1c; Fig. S3).

**Sera from vaccinated seronegative patients do not inhibit spread of cell-associated Merlin strain of HCMV in fibroblast monolayers.**

Our first assays measured the ability of sera to limit infection of cells with large titres of cell-free virus by measuring the number of IE positive cells 24hpi. To investigate whether sera had activity against cell-associated HCMV we assessed their impact on the growth of HCMV in fibroblast cultures using a viral spreading assay seeded at low MOI. To be able to ask this question, we utilised a Merlin-IE2-GFP virus engineered to grow predominantly in a cell-associated fashion (19). The expression of GFP with IE2 kinetics allows real time imaging and enumeration of the spread of the virus as we visualise in real time the increase in the number of infected cells over time and thus monitor the spread of the virus through the fibroblast monolayer (Fig. 2a). Firstly, we measured the ability of ITC88 to limit spread of this cell-associated virus. The data show that ITC88 had minimal impact on spread with the number of
infected cells increasing with culture time which would be consistent with cell-
associated virus being resistant to neutralisation (Fig. 2b). Importantly, ITC88 could
effectively limit the spread of a high passage Merlin strain that grows predominantly
cell-free and thus is functional in this viral spreading assay (Fig. 2c). Similar data
were also observed with healthy donor sera whereby seropositive sera were far less
potent against the spread of the IE2-GFP virus whereas healthy donor seronegative
sera had no effect (Fig. 2d). Having established a baseline for the assay, we next
analysed the sera from the vaccine study. The data show that seropositive sera did,
on average, impact on the spread of Merlin-IE2-GFP to similar levels to those
observed with the control sera from natural seropositives (Fig. 2e; Fig. S4a-d). In
contrast, sera from seronegative individuals had no effect on viral spread in this
assay both prior to and post vaccination (Fig. 2e; Fig. S4e-h). Furthermore, the data
also demonstrated that vaccination of the seropositives did not enhance the
moderate inhibition of viral spread observed with the seropositive sera prior to
vaccination (Fig. 2e; Fig. S4a-d).

Vaccination does not induce an antibody repertoire capable of promoting a
measurable ADCC response

A lack of evidence to support potent neutralisation led us to investigate other
antibody effector mechanisms. ADCC involves antibody recognition of an epitope
and the subsequent recruitment of cellular effector functions (e.g. NK cells) to kill the
infected cell. To allow for a high throughput screen of our sera for any potential
ADCC promoting activity we developed an in vitro assay based on a previous study
for antibodies directed against influenza proteins (20). Recombinant vaccine gB was
immobilised and incubated with PBMC from healthy donors in the presence of sera. We then analysed NK cells by flow cytometry for evidence of CD107a expression - a classic marker of degranulation of NK cells. Validation of the assay utilised PMA/IONOMYCIN - a potent activator of NK cell degranulation whereby, in the presence of these activators, CD107a surface expression on CD56+ NK cells was significantly upregulated (Fig. 3a). With this assay we could observe a differential CD107a phenotype between healthy donor seropositive and seronegative sera (Fig. 3b; Fig. S5a,b). Having established the conditions, we tested the sera from our longitudinal vaccine study. Evidence of ADCC promoting antibodies was evident in the seropositive patient sera both pre and post vaccination (Fig. S5c-e). However there was no evidence that vaccination boosted pre-existing responses in these seropositive individuals nor were levels of ADCC promoting antibodies correlated with protection from viraemia (Fig. 3c,d).

We next asked whether any effect of vaccination in seronegatives was evident. As expected, no ADCC effect was evident in the seronegative samples at baseline (i.e. pre-vaccination; Fig. 4a-d). The analysis of longitudinal samples post-vaccination revealed no evidence that vaccination consistently elicited detectable levels of anti-gB antibodies capable of inducing ADCC right up to the day of transplantation (Fig. 4a-d).

**Distinct antibody responses against gB epitopes in vaccinated individuals**

Our inability to detect evidence for neutralising or ADCC effector functions associated with protection in the seronegative vaccine recipients led us to investigate
the composition of the humoral response against key antigenic domains (AD) of HCMV. In a parallel study of seropositive individuals we have evidence that reduced viraemia post-transplant correlates with higher antibody levels against AD-2 (21) consistent with this epitope being considered an important target for antibody responses (22). Thus we asked whether vaccination of seronegatives induced specific antibody responses against known antigenic domains of gB. ELISA assays were performed on serial samples of sera from seronegatives pre and post vaccination (Fig. 5). The data show that vaccination elicited limited responses against the known ADs with no responses detectable at all against AD-2 (Fig. 5cd) nor AD-4 (Fig. 5e,f). In contrast, AD-1 and AD-5 responses were observed in certain individuals but these did not correlate with protection (Fig. 5a,b,g & h). Thus, unlike for seropositives, no direct correlate of protection could be established with well-defined ADs of gB.

Discussion

The administration of a subunit vaccine based on the key viral glycoprotein B of HCMV is a potent inducer of anti-gB antibodies (6, 9, 11). Furthermore, the level of these antibodies correlated with reduced viral load parameters in a randomised phase 2 trial in solid organ transplant recipients (9). These data support the concept that the induction of a potent humoral response against gB represents a good strategy to protect from HCMV disease. However, despite this understanding of improved clinical outcome, the mechanistic basis of protection is still not fully understood.
Classically, the induction of potent neutralising antibody responses has been considered the gold standard for evaluating any vaccine strategy (23, 24). Indeed, a number of successful vaccination programmes have utilised vaccines that do exactly this (24). However, in this study we could provide no supporting evidence for a potent neutralising antibody response as an explanation for the success of the gB HCMV vaccine. The data show that the sera of seropositive transplant recipients possessed neutralising antibodies but these were not detectably enhanced by vaccination with gB/MF59. Most likely, these potent antibodies are a composite of anti-gB and other major glycoprotein targets including the trimer gH/gL/gO and also the pentameric complex (25). Consistent with these being targets for neutralisation are data that demonstrate monoclonal antibodies directed against gH or the pentameric complex neutralise infection effectively (26-28). Recent work has demonstrated that cell-associated HCMV growth is largely resistant to the activity of Cytotect (a heterogeneous mix of anti-HCMV antibodies) presumably because the physical state of the virus denies access to neutralising antibodies (19) consistent with a previous report (29). Our data presented here support those observations; a minor effect of seropositive sera on decreasing the rate of spread in vitro could be explained by small amounts of cell-free virus made by the Merlin-IE2-GFP strain of HCMV.

It is likely then that biphasic modes of growth (i.e. cell-free and cell-associated) in vivo would argue that an effective vaccine against HCMV could be dependent on the induction of multiple humoral effector functions. Thus, while there is still a role for a vaccine that can induce neutralising antibody responses, these clinical trial data
argue that a vaccine against HCMV can be effective despite an inability to detect a potent neutralising response associated with it. More generally, they reinforce the value of assessing vaccination strategies using challenge models. A recent study in mice concluded that vaccination with AD-2 was not useful because a poor neutralising response was elicited. However, it was never addressed whether the vaccination with AD-2 was protective against CMV challenge (30). Indeed, a recent study presents data implicating a role for both neutralising and non-neutralising gB antibody responses in the MCMV challenge model (31). Furthermore, this concept may not be restricted to HCMV because human studies of a candidate HIV vaccine reported that a major component of the anti-viral humoral response correlated with ADCC (32, 33).

In contrast to acute viral infections, HCMV persists for the lifetime of the host in the face of a prodigious immune response (33). HCMV encodes multiple immune evasion genes to facilitate lifelong survival in the host and ability to re-infect new hosts even those with pre-existing natural immunity against HCMV. This illustrates the complex interactions of HCMV with the immune response and the ability of this virus to persist in the face of a potent immune response may impact on the ability to produce a sterilising vaccine based solely on the induction of neutralising antibodies. Put simply, sera from seropositives are potently neutralising in vitro but re-infection with HCMV is possible in vivo. Consequently, we investigated the ability of sera from vaccinated patients to enhance antibody dependent responses. NK cells can be recruited in an antibody dependent manner to promote cellular cytotoxicity. HCMV encodes a number of NK immune evasion genes that suggests this is an important
functional interaction (34). Furthermore, the NK cell repertoire in HCMV seropositive
individuals is dominated by subsets of NK cells – with an implication of NK cell
memory (35). Whether these NK cell subsets are elite controllers of HCMV or
instead, reflect a virally induced reprogramming remains an important open question.
Clearly, seropositives invoke anti-gB responses that could direct NK cell mediated
ADCC based on our work. However, we could not attribute the success of the
vaccine to this so that, while anti-gB antibodies exist that promote ADCC, we could
provide no evidence that this explained the protection afforded by the vaccine. The
development of antibodies that promote ADCC responses may be triggered following
initial exposure to the pathogen or a focusing of the immune response through
multiple episodes of reactivation. A vaccine clearly does not deliver these additional
exposures to the immune system. Indeed, the vaccine delivers gB in the absence of
other pathogen-encoded functions and thus, potentially, presents gB in a unique
way. Whether this allows potent anti-HCMV responses to develop more effectively
than they would in the context of infection is an important question for vaccine
studies to address. Finally, it is important to avoid suggesting that ADCC responses
have no role to play. Our data show that ADCC responses directed against gB are
not detectable (seronegative vaccinees), boosted (seropositive vaccinees) or
correlate with protection (seropositive patients cohort). However, they do not rule out
ADCC responses against other HCMV antigens being important for control in natural
infection.

Although the mechanistic correlate of protection remains to be determined, it is
evident that the gB HCMV vaccine is protective (6, 9, 11). Interestingly, the epitope
analysis points towards the exciting hypothesis that a novel epitope may be responsible. The vaccine gB is modified in the transmembrane domain as well through the loss of the furin cleavage site and is thought to exist in a post-fusion form. All these differences may result in the presentation of novel epitopes of gB not normally exposed in the virion but transiently exposed during the entry process or in HCMV infected cells. Studies are ongoing to test the hypothesis of novel epitopes being presented by the vaccine form of gB.

In conclusion, the data in this human challenge model demonstrate that the effectiveness of the gB vaccine is imparted by a novel mechanism and not wholly reliant on the classic biological activity of neutralisation.

Materials & Methods

The study was approved by the UCL Research Ethics Committee and all patients whose samples were investigated here gave written informed consent (9). To assess sera for neutralising capacity, HCMV was pre-incubated with sera for 1 hour and then the whole sample used to infect HFFs. Alternatively, virus was incubated with anti-gB antibody 2F12 (abcam) or anti-AD-2 monoclonal antibody ITC88 (22, 36) After 24 hours cells were fixed and stained for IE gene expression using anti-IE (Millipore; 1:1000) and goat anti-mouse Alexafluor 568nm (Life Technologies; 1:1000). Alternatively, an anti-pp28 antibody (Santa Cruz; 1:1000) was used to stain cells fixed at 72hpi and detected with the same secondary
antibody. Nuclei were counterstained with DAPI (SIGMA). Percentage infection was
umerated using Hermes WiScan instruments and software.

Either a high passage Merlin (grows cell free) or an IE2-GFP virus engineered to
grow predominantly cell associated (a kind gift of Richard Stanton (19)) was used to
infect HFFs at an MOI of 0.01. Cells were either fixed and stained for IE (Merlin) or
visualised for GFP expression (IE2-GFP) between 1-14 days post infection. Nuclei
were counterstained with DAPI (SIGMA). Percentage infection was enumerated
using Hermes WiScan instruments and software.

To assay for ADCC promoting antibodies, total PBMC or purified NK cells (MACS
NK cell isolation kit II; Miltenyi Biotec) from seronegative healthy donors was used.
Briefly, 96 well plates were coated with gB vaccine protein (0.75ug/well) and then
incubated with sera diluted in PBS as described. Either PBMC or NK cells were
added to the wells and, 48 hours later, the cells harvested and stained for CD3,
CD56 and CD107a expression (BD biosciences) and enumerated by Flow cytometry.
Stimulation with PMA and Ionomycin was used as a positive control and healthy
seronegative donor sera as a negative. Additionally, sera isolated pre-vaccination
from seronegatives was used as a baseline negative response.

ELISAs for AD1,2, 4 and 5 have been described previously (15). AD1 and AD2 are
non-structured epitopes and it is well established that the peptides are recognised by
AD1 and AD2 antibody responses. The recombinant AD4 used has been shown to
be recognised by known AD4 conformational antibodies and the structure of the AD5
antigen has been shown to have the same structure as AD5 in gB (15, 37, 38).
Briefly, sera was diluted in PBS as described and then incubated with peptide coated
96 well plates. Healthy seropositive and seronegative sera were used as controls.
Anti-human IgG conjugated to HRP was used to detect CMV antibodies and visualised using TMB substrate. OD was measured at 450nm. Visit 1 (e.g. pre-vaccination of seronegative patients) was set as background/baseline.

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Figure Legends

Figure 1 Vaccination does not promote neutralising antibody responses in seronegatives a) HCMV was incubated for 1 hour with different concentrations of monoclonal antibodies against gB (ITC88 and 2F12), IgG1 isotype control or media and then used to infect HFFs (MOI=0.5). Percentage IE positivity was scored 24hpi. n=3. b) HCMV was incubated for 1 hour with sera from seropositive or seronegative patients either vaccinated with gB or given placebo and then used to infect HFFs (MOI=1). The analysis was performed on sera isolated pre-vaccination and at day of transplant (post vaccination). Percentage IE positivity was scored 24hpi and further stratified into patients who developed viraemia. n=3. c) HCMV was incubated for 1 hour with sera from seronegative patients either vaccinated with gB or given placebo
and then used to infect HFFs (MOI=1). The analysis was performed on sera isolated pre-vaccination and at day of transplant (post vaccination). Percentage pp28 positivity was scored 120hpi and further stratified into patients who developed viraemia. n=3.

Figure 2 Sera from vaccinated seronegatives does not control the spread of cell associated HCMV in vitro a) HFFs were infected with Merlin-IE2-GFP (MOI=0.01) and progress of infection monitored for two weeks. Representative images of GFP expression at 1, 6, 9 and 14 days post infection are shown. Cells were counterstained with DAPI to show cell layers. b-c) HFFs infected with Merlin-IE2-GFP (B) or Merlin (C) were, 24hpi, incubated with ITC88 (100ug/ml) and viral spread assay 2 weeks post infection. GFP (Cell associated) or IE immunostaining (Cell Free) was used to calculate percentage infection. n=3. d) HFFs infected with Merlin (cell free) or Merlin-IE2-GFP (cell associated) were, 24hpi, incubated with no sera (control), seropositive or seronegative sera. After 2 weeks GFP (Cell associated) or IE immunostaining (Cell Free) was used to calculate percentage infection. n=3. e) HFFs infected with Merlin-IE2-GFP (cell associated) were, 24hpi, incubated with no sera (infected cells), healthy donor seropositive (HCMV(+)) or seronegative (HCMV(-)) sera. Alternatively, they were incubated with sera from either seropositive or seronegative patients given gB vaccine or placebo. Sera pre-vaccination and at day of transplant (post-vaccination) was analysed. After 2 weeks GFP (Cell associated) was used to calculate percentage infection. n=3. Patients were further stratified into those who experienced viraemia versus those that did not.
**Figure 3.** Increased ADCC antibody responses against gB are not detected in seropositives a) Gating strategy to study evidence of ADCC activity. NK cells defined as CD56+CD3- were then assayed for CD107a expression or IFNγ. PMA/Ionomycin was used as a positive control. b) Titration of healthy donor sera from seropositive and seronegative donors for ability to promote CD107a expression on NK cells. c-d) Summary of data of ADCC responses in seropositive liver and kidney organ recipients at time of transplant. Comparisons between placebo and vaccination or viraemia or no viraemia shown. n=3

**Figure 4.** Vaccination does not induce detectable ADCC antibody responses against gB in seronegatives a-d) Longitudinal sera samples from multiple visits were analysed for ADCC promoting activity. Samples were pre-vaccination (v#1), or 1 (v#2), 2 (v#3), 6 (v#4), 7 (v#5) months post vaccination or time of transplant (d0) or 7 days post transplant (d7). Baseline negative controls are shown using unstimulated cells or healthy donor seronegative sera and PMA/Ionomycin served as positive control.

**Figure 5.** Vaccination induces a pattern of epitope responses distinct from natural infection A-H) ELISA assays were performed on sera pre-vaccination (0 months) or 1,2,6 and 7 months post vaccination. Pre-vaccination represents background. ELISA ODs for anti-AD1 (a), AD2 (c), AD4 (e) and AD5 (g) responses are shown. Alternatively, data was stratified using outcome post transplant (b,d,f,h) to assess impact of responses on viraemia. Statistical significance was measured using non-parametric Mann-U Whitney test. N.S. = non significant.


Figure S2: Healthy Donor Control

% IE positive cells

- vac
- placebo

Comparison with different treatments: Control, Seroneg + C, Seroneg + C, Seropos + H1, Seropos + H1 + C
Figure S3
Figure S5
**e)** Renal Transplant Patients, HCMV seropositive (II)

- **Vaccinated:**
  - Patient 6
  - Patient 7
- **Placebo:**
  - Patient 5
  - Patient 6
  - Patient 7
  - Patient 8
  - Patient 9
  - PMA/I
  - Unstimulated
  - Autologous

**f)** Renal Transplant Patients, HCMV seropositive (III)

- **Vaccinated:**
  - Patient 8
  - Patient 9
  - Patient 10
- **Placebo:**
  - Patient 10
  - Patient 11
  - Patient 12
  - Patient 13
  - Patient 14
  - Patient 15
  - PMA/I
  - Unstimulated control
  - Autologous

Figure S5
Supplementary Figure Legends

Figure S1. Vaccination does not induce detectable neutralizing antibody responses in seronegative vaccine recipients and does not boost pre-existing neutralizing antibody responses in seropositive vaccinees. Merlin was incubated with sera from seronegative (a-d) and seropositive (e-h) renal (a,b,e,f) and liver (c,d,g,h) transplant recipients or an ITC88 positive control (green bar), and used to inoculate HFFs in vitro (MOI=1). Infection was assayed by IE immunostaining 24hpi and the proportion of infected cells calculated by counterstaining nuclei with DAPI. Sera isolated pre-vaccination (baseline – blue bars) or post vaccination (day of transplant – red bars) was tested in triplicate. Sera from patients vaccinated with gB (a,c,e,g) or placebo (b,d,f,h) are shown and are subdivided into patients who went onto display evidence of viraemia post-transplant. The mean values of the percentage of infection are presented with the error bars indicating the standard deviation (SD).

Figure S2. Exogenous Complement does not promote neutralisation with sera from seronegative vaccinees. HCMV Merlin was incubated with media, heat inactivated sera from seronegative patients given gB vaccine (vac) or placebo and then additionally incubated with guinea pig complement (+C) at 1:2 for 3 hours. As a control, healthy donor seropositive sera was used fresh or heat inactivated and heat inactivated with the addition of complement (1:2). HFFs were then infected, immunostained for IE 24hpi and infection scored for % infection.
Figure S3. Pre-incubation of HCMV with seronegative sera does not routinely reduce the frequency of pp28 positive cells 96hpi. Merlin was incubated with sera from seronegative renal transplant recipients, or an ITC88 positive control, and used to inoculate HFFs in vitr o (MOI=1). Infection was assayed by pp28 immunostaining 96hpi and the proportion of infected cells calculated by counterstaining nuclei with DAPI. Sera isolated pre-vaccination (baseline – blue bars) or post vaccination (day of transplant – red bars) was tested in triplicate. Sera from patients vaccinated with gB, n=12 (A) or placebo, n=8 (B) are shown and are subdivided into patients who went onto display evidence of viraemia post-transplant. The mean values of the percentage of infection are presented with the error bars indicating the standard deviation (SD).

Figure S4. Vaccination does not promote an ability of sera from seronegatives to limit cell to cell spread of HCMV in vitro. IE2-GFP tagged Merlin was incubated with sera from seropositive liver (a,b) and renal (c,d) transplant patients and seronegative liver (e,f) and renal (g,h) transplant patients, healthy donor sera (seronegative and seropositive individual) or an ITC88 positive control, and used to inoculate HFFs in vitro (MOI=0.25). Infection was assayed by GFP positivity at 14dpi and the proportion of infected cells calculated by counterstaining nuclei with DAPI. Sera isolated pre-vaccination (baseline – red bars) or post vaccination (day of transplant – blue bars) was tested in triplicate. Sera from patients vaccinated with gB (a,c,e,g) or placebo (b,d,f,h) are shown and are subdivided into patients who went onto display evidence of viraemia post-transplant. The mean values of the
percentage of infection are presented with the error bars indicating the standard deviation (SD).

**Figure S5.** ADCC promoting antibodies can be detected specifically in seropositive sera from healthy donors and transplant patients but levels are **not affected by vaccination.** a-b) PBMC isolated from a healthy seronegative donor was incubated with sera isolated from 10 seropositive (a) or seronegative (b) donors or with PMA/Ionomycin positive control and analysed for CD107a expression on NK cells (CD3-CD56+). c-f) PBMC from a healthy seronegative donor was incubated with PMA/Ionomycin, healthy seronegative donor sera (autologous sera) or left unstimulated and NK cells analysed for evidence of CD107a surface expression by FACS. Alternatively, PBMC was incubated with longitudinal serum samples from liver (c) and renal (d-f) where v#1 is pre-vaccination, v#2 is 1 month post vaccination, v#3 is two months post vaccination, v#4 is 6 months post vaccination, v#5 is 7 months post vaccination, d0 is day of transplant and d7 is 7 days post transplant.