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Original antigenic sin shapes the immunological repertoire evoked by HCMV gB-MF59 vaccine in seropositive recipients

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Short Communication

Original antigenic sin shapes the immunological repertoire evoked by HCMV gB-MF59 vaccine in seropositive recipients

Running title: Original antigenic sin impacts CMV vaccination

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Summary:

Vaccination of HCMV infected individuals with the glycoprotein B vaccine boosts pre-existing immune responses against gB but fails to induce new responses against novel linear epitopes within gB in seropositive individuals.

Abstract:

A cytomegalovirus (CMV) vaccine is urgently needed to protect against primary infection and enhance existing immunity in CMV infected individuals (CMV+). Using sera from CMV+ gB/MF59 vaccine recipients prior to transplant we investigated the composition of the immune response. Vaccination boosted pre-existing humoral responses in our CMV+ cohort but did not promote *de-novo* responses against novel linear epitopes. This suggests that prior natural infection has a profound effect on shaping the antibody repertoire and subsequent response to vaccination ('original antigenic sin'). Thus vaccination of CMV+ may require strategies of epitope presentation distinct from those intended to prevent primary infection.

Abstract word count: 99/100

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Main text:

Background:

Human Cytomegalovirus (HCMV) infection is common, with seroprevalence ranging from 60 to 100% (1). HCMV can promote substantial mortality and morbidity in immunocompromised individuals, including solid organ transplant (SOT) recipients (2). In these patients, CMV end-organ disease results from primary infection, reinfection or reactivation (3). The most successful vaccine studied to date is recombinant glycoprotein-B (gB) with MF59 adjuvant, which demonstrated partial efficacy in reducing viraemia after SOT and similar efficacy in preventing primary infection in women and adolescents (4, 5). While the mechanism of protection is not fully understood we have previously reported that higher levels of total anti-gB IgG antibody correlated with a shorter duration of post transplantation viraemia (6).

In CMV+ individuals the vaccine clearly boosted pre-existing antibody responses (7). Furthermore, detailed analyses of humoral responses against well-defined antigenic domains (AD1, AD2, AD4, and AD5) in seropositive individuals revealed that only anti-AD2 antibody responses correlated with protection from post-transplantation viremia. Importantly, vaccination only boosted AD2 responses in the 50% of CMV+ individuals with a pre-existing response and did not induce a new AD2 response in those who lacked AD2 antibodies following natural infection. Although there was no evidence that the potent responses towards AD1, AD4 and AD5 impaired protection from AD2, it is possible that a large proportion of the antibodies elicited by natural infection (and thus boosted by vaccination) are non-protective (7, 8). We hypothesized that highly immunogenic domains that induce non-protective responses might facilitate CMV replication by diverting immune system resources

away from domains that might induce more protective responses (7, 9, 10). To begin addressing this interesting question we used peptide array technology for scanning antibody responses to linear gB epitopes across all protein domains in six CMV+ SOT recipients.

Methods:

Patient population

The sub-population from whom samples have been evaluated and described in this work are the cohort of solid organ transplant patients who were enrolled in the phase-2 randomised and double-blinded placebo controlled cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant trial. This trial was registered with ClinicalTrials.gov, NCT00299260 (6). The vaccine or placebo was given in three doses: at Day 0 (baseline), 1 month and 6 months later. Following vaccination, the blood samples from patients were obtained consecutively. The first five blood samples were collected before transplantation in order to measure antibodies (qualitatively and quantitatively) at baseline, and after 1, 2, 6 and 7 months. The patients who subsequently underwent transplantation were followed up for 90 days during which serial blood samples were obtained around days 0, 7, 35, 63, 90 post-transplant. The level of viral DNA was also tested by measuring CMV DNA by real-time quantitative PCR (RTqPCR) (6). Exclusion criteria included: pregnancy (a negative pregnancy test was required before each vaccine dose); receipt of blood products (except albumin) in the previous 3 months, and simultaneous multi-organ transplantation (6). The study was approved by the Research Ethics Committee and all patients gave written informed consent (6).

gB peptide microarray

To identify linear gB epitope binding, 15-mer peptides covering the entire gB open reading frame (Towne strain), and overlapping with neighbouring peptides by 10 residues (total of 188 peptides) were synthesized and printed to a PepStar multiwell array (JPT Peptide) in triplicate. Microarray binding was performed manually using individual slides immobilized in the ArraySlide 24-4 chamber (JPT Peptide). First, arrays were incubated for 1 hour with sera diluted 1:200 in blocking buffer (Superblock T20 (TBS), ThermoFisher Scientific) followed by a 1 hour incubation with anti-human IgG conjugated to AF647 (Jackson ImmunoResearch) diluted in blocking buffer (0.1 µg/mL). Following each incubation step, arrays were washed 5x in wash buffer (1x TBS buffer + 0.1% Tween) using an automated plate washer (Wellwash Versa). Array was then dried by centrifugation and scanned at a wavelength of 635 nm using an Axon Genepix 4300 SL50 scanner (Molecular Devices) at a PMT setting of 650 and 100% laser power. Images were analysed using Genepix Pro 7 software (Molecular Devices). Images were reviewed manually for accurate automated peptide identification. For each spot, mean signal intensity was extracted. For each peptide, the MMC2 values were calculated (the mean values of all three instances on the microarray, except when the coefficient of variation (CV) was larger than 0.5. In this case the mean of the two closest values (MC2) was assigned to MMC2). Data analysis and graphical presentations were made using the software R.

Results:

To characterise the antibody profile against linear epitopes of gB the sera of six CMV+ gB/MF59 vaccine recipients were analysed pre and post-vaccination (Fig.1; Fig.S.1; Fig.S.3).

This allowed the identification of epitopes recognised during natural infection as well those induced or boosted by vaccine. Responses to several previously reported epitopes were observed including some located in the Cytosolic Terminal Domain (CTD). Studies of the serological responses to this region are limited with two studies from the early 90s showing high serum reactivity to this region, subsequently called “AD3” (11, 12). It was speculated that, due to its location on the intraluminal, cytosolic part of gB, antibodies against this region will be most likely non-neutralising and non-protective. Perhaps this assumption explains why AD3 has not been given sufficient attention as a potential antibody target in the past. However, Nelson et al (13) recently analysed sera from a cohort of CMV- post-partum women vaccinated with gB/MF59 and subsequently found that 76% of the vaccine-induced linear IgG response recognized CTD/AD3.

Our work with CMV+ sera shows that this also happens after natural infection demonstrating that an overwhelming majority of all anti-gB antibodies against linear epitopes were specific for this region (Fig.1.B). Interestingly, vaccination boosted pre-existing anti-CTD responses to an extremely high level in three patients, dwarfing the responses observed to other ADs (Fig.1.C and Fig. S1). The same three patients experienced post-transplantation CMV viraemia. In direct contrast the remaining three patients who had not developed these antibody responses

127 subsequently following vaccination and had no evidence of post-transplantation
128 viraemia (Fig.1.D).

129 Next, we sought to investigate how such potent response towards CTD in these
130 three individuals correlated with production of antibodies towards other regions
131 (Fig.2.) Interestingly we could see that high level of antibodies to AD2 and CTD are
132 mutually exclusive. This could potentially suggest that high level of anti-CTD
133 antibodies could hinder generation of anti-AD2 responses, a response that we and
134 others have previously demonstrated to be correlated with protection (Fig 2B) (8).
135 Although such a small number of individuals preclude definite conclusions, our
136 results argue that future studies should further investigate this highly immunogenic,
137 cytosolic region of gB and its relationship with other antigenic domains of gB.

Discussion:

Based on this study of linear epitopes, our data suggest that vaccinating CMV+ individuals with the gB/MF59 vaccine predominantly boosts pre-existing antibody responses rather than inducing *de novo* responses. It is intriguing that while CTD is highly immunogenic, responses to this region appear to inversely correlate with protection from viraemia. One hypothesis is that inducing a humoral response against CTD CMV diverts the immune response away from targets more likely to induce protective antibody responses i.e. AD2. A competition model is not unique in HCMV whereby it is argued AD1 responses may interfere with protective AD2 responses - although in our patient cohort we did not observe a correlation between AD1 responses and the presence/absence of post-transplantation viremia (8). Additionally, we cannot rule out the reason for differences in protection are related to differences in the responses to other important targets for neutralisation (e.g. gH/gL complexes).

An important implication of this study is that vaccination of CMV+ individuals with gB/MF59 might simply boost the pre-existing antibody responses and, furthermore, in some individuals these might be non-protective. This concept is consistent with the paradigm of “original antigenic sin”, which describes the tendency of the immune system to preferentially utilize immunological memory originating from a previous antigen encounter. Thus, the ‘original antigenic sin’ might be responsible for shaping the repertoire of immunological responses evoked by either vaccination or secondary exposure to different versions of the same pathogen (e.g. a different strain, or a recombinant protein subunit). As a result, pre-existing responses are boosted

instead of vaccination promoting the development of novel protective responses that may occur in response to a newly encountered antigen. This phenomenon is well established with studies of Influenza, Dengue, and HIV, and considered to be a substantial obstacle to successful vaccine development (14). In this report we show, for the first time, that this immunological phenomenon could also hamper the success of the HCMV gB/MF59 vaccine in certain individuals. This becomes prescient if we consider that a successful vaccine against this highly prevalent pathogen should not only protect against primary infection but also re-infection with a different strain of the virus as well as re-activation of latent infection (1, 15).

We believe that this observation – albeit based on small numbers – illustrates the complexity of developing a universal vaccine strategy against a persistent viral infection highly prevalent in the population. It also supports the hypothesis that deletion of specific regions of gB, or alternative strategies to present gB, may be important – particularly in individuals with prior exposure to HCMV.

Figure 1. Responses against cytosolic terminal domain (CTD, AD3) in seropositive individuals are dominant and non-protective.

A) Linear structure of defined glycoprotein B antigenic domains. The entire open reading frame (ORFs) of HCMV gB are shown. The four distinct regions of the gB structure are indicated by black bars at the base of the figure, including the ectodomain, membrane proximal domain (MPD), transmembrane domain (TM), and the cytoplasmic domain. Major antigenic regions indicated include AD1 (orange), AD2 site 1 (red), AD2 site 2 (yellow), AD3 (purple), AD4 (Domain II) (green), and AD5 (Domain I) (blue). Numbers indicate approximate amino acid residues dividing each region of interest. Diagram was adapted from Burke et al., Plos Pathogens, 2015 and Nelson et al., PNAS, 2018. B-C). The highest values of antibody responses against these five major antigenic domains prior to vaccination (B) and following vaccination (C) are shown for each naturally seropositive SOT patient from R+ group. D) The highest value of IgG antibody responses against immunodominant AD3 region are shown for each patient prior to vaccination and post-vaccination. Median values of antibody responses are depicted by horizontal lines. Patients were further stratified for viraemia post-transplant (>200 viral genomes/ml of whole blood).

Fig.2. High level of antibodies to AD2 and CTD (AD3) are mutually exclusive.

A-D) The highest IgG response against AD1 (A), AD2 (B), AD4 (C) and AD5 (D) was plotted alongside the respective responses against cytoplasmic terminal domain (CTD/AD3); (n=6).

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257

Figure S1. **The linear epitope binding responses against cytosolic antigenic domain 3 (AD3) in naturally seropositive individuals is not correlated with protection.**

The binding magnitude of antibody responses of six HCMV seropositive SOT patients pre- and post-vaccination and two HCMV seronegative recipients of placebo as a control were assessed against a 15-mer peptide library spanning the cytoplasmic terminal domain (CTD, AD3). The negative cut-off values were set as the highest responses in the sera from seronegative placebo recipients.

Figure S2. General principle of epitope detection using overlapping peptide scans.

JPT's PepStarTM Peptide Microarrays are designed for detecting potential biomarkers for infectious diseases, autoimmune diseases, cancer and allergies and to elucidate protein-protein interactions. Each spot in the microarray represents a single individual peptide. After incubation of the peptide microarray with serum or antibody samples, bound antibodies or proteins can be detected using fluorescently labeled secondary antibodies. Resulting antibody signatures represent unique insights into the properties of samples studied.

Figure S3: **Heatmap diagram.**

Heatmap diagram showing all incubations of the serum samples (HCMV seropositive SOT patients, pre- and post-vaccination) and controls (HCMV seronegative SOT patients, placebo); y-axis represents peptide sequences in the library, x-axis shows samples applied. Each column indicates a single patient (pre- or post-vaccination). The binding magnitude is indicated as the MMC2 value (light units) calculated from three spot replicates of each peptide. These values are shown as colour coded ranging from white (0 or low intensity) over yellow (middle intensity) to red (high intensity).

Footnotes:

Funding:

This study was supported by the Rosetrees and Stoneygate Trusts (A1601) and the Royal Free Charity; M.B.R. was also supported by an MRC Fellowship (G:0900466). The original clinical trial of gB/MF59 was supported the National Institute of Allergy and Infectious Diseases (R01AI051355) and Sanofi Pasteur.

Conflict of Interest:

Funding sources (Rosetrees Trust, Stoneygate Trust, Royal Free charity and MRC) had no role in the study design, data collection, data analysis, data interpretation, writing of the manuscript, or in the decision to submit to publication. F.K. and P.H. are employees of JPT. All authors have submitted ICMJE forms for disclosure of potential Conflicts of Interest.

Ethics statement:

The study was approved by the Research Ethics Committee and all patients whose samples were investigated here gave written informed consent (6).

Meeting(s) where the information has previously been presented:

n/a

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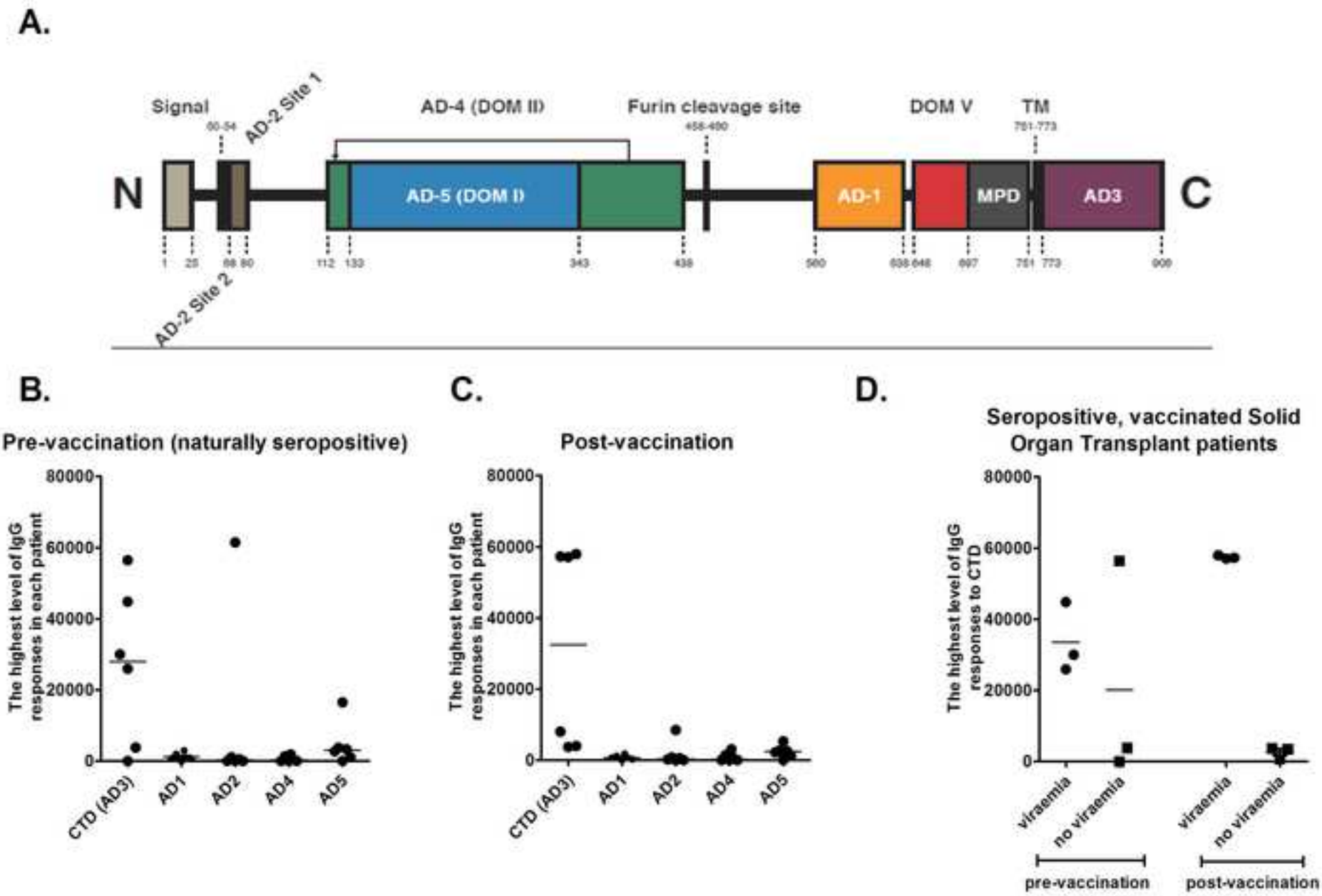

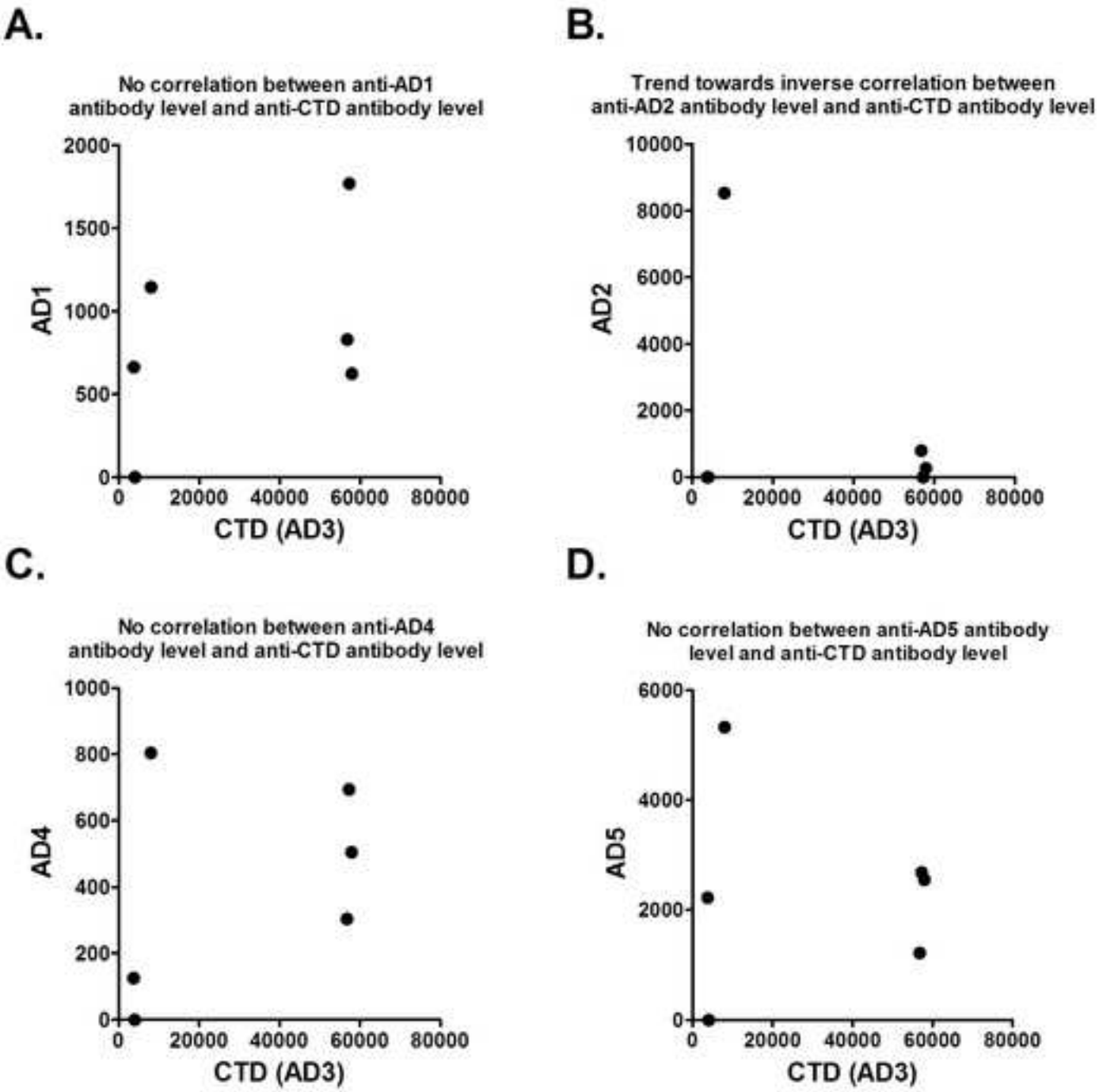

supplementary figure 1

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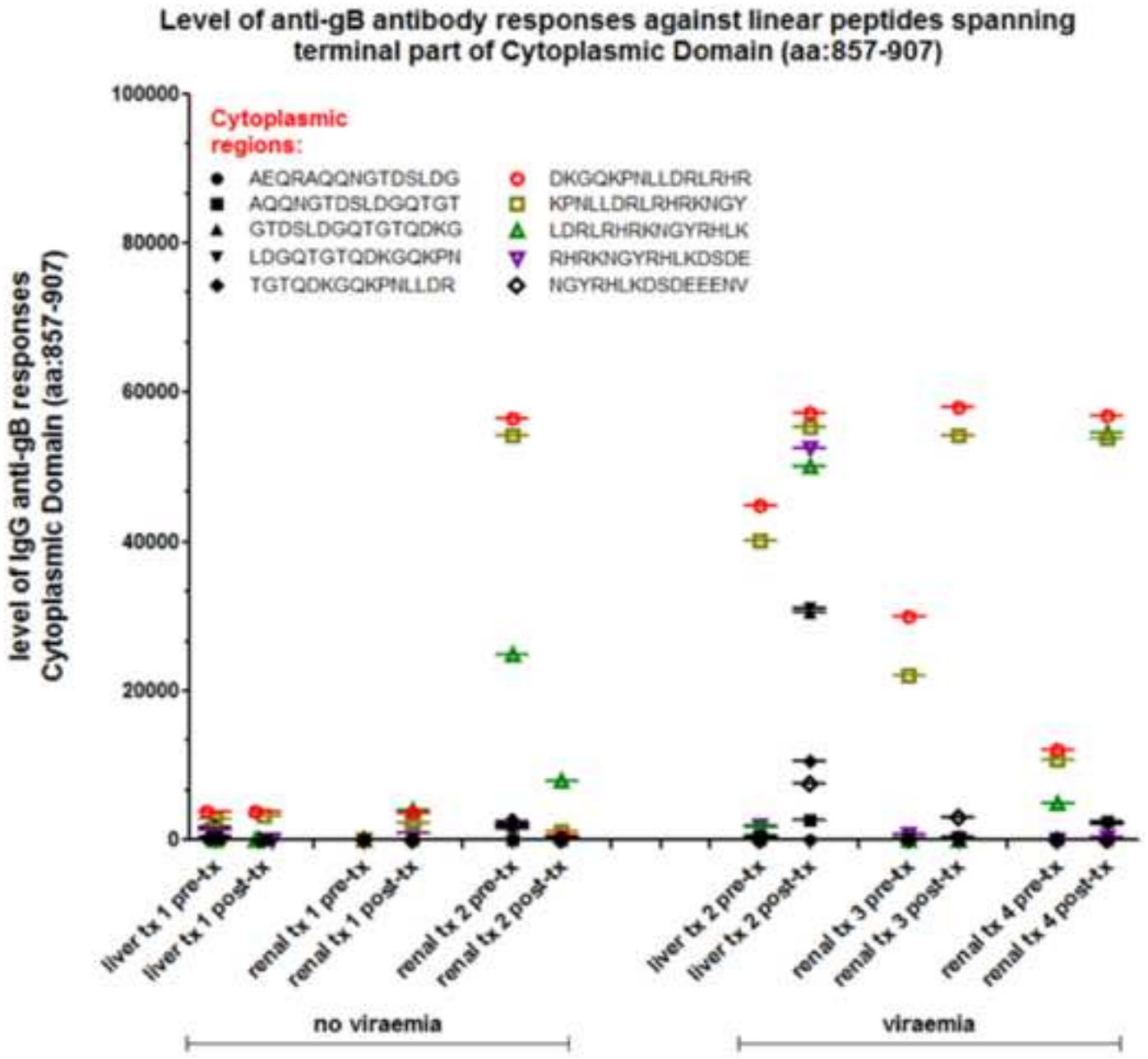

supplementary figure 2

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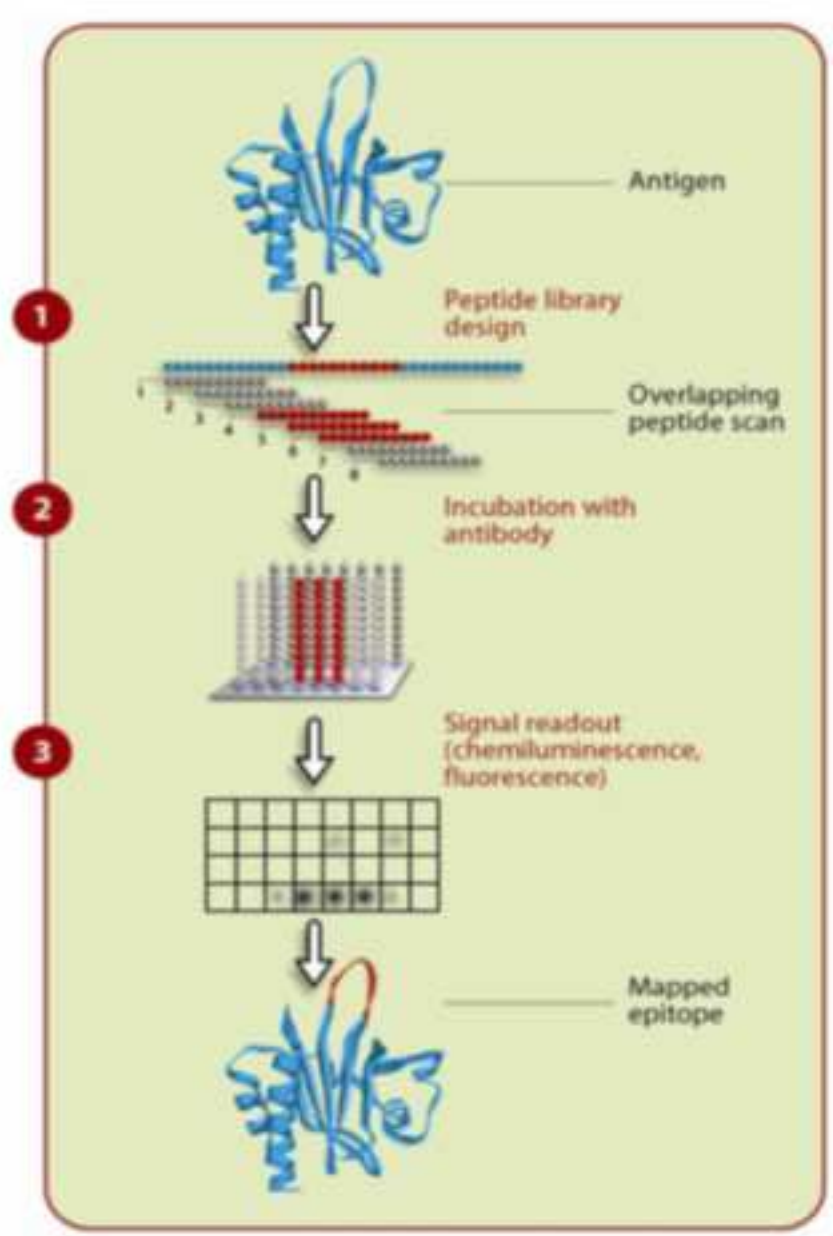

supplementary figure 3

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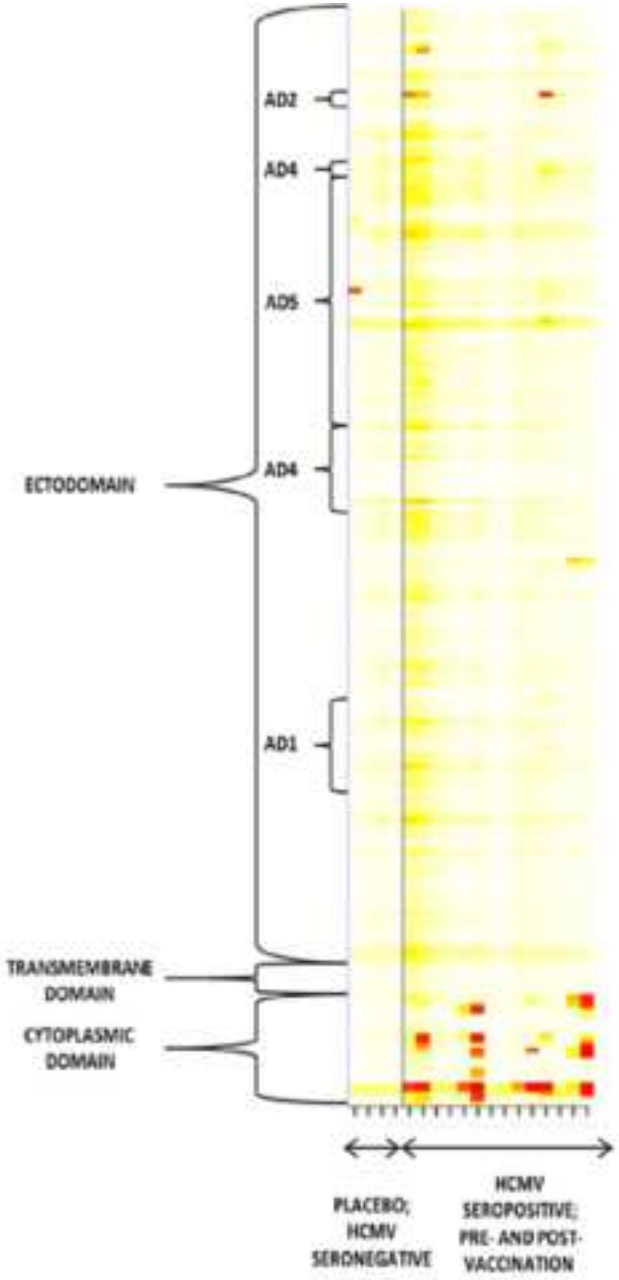

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Editorial Board of Journal of Infectious Diseases

Please find enclosed our revised manuscript entitled 'Original antigenic sin shapes the immunological repertoire evoked by HCMV gB-MF59 vaccine in seropositive recipients ' by Baraniak et al for consideration at the Journal of Infectious Diseases.

We appreciate the positive responses of the reviewers and have either incorporated suggestions into the manuscript or, where the information is already included, highlighted this.

The authors declare no competing financial interests and all authors have read and approve the submission of the manuscript.

Thank you for your consideration of our manuscript

Ilona Baraniak
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Senior Lecturer in Virology : Dr Matthew Reeves *:matthew.reeves@ucl.ac.uk*

Response to Reviewers

We thank Reviewer 1 for their positive comments regarding our track record in this area of research.

Reviewer 2 makes 4 points which we respond to below:

First, as the authors state, the sample size is small.
As the reviewer correctly points out the sample size is small. However, we felt that even from this small sample set there were potentially important observations that, if highlighted now, could inform the design of future vaccine studies with larger sample sizes and thus be of value to the field.

Second, no comparative data are provided on samples from seropositive gB vaccinees who were protected.
In the study we did highlight that 3 of the 6 seropositives experienced viraemia post-transplant (Ln119 and Figure 1D). However, we were wary (in light of point 1 above) of over-interpreting a small sample size to make conclusions about protection and so have not made this a major point.

Third, antibodies were studied only for binding and no account is taken of non-neutralizing functional responses correlating with protection, as studied by Nelson et al.
The question the reviewer raises is an important one that is an ongoing area of study in our lab. The Nelson study along with our own published jointly with it (Baraniak et al, 2018, PNAS) both sought to investigate the mechanism of protection of the vaccine. It is worth noting that both these studies were seeking to understand the protection observed in seronegative vaccine recipients.

The current study is addressing a different question using seropositives. It is essentially aimed at understanding what happens to the gB antibody response following vaccination and, specifically, if there is any evidence any new antibody responses developing in individuals who have been infected with the virus prior to vaccination. We are making no claims about the functionality of the antibody responses. Unfortunately many of the assays used in the papers above are not applicable here due to the complication of these patient samples being from seropositive individuals – and thus have antibodies against multiple CMV epitopes.

Given the low numbers analysed as discussed above it would not be prudent to make claims of correlates of protection. Indeed throughout the text we have tried to make it clear that any interpretations and suggestions are made on low numbers.

Fourth, no data are provided on responses to the pentamer proteins that are considered to have a role in protection through neutralization.

No data are provided on pentamer because the focus of the study was to understand in more detail the nature of the response to gB because this is what the patients were vaccinated with. It is unlikely that changes to the pentamer response would be evident. However, please note that we have analysed these sera previously (Baraniak et al, 2018, PNAS), shown they have neutralising

activity which is not affected by gB vaccination and suggested this activity was due to antibodies that recognise pentamer. To clarify this, we have added a sentence to the discussion to state that differences in neutralising antibody responses against other targets could explain why some patients were protected and others were not (Ln 143).

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The sub-population from whom samples have been evaluated and described in this work are the cohort of solid organ transplant patients who were enrolled in the phase-2 randomised and double-blinded placebo controlled cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant trial. This trial was registered with ClinicalTrials.gov, NCT00299260 (6). The vaccine or placebo was given in three doses: at Day 0 (baseline), 1 month and 6 months later. Following vaccination, the blood samples from patients were obtained consecutively. The first five blood samples were collected before transplantation in order to measure antibodies (qualitatively and quantitatively) at baseline, and after 1, 2, 6 and 7 months. The patients who subsequently underwent transplantation were followed up for 90 days during which serial blood samples were obtained around days 0, 7, 35, 63, 90 post-transplant. The level of viral DNA was also tested by measuring CMV DNA by real-time quantitative PCR (RTqPCR) (6). Exclusion criteria included: pregnancy (a negative pregnancy test was required before each vaccine dose); receipt of blood products (except albumin) in the previous 3 months, and simultaneous multi-organ transplantation (6). The study was approved by the Research Ethics Committee and all patients gave written informed consent (6).

gB peptide microarray

To identify linear gB epitope binding, 15-mer peptides covering the entire gB open reading frame (Towne strain), and overlapping with neighbouring peptides by 10 residues (total of 188 peptides) were synthesized and printed to a PepStar multiwell array (JPT Peptide) in triplicate. Microarray binding was performed manually using individual slides immobilized in the ArraySlide 24-4 chamber (JPT Peptide). First, arrays were incubated for 1 hour with sera diluted 1:200 in blocking buffer (Superblock T20 (TBS), ThermoFisher Scientific) followed by a 1 hour incubation with anti-human IgG conjugated to AF647 (Jackson ImmunoResearch) diluted in blocking buffer (0.1 µg/mL). Following each incubation step, arrays were washed 5x in wash buffer (1x TBS buffer + 0.1% Tween) using an automated plate washer (Wellwash Versa). Array was then dried by centrifugation and scanned at a wavelength of 635 nm using an Axon Genepix 4300 SL50 scanner (Molecular Devices) at a PMT setting of 650 and 100% laser power. Images were analysed using Genepix Pro 7 software (Molecular Devices). Images were reviewed manually for accurate automated peptide identification. For each spot, mean signal intensity was extracted. For each peptide, the MMC2 values were calculated (the mean values of all three instances on the microarray, except when the coefficient of variation (CV) was larger than 0.5. In this case the mean of the two closest values (MC2) was assigned to MMC2). Data analysis and graphical presentations were made using the software R.

Results:

To characterise the antibody profile against linear epitopes of gB the sera of six CMV+ gB/MF59 vaccine recipients were analysed pre and post-vaccination (Fig.1; Fig.S.1; Fig.S.3).

This allowed the identification of epitopes recognised during natural infection as well those induced or boosted by vaccine. Responses to several previously reported epitopes were observed including some located in the Cytosolic Terminal Domain (CTD). Studies of the serological responses to this region are limited with two studies from the early 90s showing high serum reactivity to this region, subsequently called “AD3” (11, 12). It was speculated that, due to its location on the intraluminal, cytosolic part of gB, antibodies against this region will be most likely non-neutralising and non-protective. Perhaps this assumption explains why AD3 has not been given sufficient attention as a potential antibody target in the past. However, Nelson et al (13) recently analysed sera from a cohort of CMV- post-partum women vaccinated with gB/MF59 and subsequently found that 76% of the vaccine-induced linear IgG response recognized CTD/AD3.

Our work with CMV+ sera shows that this also happens after natural infection demonstrating that an overwhelming majority of all anti-gB antibodies against linear epitopes were specific for this region (Fig.1.B). Interestingly, vaccination boosted pre-existing anti-CTD responses to an extremely high level in three patients, dwarfing the responses observed to other ADs (Fig.1.C and Fig. S1). The same three patients experienced post-transplantation CMV viraemia. In direct contrast the remaining three patients who had not developed these antibody responses

subsequently following vaccination and had no evidence of post-transplantation viraemia (Fig.1.D).

Next, we sought to investigate how such potent response towards CTD in these three individuals correlated with production of antibodies towards other regions (Fig.2.) Interestingly we could see that high level of antibodies to AD2 and CTD are mutually exclusive. This could potentially suggest that high level of anti-CTD antibodies could hinder generation of anti-AD2 responses, a response that we and others have previously demonstrated to be correlated with protection (Fig 2B) (8). Although such a small number of individuals preclude definite conclusions, our results argue that future studies should further investigate this highly immunogenic, cytosolic region of gB and its relationship with other antigenic domains of gB.

Discussion:

Based on this study of linear epitopes, our data suggest that vaccinating CMV+ individuals with the gB/MF59 vaccine predominantly boosts pre-existing antibody responses rather than inducing *de novo* responses. It is intriguing that while CTD is highly immunogenic, responses to this region appear to inversely correlate with protection from viraemia. One hypothesis is that inducing a humoral response against CTD CMV diverts the immune response away from targets more likely to induce protective antibody responses i.e. AD2. A competition model is not unique in HCMV whereby it is argued AD1 responses may interfere with protective AD2 responses - although in our patient cohort we did not observe a correlation between AD1 responses and the presence/absence of post-transplantation viremia (8). Additionally, we cannot rule out the reason for differences in protection are related to differences in the responses to other important targets for neutralisation (e.g. gH/gL complexes).

An important implication of this study is that vaccination of CMV+ individuals with gB/MF59 might simply boost the pre-existing antibody responses and, furthermore, in some individuals these might be non-protective. This concept is consistent with the paradigm of “original antigenic sin”, which describes the tendency of the immune system to preferentially utilize immunological memory originating from a previous antigen encounter. Thus, the ‘original antigenic sin’ might be responsible for shaping the repertoire of immunological responses evoked by either vaccination or secondary exposure to different versions of the same pathogen (e.g. a different strain, or a recombinant protein subunit). As a result, pre-existing responses are boosted

instead of vaccination promoting the development of novel protective responses that may occur in response to a newly encountered antigen. This phenomenon is well established with studies of Influenza, Dengue, and HIV, and considered to be a substantial obstacle to successful vaccine development (14). In this report we show, for the first time, that this immunological phenomenon could also hamper the success of the HCMV gB/MF59 vaccine in certain individuals. This becomes prescient if we consider that a successful vaccine against this highly prevalent pathogen should not only protect against primary infection but also re-infection with a different strain of the virus as well as re-activation of latent infection (1, 15).

We believe that this observation – albeit based on small numbers – illustrates the complexity of developing a universal vaccine strategy against a persistent viral infection highly prevalent in the population. It also supports the hypothesis that deletion of specific regions of gB, or alternative strategies to present gB, may be important – particularly in individuals with prior exposure to HCMV.

Figure 1. Responses against cytosolic terminal domain (CTD, AD3) in seropositive individuals are dominant and non-protective.

A) Linear structure of defined glycoprotein B antigenic domains. The entire open reading frame (ORFs) of HCMV gB are shown. The four distinct regions of the gB structure are indicated by black bars at the base of the figure, including the ectodomain, membrane proximal domain (MPD), transmembrane domain (TM), and the cytoplasmic domain. Major antigenic regions indicated include AD1 (orange), AD2 site 1 (red), AD2 site 2 (yellow), AD3 (purple), AD4 (Domain II) (green), and AD5 (Domain I) (blue). Numbers indicate approximate amino acid residues dividing each region of interest. Diagram was adapted from Burke et al., Plos Pathogens, 2015 and Nelson et al., PNAS, 2018. B-C). The highest values of antibody responses against these five major antigenic domains prior to vaccination (B) and following vaccination (C) are shown for each naturally seropositive SOT patient from R+ group. D) The highest value of IgG antibody responses against immunodominant AD3 region are shown for each patient prior to vaccination and post-vaccination. Median values of antibody responses are depicted by horizontal lines. Patients were further stratified for viraemia post-transplant (>200 viral genomes/ml of whole blood).

Fig.2. High level of antibodies to AD2 and CTD (AD3) are mutually exclusive.

A-D) The highest IgG response against AD1 (A), AD2 (B), AD4 (C) and AD5 (D) was plotted alongside the respective responses against cytoplasmic terminal domain (CTD/AD3); (n=6).

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Figure S1. **The linear epitope binding responses against cytosolic antigenic domain 3 (AD3) in naturally seropositive individuals is not correlated with protection.**

The binding magnitude of antibody responses of six HCMV seropositive SOT patients pre- and post-vaccination and two HCMV seronegative recipients of placebo as a control were assessed against a 15-mer peptide library spanning the cytoplasmic terminal domain (CTD, AD3). The negative cut-off values were set as the highest responses in the sera from seronegative placebo recipients.

Figure S2. General principle of epitope detection using overlapping peptide scans.

JPT's PepStarTM Peptide Microarrays are designed for detecting potential biomarkers for infectious diseases, autoimmune diseases, cancer and allergies and to elucidate protein-protein interactions. Each spot in the microarray represents a single individual peptide. After incubation of the peptide microarray with serum or antibody samples, bound antibodies or proteins can be detected using fluorescently labeled secondary antibodies. Resulting antibody signatures represent unique insights into the properties of samples studied.

Figure S3: **Heatmap diagram.**

Heatmap diagram showing all incubations of the serum samples (HCMV seropositive SOT patients, pre- and post-vaccination) and controls (HCMV seronegative SOT patients, placebo); y-axis represents peptide sequences in the library, x-axis shows samples applied. Each column indicates a single patient (pre- or post-vaccination). The binding magnitude is indicated as the MMC2 value (light units) calculated from three spot replicates of each peptide. These values are shown as colour coded ranging from white (0 or low intensity) over yellow (middle intensity) to red (high intensity).

Footnotes:

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Conflict of Interest:

Funding sources (Rosetrees Trust, Stoneygate Trust, Royal Free charity and MRC) had no role in the study design, data collection, data analysis, data interpretation, writing of the manuscript, or in the decision to submit to publication. F.K. and P.H. are employees of JPT. All authors have submitted ICMJE forms for disclosure of potential Conflicts of Interest.

Ethics statement:

The study was approved by the Research Ethics Committee and all patients whose samples were investigated here gave written informed consent (6).

Meeting(s) where the information has previously been presented:

n/a

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