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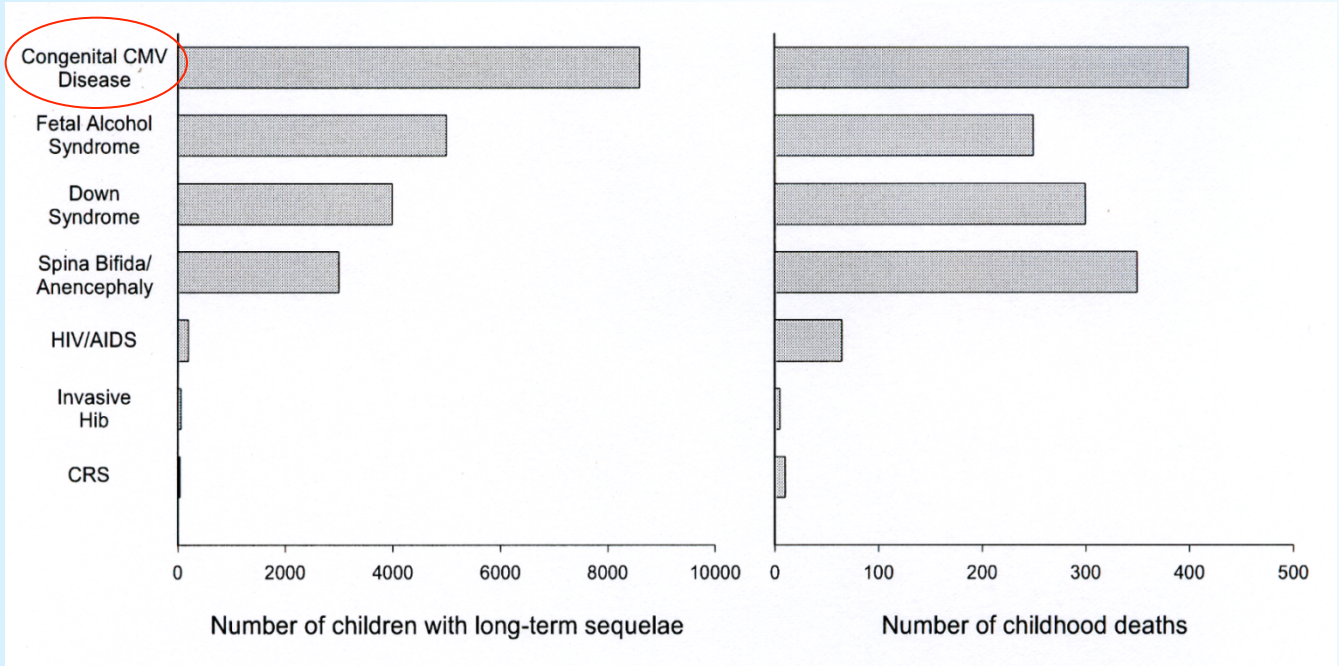
Antibody-Driven Vaccine Design: Identification of the Human Cytomegalovirus gHgLpUL128L Complex as a Subunit Vaccine.

Daniele Lilleri

Estimates of the annual burden of prominent childhood diseases and syndromes in the US



Congenital CMV disease is the most common fetal/neonatal disease.
(Annual caring costs:
$1-$2 billion)

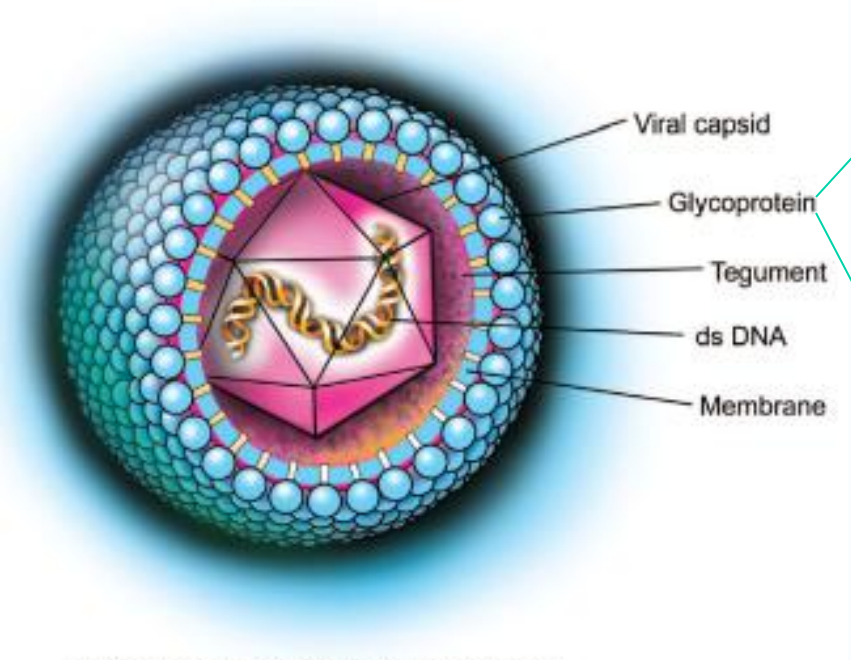
Cannon MJ, Davis KF. BMC Public Health 2005;5:70.

The development of a CMV vaccine was listed as a top priority by the Institute of Medicine of the National Academy of Sciences (USA).

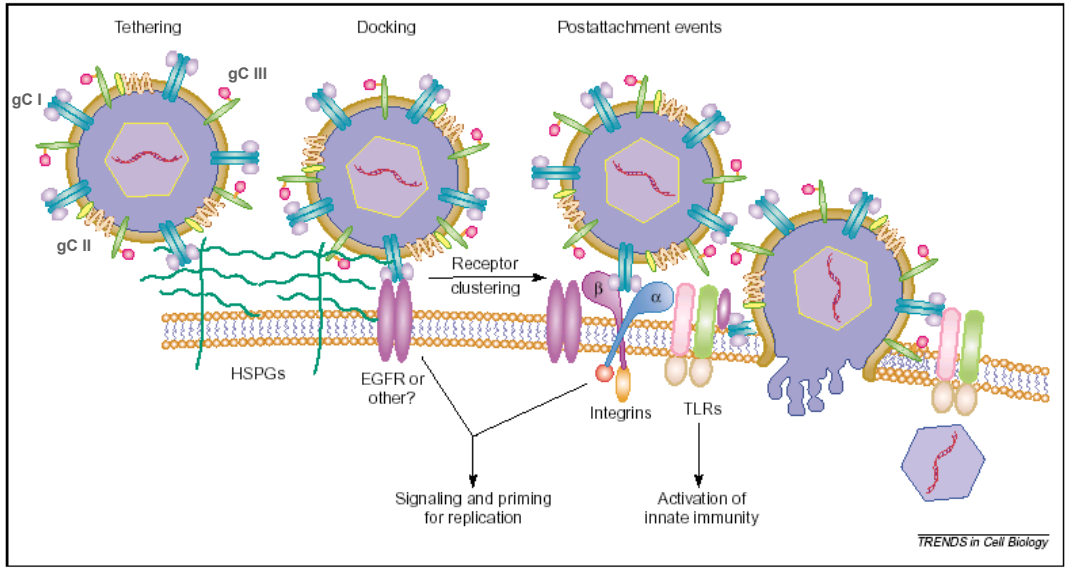
The National Vaccine Advisory Committee emphasize the financial and humanitarian justifications for developing such a vaccine.
(Stratton K et al. Washington DC, National Academy Press, 2001)

- **HCMV surface glycoproteins as candidate subunit vaccines**

HCMV surface glycoprotein complexes

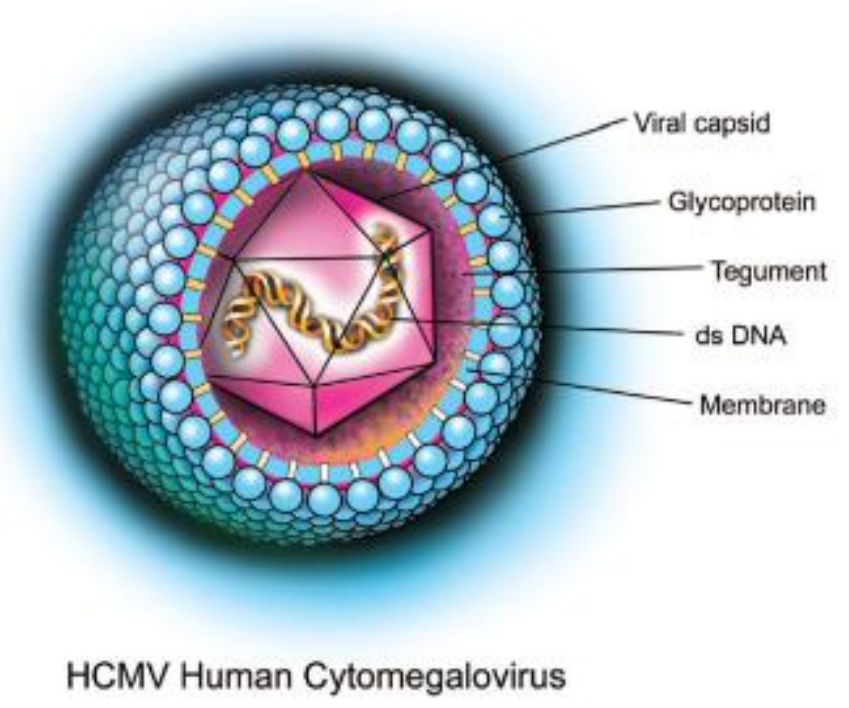


Surface glycoprotein complexes	
gC I	gB omodimer
gC II	gM/gN
gC III	-gH/gL -gH/gL/pUL128/pUL130/pUL131



- Essential for virus replication *in vitro*
- Presence in human sera of neutralizing antibodies directed against gB, gH or gM/gN viral glycoproteins (Rasmussen et al., 1991; Urban et al., 1992; Shimamura et al., 2006; Funaro et al., 2008).

HCMV surface glycoprotein complexes



HCMV Human Cytomegalovirus

Surface glycoprotein complexes	
gC I	gB omodimer
gC II	gM/gN
gC III	-gH/gL -gH/gL/pUL128/pUL130/pUL131

JOURNAL OF VIROLOGY, Sept. 2004, p. 10023–10033 Vol. 78, No. 18
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.18.10023–10033.2004

Human Cytomegalovirus UL131-128 Genes Are Indispensable for Virus Growth in Endothelial Cells and Virus Transfer to Leukocytes

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Dendritic-cell infection by human cytomegalovirus is restricted to strains carrying functional UL131–128 genes and mediates efficient viral antigen presentation to CD8$^+$ T cells

Giuseppe Gerna,[1] Elena Percivalle,[1] Daniele Lilleri,[1] Laura Lozza,[1] Chiara Fornara,[1] Gabriele Hahn,[2] Fausto Baldanti[1] and M. Grazia Revello[1]

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JOURNAL OF VIROLOGY, Jan. 2008, p. 60–70 Vol. 82, No. 1
0022-538X/08/$08.00+0 doi:10.1128/JVI.01910-07

Characterization of the Human Cytomegalovirus gH/gL/UL128-131 Complex That Mediates Entry into Epithelial and Endothelial Cells$^{\nabla}$

Brent J. Ryckman,[1]* Barb L. Rainish,[1] Marie C. Chase,[1] Jamie A. Borton,[1,2] Jay A. Nelson,[1,2] Michael A. Jarvis,[1,2] and David C. Johnson[1]

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- Dispensable for infection of fibroblasts
- Consistently mutated in laboratory adapted strains
- Essential for infection of epithelial, endothelial, dendritic cells and virus transfer to leukocytes

The gB vaccine

The NEW ENGLAND JOURNAL of MEDICINE

N Engl J Med 2009;360:1191-9.

ORIGINAL ARTICLE

Vaccine Prevention of Maternal Cytomegalovirus Infection

Robert F. Pass, M.D., Changpin Zhang, M.D., Ashley Evans, M.D., Tina Simpson, M.D., William Andrews, M.D., Meei-Li Huang, Ph.D., Lawrence Corey, M.D., Janie Hill, R.N., Elizabeth Davis, R.N., M.P.H., Cynthia Flanigan, B.S., and Gretchen Cloud, M.S.

From the Departments of Pediatrics (R.F.P., C.Z., T.S., J.H.), Obstetrics and Gynecology (W.A.), and Medicine (C.F., G.C.), University of Alabama at Birmingham, Birmingham; the Department of Pediatrics, University of Alabama College of Community Health Sciences, Tuscaloosa (A.E., E.D.); and the Departments of Laboratory Medicine (M.-L.H., L.C.) and Medicine (L.C.), University of Washington; and the Fred Hutchinson Cancer Research Center (M.-L.H., L.C.) — both in Seattle. Address reprint requests to Dr. Pass at Children's Hospital, 1600 7th Ave. S., CHB 309, Birmingham, AL 35233, or at rpass@peds.uab.edu.

Rationale for the use of gB as a vaccine:

-major envelope constituent;
-important role in virus entry, cell-to-cell spread and fusion of infected cells;
-target of neutralizing antibody;
-target of T-cell immunity.

gB vaccine showed 50% efficacy

The gB vaccine

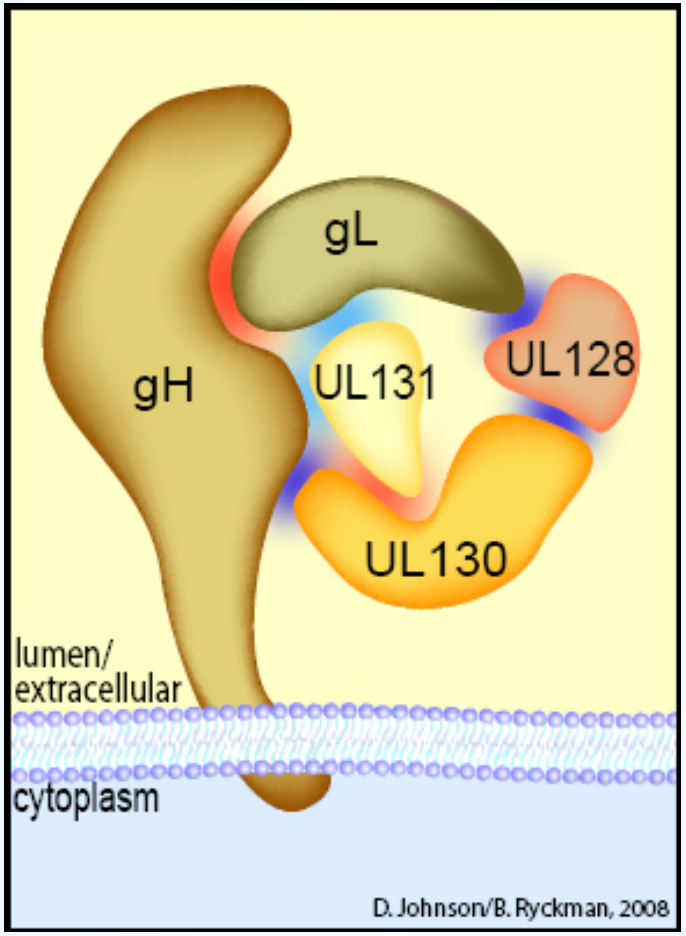
Lancet 2011; 377: 1256–63

Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: a phase 2 randomised placebo-controlled trial

Paul D Griffiths, Anna Stanton, Erin McCarrell, Colette Smith, Mohamed Osman, Mark Harber, Andrew Davenport, Gareth Jones, David C Wheeler, James O'Beirne, Douglas Thorburn, David Patch, Claire E Atkinson, Sylvie Pichon, Paul Sweny, Marisa Lanzman, Elizabeth Woodford, Emily Rothwell, Natasha Old, Ruth Kinyanjui, Tanzina Haque, Sowsan Atabani, Suzanne Luck, Steven Prideaux, Richard S B Milne, Vincent C Emery, Andrew K Burroughs

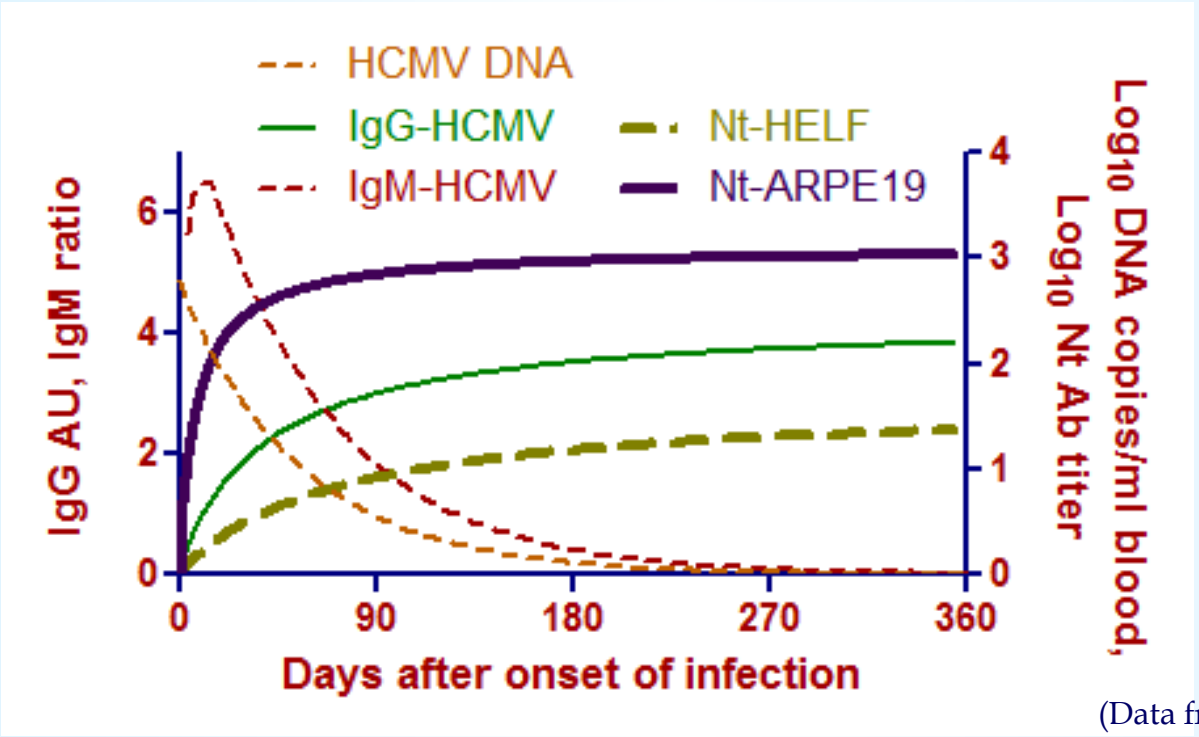
In the seronegative patients with seropositive donors, the duration of viraemia and number of days of ganciclovir treatment were reduced in vaccine recipients.

ntibody response to HCMV: rationale for gH/gL/UL128 UL130/UL131 as a candidate vaccine



Predominance in human sera of the neutralizing activity against endothelial/epithelial cell infection

- Higher titers and earlier appearance of antibodies neutralizing infection of endothelial/epithelial cells as compared to fibroblasts after natural infection (Gerna et al., J Gen Virol 2008) .



(Data from 45 pregnant women with primary HCMV infection)

- The Towne vaccine and the gB/MF59 subunit vaccine induced epithelial entry-specific neutralizing activities that were on average 28-fold (Towne) or 15-fold (gB/MF59) lower than those observed following natural infection (Cui e al., Vaccine 2008).

Characterization the potent neutralizing antibodies that inhibit infection of endothelial/epithelial cells

JOURNAL OF VIROLOGY, Jan. 2010, p. 1005–1013 Vol. 84, No. 2
0022-538X/10/$12.00 doi:10.1128/JVI.01809-09

Isolation of Human Monoclonal Antibodies That Potently Neutralize Human Cytomegalovirus Infection by Targeting Different Epitopes on the gH/gL/UL128-131A Complex▽

Annalisa Macagno,[1]* Nadia L. Bernasconi,[1] Fabrizia Vanzetta,[1] Erica Dander,[1] Antonella Sarasini,[2] Maria Grazia Revello,[2] Giuseppe Gerna,[2] Federica Sallusto,[1] and Antonio Lanzavecchia[1]*

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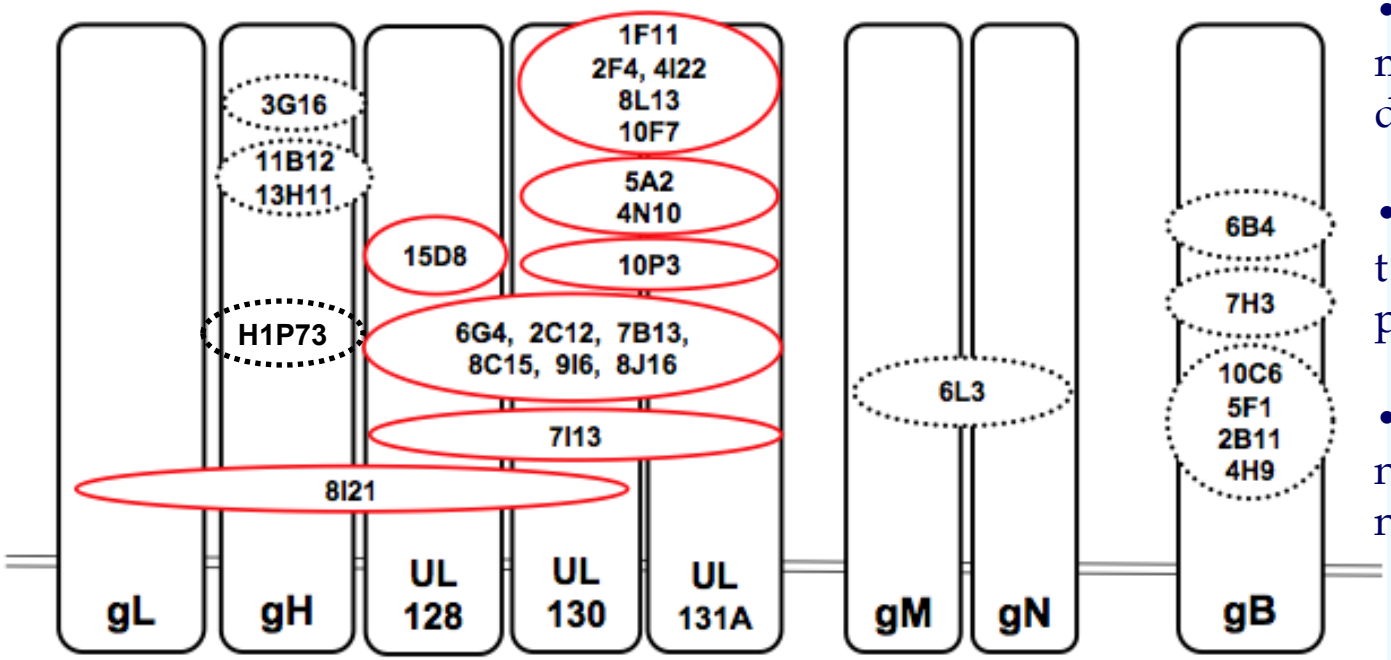
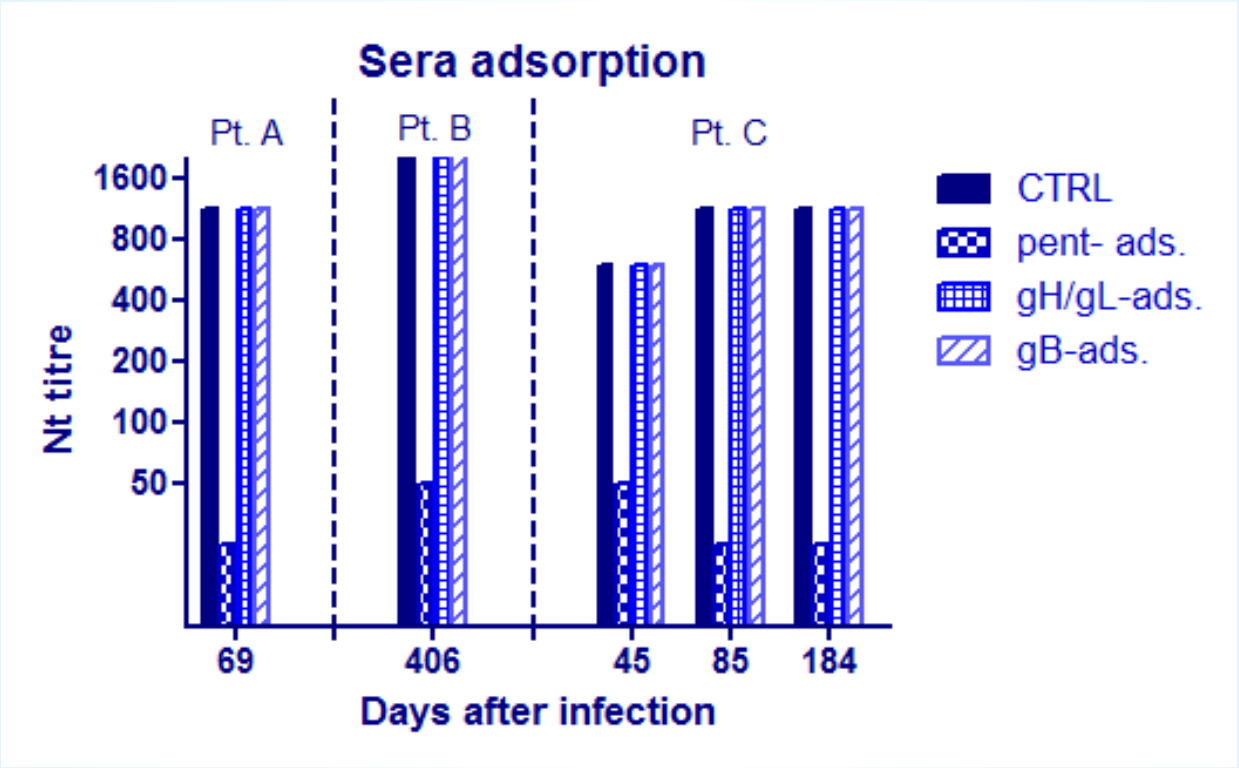


- Each mAb or group of mAbs in circle define distinct antigenic sites.
- Red circles: potent mAbs that neutralize in the picomolar range.
- Dotted circles: mAbs that neutralize in the nanomolar range.

Antibodies anti-gH/gL/UL128-131 and neutralization of epithelial cell infection



•Adsorption of sera with the pentamer complex nearly abolishes ARPE infection neutralizing activity.

•The gB/MF59 subunit vaccine induced epithelial entry-specific neutralizing activities that were on average 15-fold lower than those observed following natural infection (Cui e al., Vaccine 2008).

Antibodies anti-gH/gL/UL128-131 and neutralization of epithelial cell infection

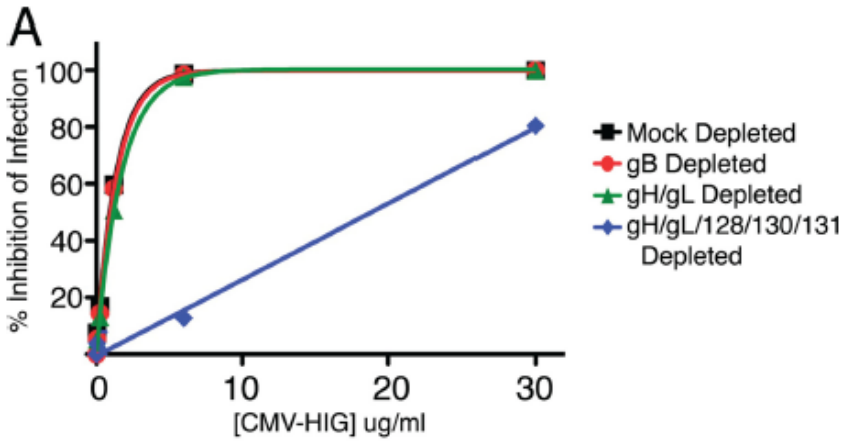
Journal of Virology p. 7444–7447 July 2012 Volume 86 Number 13

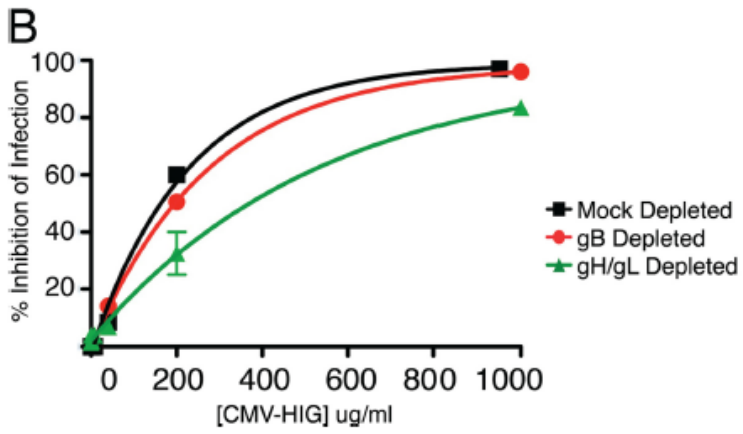
Antibodies against the gH/gL/UL128/UL130/UL131 Complex Comprise the Majority of the Anti-Cytomegalovirus (Anti-CMV) Neutralizing Antibody Response in CMV Hyperimmune Globulin

Ashley E. Fouts,[a] Pamela Chan,[b] Jean-Philippe Stephan,[c] Richard Vandlen,[c] and Becket Feierbach[a]

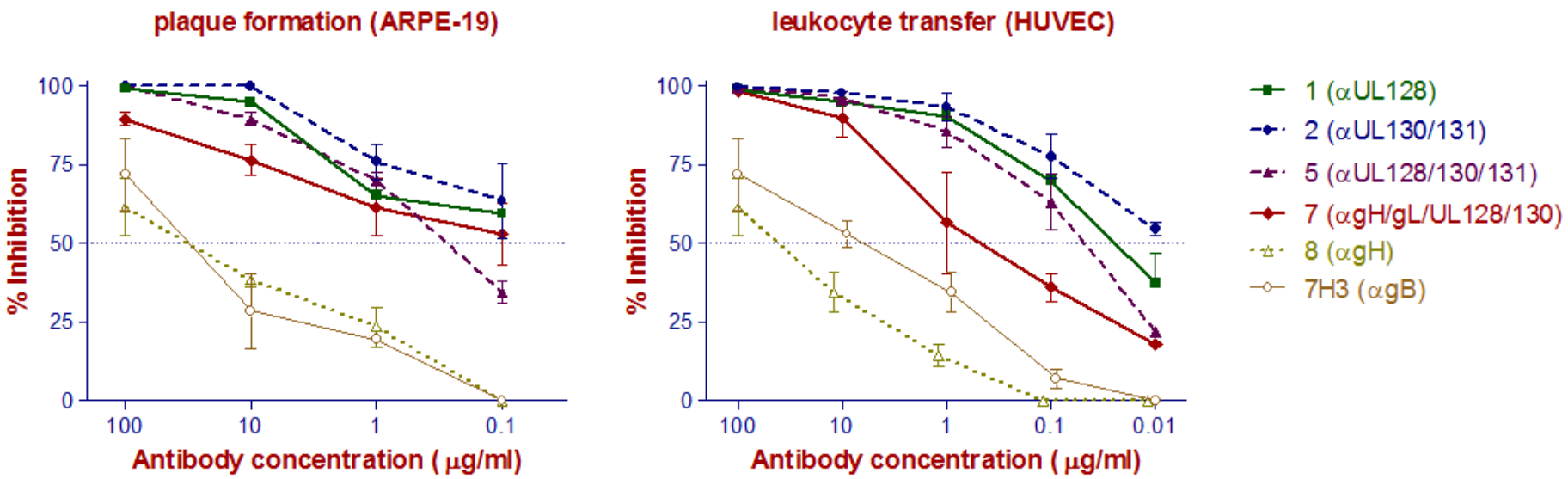
Departments of Infectious Diseases,[a] Biochemical and Cellular Pharmacology,[b] and Protein Chemistry,[c] Genentech, Inc., South San Francisco, California, USA

Anti-cytomegalovirus (anti-CMV) hyperimmune globulin (HIG) has demonstrated efficacy in preventing CMV disease in solid-organ transplant patients as well as congenital disease when administered to pregnant women. To identify the neutralizing component of cytomegalovirus hyperimmune globulin (CMV-HIG), we performed serial depletions of CMV-HIG on cell-surface-expressed CMV antigens as well as purified antigens. Using this approach, we demonstrate that the major neutralizing antibody response is directed at the gH/gL/UL128/UL130/UL131 complex, suggesting little role for anti-gB antibodies in CMV-HIG neutralization.





Inhibition of cell-to-cell HCMV spreading in epithelial cells and virus transfer to leukocytes



mAbs to gHgLpUL128-131 block the routes of virus dissemination

Antibody response to HCMV glycoproteins

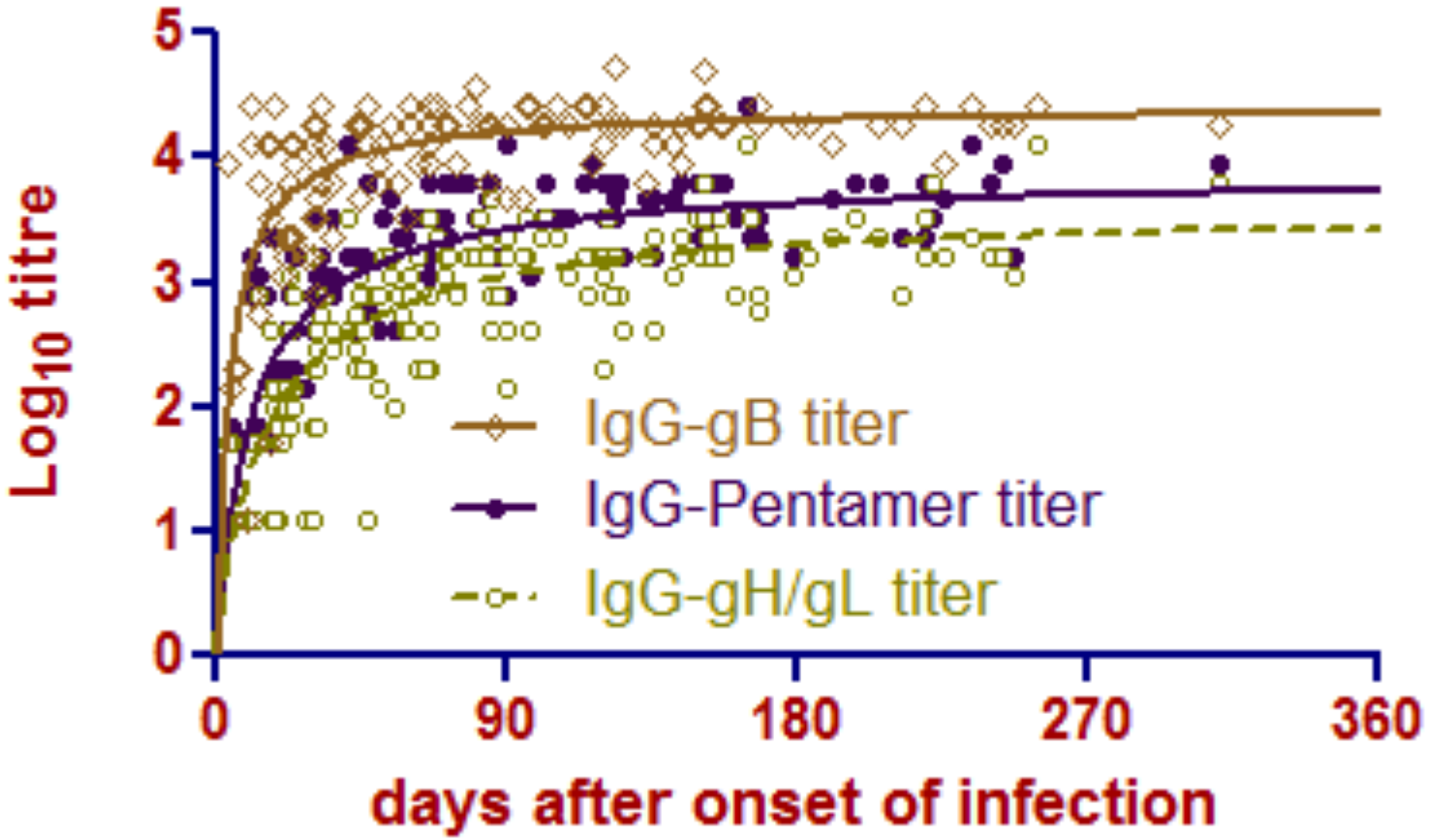
Analysis of the antibody response to:

- **gB**
- **gH/gL**
- **gH/gL/UL128-UL131**

during primary infection of 23 pregnant women

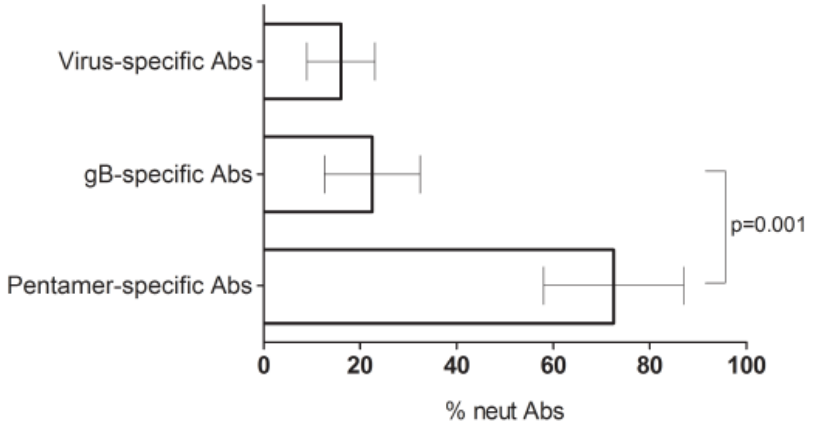
-11 non-transmitting the infection to the fetus
-12 transmitting the infection to the fetus

Development antibodies to the HCMV glycoprotein complexes after primary infection



Neutralizing vs non-neutralizing antibodies elicited by HCMV glycoproteins after natural infection

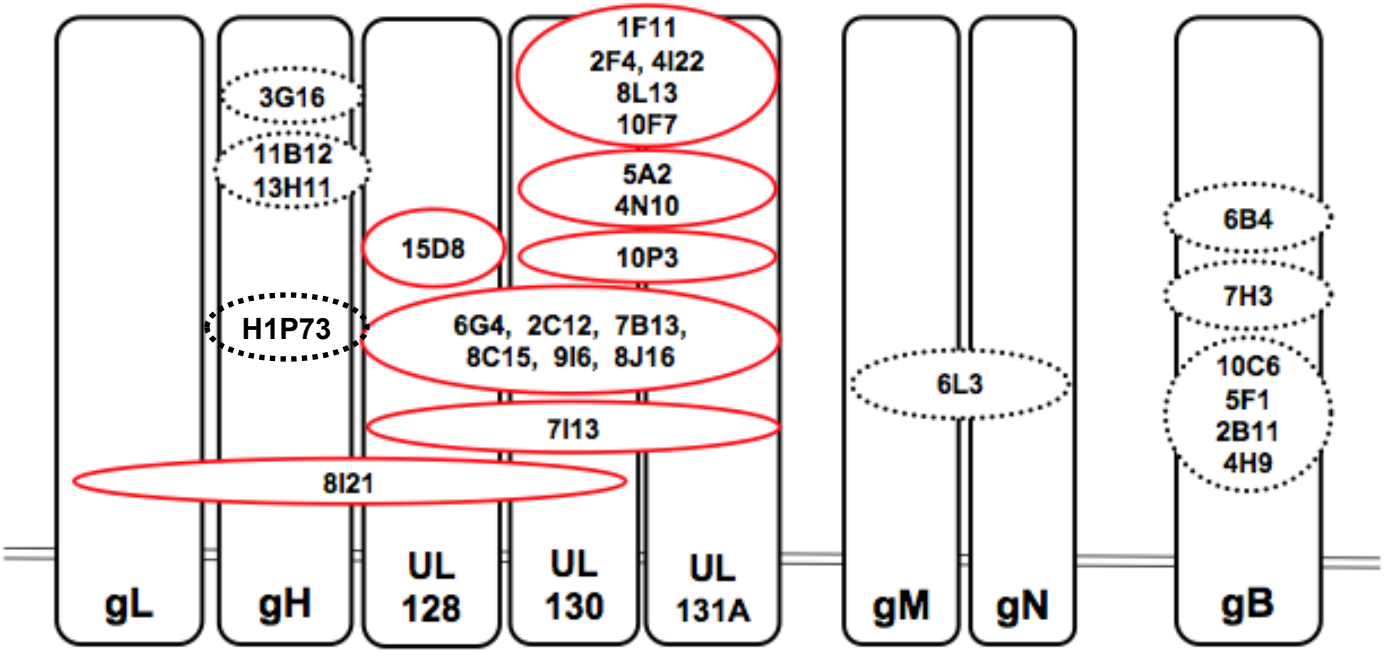
a



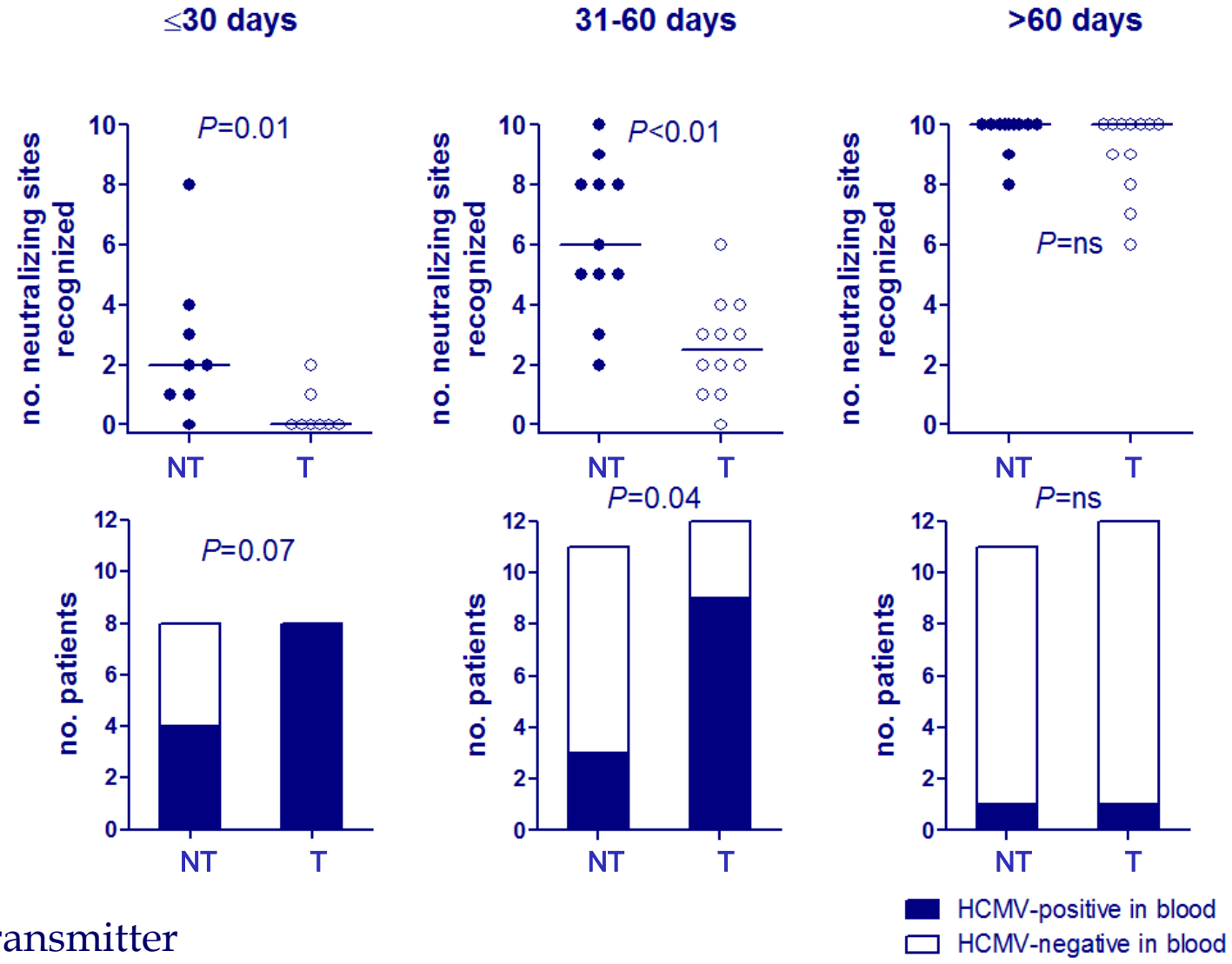
Antibody specificity		neutralizing Abs / non-neutralizing Abs	% neutralizing Abs
virus, all antigens	patient 1	6 / 49	11%
	patient 2	7 / 54	11%
	patient 3	32 / 90	26%
	patient 4	7 / 38	16%
gB	patient 1	2 / 11	15%
	patient 2	3 / 14	18%
	patient 3	8 / 32	20%
	patient 4	3 / 5	37%
gHgLpUL	patient 1	6 / 5	55%
	patient 2	12 / 3	80%
	patient 3	29 / 4	87%
	patient 4	4 / 2	67%

Most gH/gL/pUL128-131-specific antibodies are neutralizing, whereas most gB-specific antibodies are non-neutralizing

Dissection of the antibody response to gH/gL/ pUL128-131 during primary infection



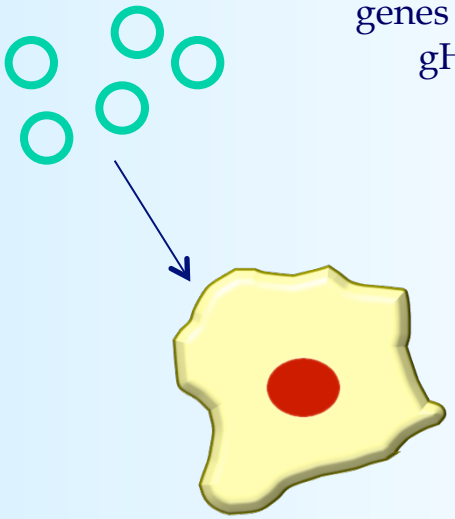
Breadth of the neutralizing response to gH/gL/ UL128L and HCMV transmission to the fetus

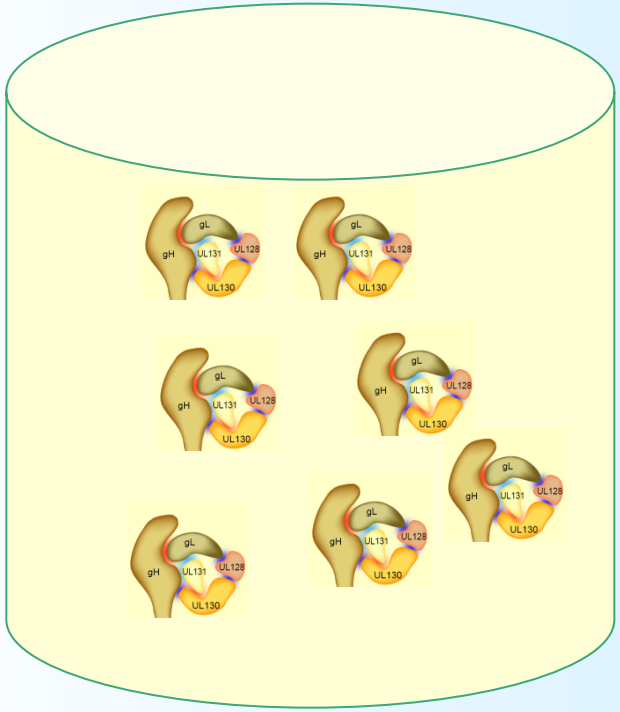


NT, non-transmitter
T, transmitter

Production of gHgLpUL128L (and gB and gHgL) for mice immunization

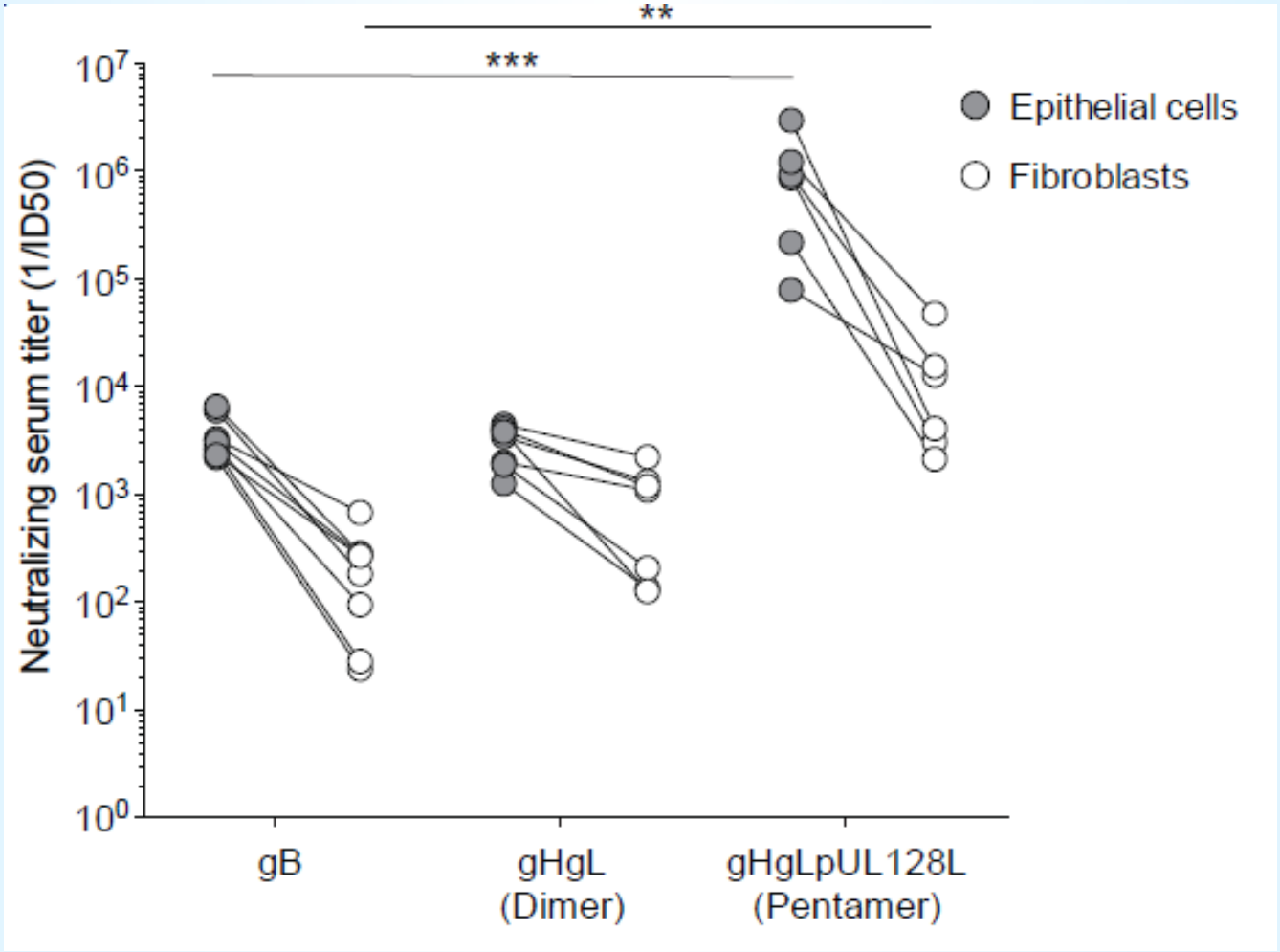
Transfection of HEK293 cells with DNA encoding for the 5 genes of the pentamer gHgLpUL128L





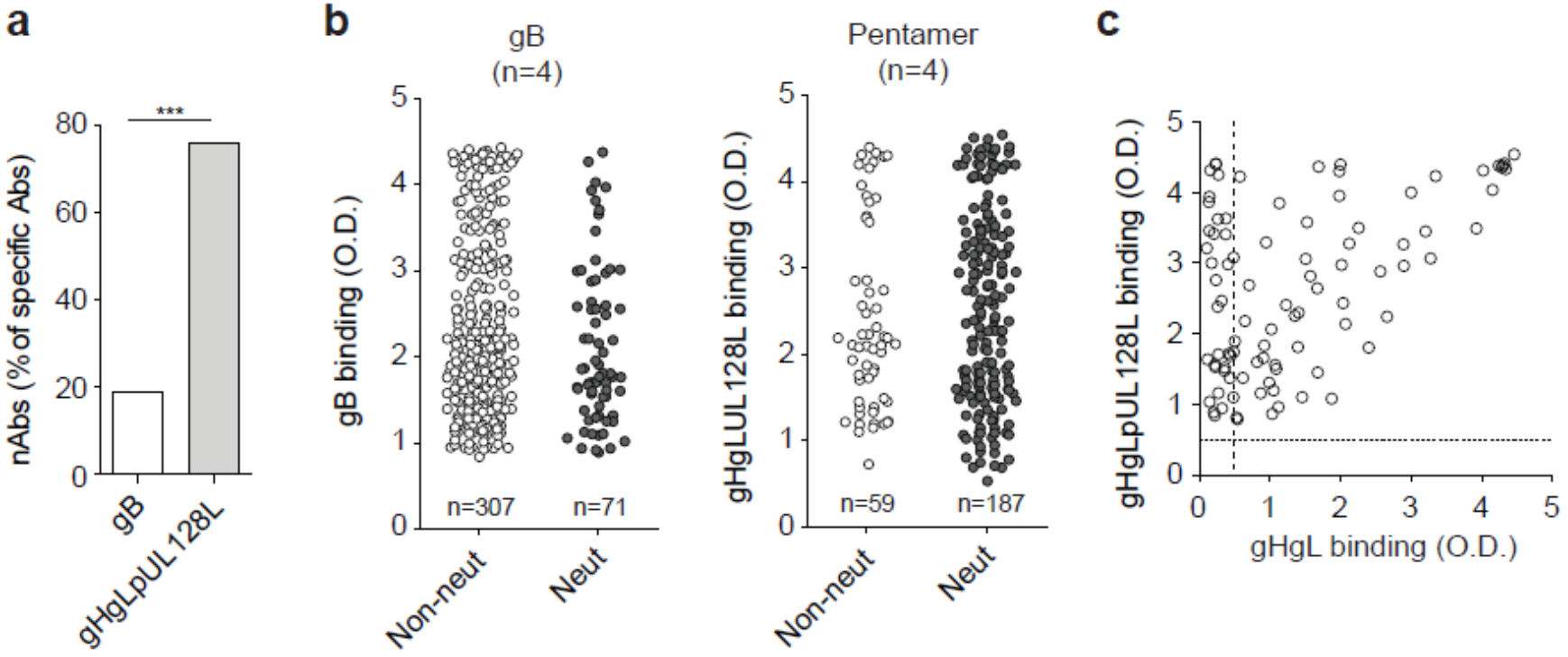
Synthesis of the pentamer gHgLpUL128L by the transfected cells

Neutralizing titers after mice immunization with soluble HCMV glycoproteins (+Carbopol)



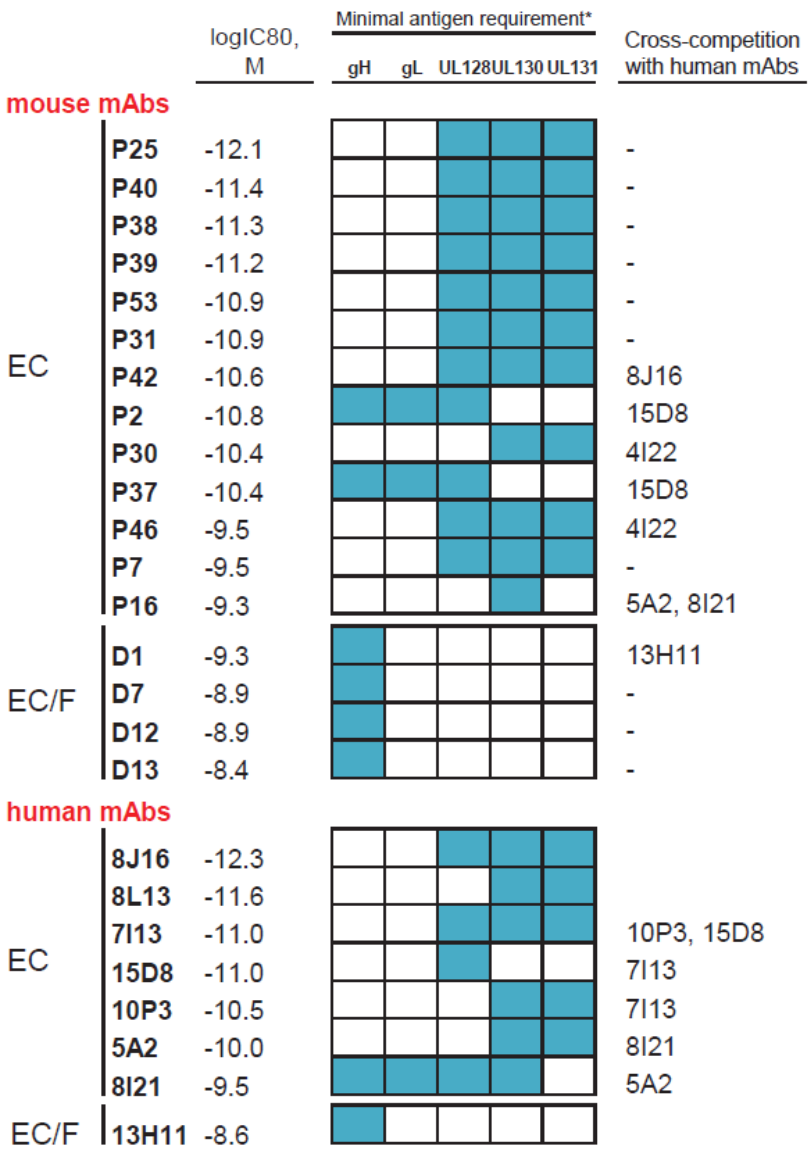
Higher neutralizing titers are observed after immunization with gHgLpUL128L pentamer.

Characterization of monoclonal antibodies from gB- and gHgLpUL128L-immunized mice



As observed after natural infection of humans, most gH/gL/pUL128-131-specific antibodies are neutralizing, whereas most gB-specific antibodies are non-neutralizing

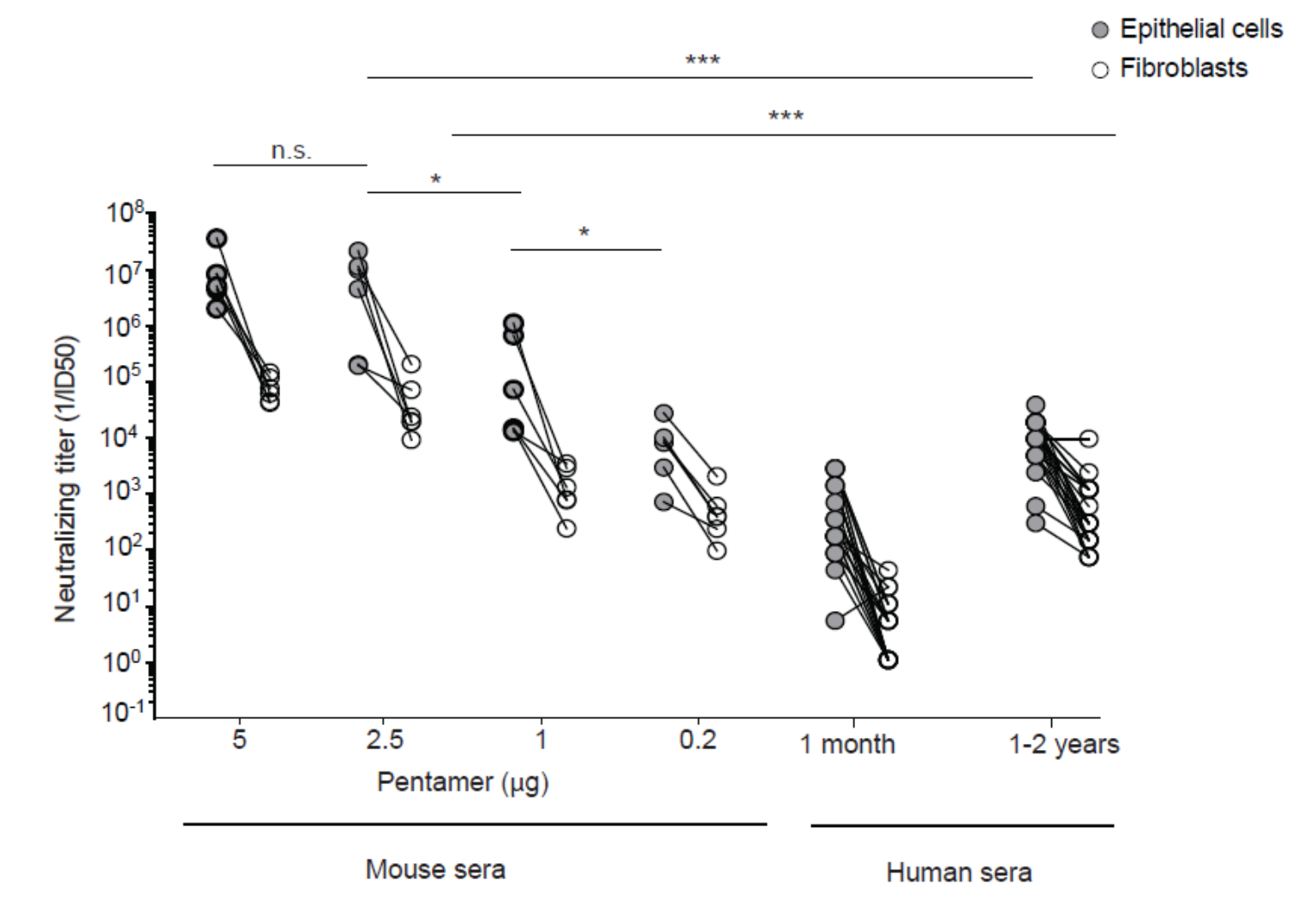
Characterization of monoclonal antibodies from gHgLpUL128L-immunized mice



Mouse mAbs show the same potency of human mAbs, and target previosly described and additional sites.

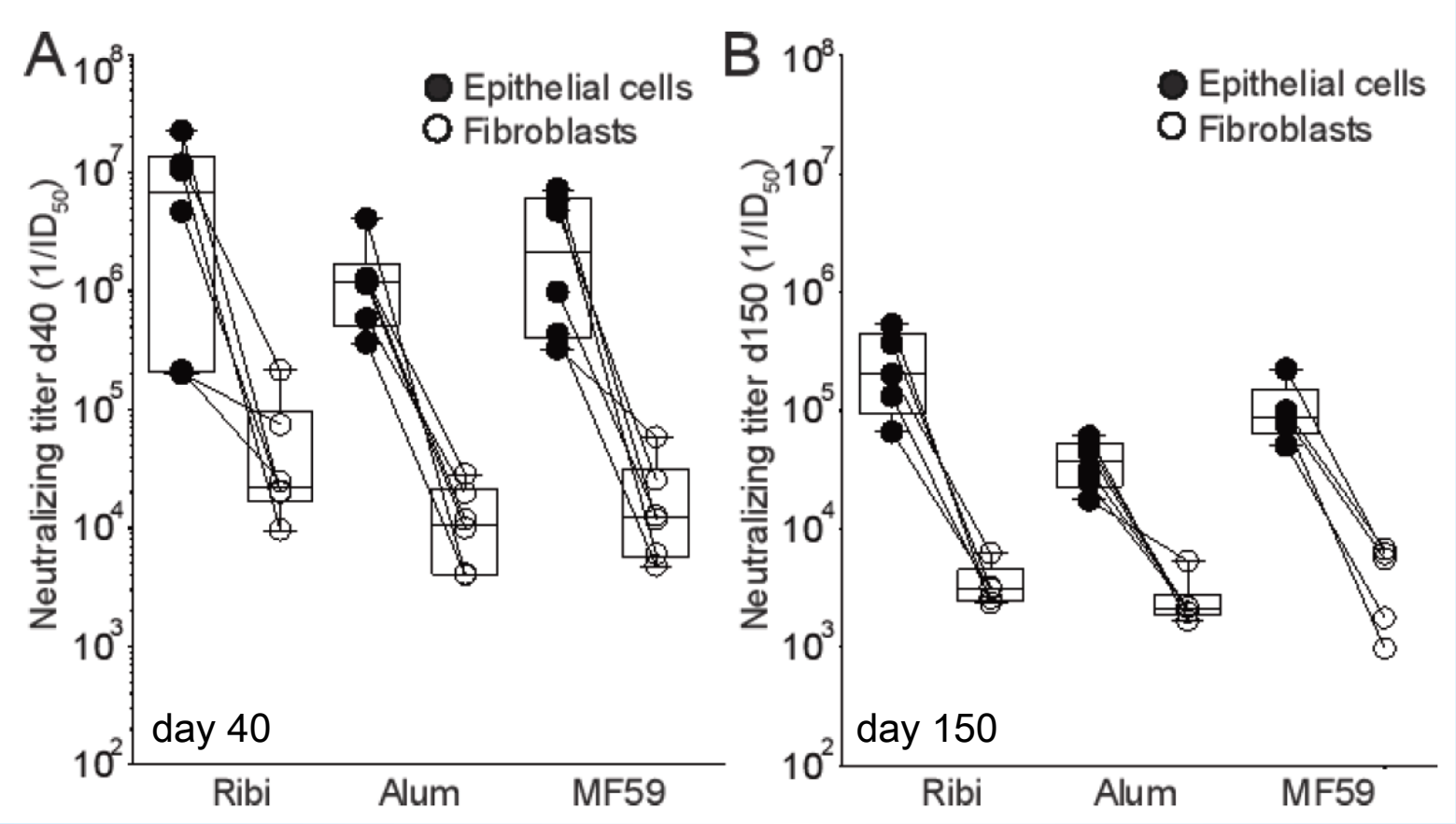
The analysis performed at the clonal level reveal a striking parallel between the antibody response induced by natural infection and immunization of mice with recombinant proteins.

Neutralizing titers in mice immunized with of gHgLpUL128L pentamer (+Ribi) produced in stably transfected CHO cells.



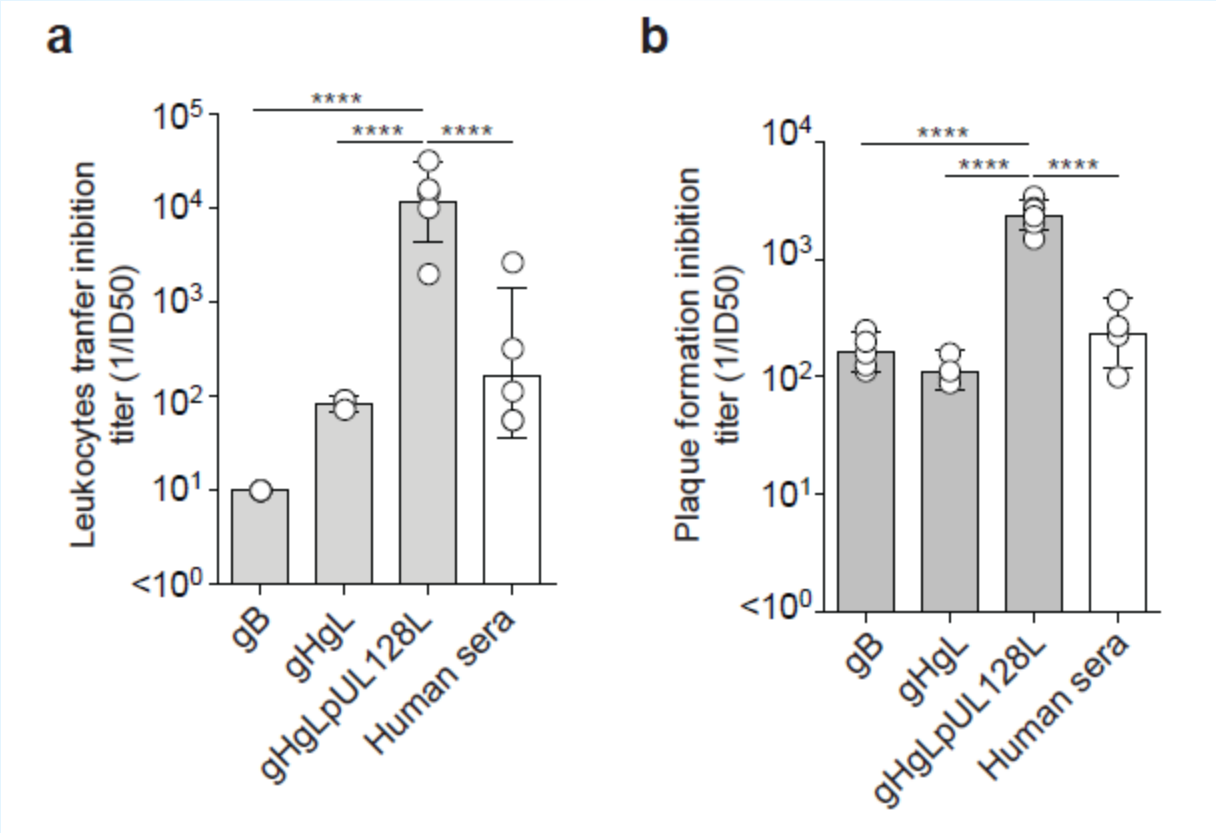
The neutralizing titers *vs* epithelial cells and fibroblasts elicited by the 5 and 2.5 µg dose were higher that those found in the sera of donors early and late after HCMV infection.

Magnitude and persistence of neutralizing titers in mice immunized with gHgLpUL128L pentamer formulated with three adjuvants approved for human use.



The three adjuvants were comparable in eliciting high serum neutralizing titers. High neutralizing titers are present 5 months after immunization.

Dissemination inhibiting activities of the antibodies induced by the gHgLpUL128L pentamer vaccine.



Sera from pentamer-immunized mice displayed a high potency in blocking plaque formation (i.e. cell-to-cell virus spreading) as well as virus transfer from infected endothelial cells to leukocytes.

Conclusions

- Novel approach to vaccine design: analysis of the human neutralizing antibody response to the pathogen to identify and formulate an antigen capable of eliciting the most effective antibody response.

- A soluble gHgLpUL128L pentameric complex elicited an antibody response that potently neutralizes HCMV infection of all the known cellular targets.

- The elicited antibody response showed a potential activity in blocking virus dissemination *in vivo* (i.e. inhibition *in vitro* of HCMV spreading in epithelial cells and transfer from endothelial cells to leukocytes).

- Pentamer-based vaccines eliciting antibodies limiting the spreading of either cell-free and cell-associated virus, may control HCMV infection in immunocompromised patients or provide sterilizing immunity in healthy individuals.

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