NOVEL ANTIGEN ELICITS BROADLY NEUTRALIZING ANTI-HIV MONOCLONAL ANTIBODIES IN MICE

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ABSTRACT

Background: Although broadly neutralizing HIV antibodies are detected in infected (and sometimes uninfected) humans, immunization with HIV-1 antigens generally do not elicit broadly neutralizing antibodies. Clinical studies found that Human Pegivirus (HPgV; previously known as GBV-C or HGV) E2 antibody is associated with improved survival in HIV and HPgV co-infected individuals, and envelope protein (E2) antibody neutralizes HIV-1 infectivity by reducing virus entry *in vitro*. To determine if HPgV E2 elicits broadly neutralizing HIV monoclonal antibodies (MAbs), mice were immunized with E2 (lacking C-terminal transmembrane domain) or a synthetic peptide we previously demonstrated is involved in HIV entry and generated anti-E2 MAbs against both antigens. The interactions of these MAbs were examined for HIV-1 infectivity inhibition, HIV-1 particle precipitation, and HIV-1 protein binding.

Methods: HPgV E2 protein was expressed in CHO cells, and a 17 amino acid synthetic peptide previously shown to inhibit HIV entry was generated. Mice were immunized with 25 ug E2 protein or peptide in IFA four times prior to sacrifice, and hybridomas generated. One anti-E2 and one anti-peptide hybridoma cell line (8H2 and 1C4 respectively) were identified from more than 2,000 independent cultures using a capture E2 ELISA. HIV-1 envelope proteins (gp120, gp140 and gp41), gp41 peptides (MPER and T-20), and X4- and R5-tropic HIV-1 isolates representing clades A, B, and D were studied.

Results: Both HPgV and synthetic peptide E2 MAbs precipitated HIV-1 particles and neutralized X4 and R5-tropic HIV isolates from diverse geographic regions representing three clades (IC_{50} ranging from 2.5 to 7.5 ug/mL). These antibodies did not neutralize mumps or yellow fever viruses, demonstrating specificity. 8H2 reacted with HIV-1 gp140 and gp41 proteins, but not gp120 using two types of ELISA methods. Both 8H2 and 1C4 recognized the HIV-1 gp41 fusion (MPER) peptide in immunoblot assays.

Conclusion: Although HIV-1 antigens do not elicit broadly neutralizing HIV-1 antibodies, HPgV (GBV-C) E2 and an E2 peptide elicited HIV-1 neutralizing MAbs in mice. Since HPgV E2 antibodies are associated with prolonged survival in three clinical studies, these data illustrate the potential for a novel antigen to incorporate into HIV-1 vaccine strategies.

INTRODUCTION

The human Pegivirus (HPgV or GB virus C) is a human flavivirus that is transmitted by sexual, blood, and maternal fetal routes. HPgV infects up to one third of humans globally, and thus contributes to the microbiome. The viral RNA genome encodes a single polyprotein which is post-translationally cleaved into structural and nonstructural proteins. The envelope glycoprotein (E2) is the major viral surface protein. HPgV E2 inhibits HIV replication and has been shown to block HIV gp41-mediated fusion *in vitro* (1-5). Persistent HPgV infection can occur; however, individuals who clear viremia do so concurrently with the development of E2 antibodies. Although persistent HPgV viremia is associated the best prolonged survival in HIV-infected people, an association between HPgV E2 antibodies with survival compared to those without viremia or antibody has been described (6,7; Figure 1). We found that HPgV E2 antibodies neutralize HIV infectivity early in infection by interacting with a cellular antigen displayed on HIV particles (3). E2 antibody cross-reacted with HIV gp41 MPER and anti-MPER antibodies cross-reacted with HPgV E2. In addition, peptides within the HPgV E2 region were shown to bind gp41 and inhibit HIV-1 replication. Although several commercial E2 MAbs and an E2 antibody detection kit were described previously, these reagents are no longer available. A MAb generated against E2 (8H2), against a 17mer E2 peptide (1C4) were generated and characterized for their ability to interact with HIV-1 proteins and inhibit HIV-1 infectivity.

Survival Curves for HPgV RNA and E2 antibody vs. neither

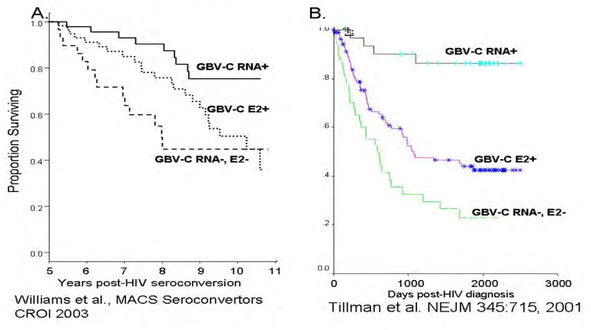


Figure 1. Survival in HIV-infected individuals with persistent HPgV viremia (RNA+), without viremia with anti-HPgV E2 antibodies (HPgV E2+) or without (HPgV RNA-, E2-). Survival was best in those with HPgV viremia. However, among those without viremia, HPgV E2 antibody was associated with prolonged survival.

RESULTS

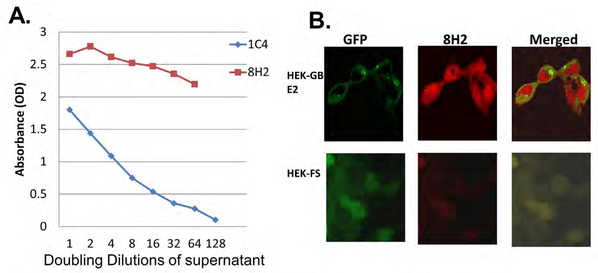


Figure 2. HPgV 8H2 E2 MAbs react with E2 protein in ELISA and IFA experiments. *Panel A:* 8H2 and 1C4 culture supernatants reacted with recombinant HPgV E2 protein by ELISA, and 8H2 reacted specifically with recombinant E2 in Cho cells expressing E2 by confocal microscopy. The negative control cells transcribe HPgV RNA encoding E2 but with a frame-shift introduced (FS) (8). Both constructs express GFP on the same mRNA with translation directed by the EMC IRES. Note: 1C4 reacted with E2 by western blot (not shown), and 8H2 did not react with western blot, indicating that it recognizes a conformational epitope on E2.

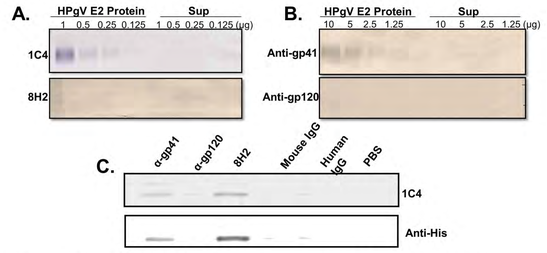


Figure 3 . A murine HPgV E2 MAb and anti-HIV gp41 MAb interact with recombinant HPgV E2 protein (rE2). *Panel A:* Both 8H2 and 1C4 anti-E2 MAbs reacted with HPgV E2 protein (His-tagged) in cell culture supernatant in immunoblots. Cell culture supernatant from cells not expressing E2 served as control. The protein content = total supernatant protein content. *Panel B:* HPgV E2 protein was also bound by 2F5 (anti-gp41 Mab), but not by an anti-gp120 Mab (2G12). *Panel C:* rHPgV E2 (His tagged; 10µg) was mixed with antibodies illustrated (5µg/ml), and following incubation with pansorbin, E2 was precipitated by the Mabs illustrated, but not anti-gp120 or IgG controls. 1C4 and anti-His detected rE2 after precipitation.

Figure 4. The HPgV rE2 elicited MAb 8H2 reacts with HIV-1 gp41. *Panel A:* HIV gp41 (starting concentration 5µg/ml) obtained from the NIH AIDS reference reagent program reacted with the anti-HPgV E2 MAb (8H2) in ELISA, but not with gp120 or gp140. *Panel B:* 8H2 MAb bound the HIV glycoproteins and HPgV E2 in immunodot blot assays, and HPgV E2 MAb 8H2 and 1C4 (5µg/ml) as shown in panel B. Proteins on blot represent 1ug protein. The two HPgV E2 proteins were expressed in either CHO-S (#1) or Jurkat Tet-off (#2) cells as described.

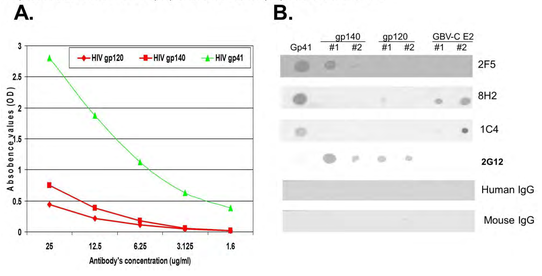


Figure 5. The converse – HPgV E2 MAb (8H2) reacts with the gp41 MPER peptide. *Panel A:* HPgV 8H2 and 1C4 anti-E2 MAbs and anti-gp41 MAb (2F5) reacted with gp41MPER. Anti-gp120 did not in ELISAs. *Panel B:* HIV (2F5, 2G12) and HPgV (8H2 and 1C4) anti-E2 MAbs reactivity with HIV (MPER/T-20), and HPgV (M6, NS5a) peptides in an immunodot blot assay. Isotype IgGs served as the controls. All antibody concentrations were 5ug/ml. M6 (E2 17mer peptide [5]), and NS5A 14mer peptide (10) were synthesized.

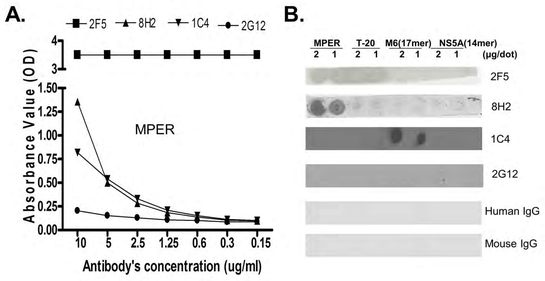


Figure 6. 8H2 and anti-HIV-1 gp41 HumAb precipates HPgV E2 protein. HPgV rE2 (10ug/mL) was incubated with anti-gp41 (F240), anti-gp120 (2G12) or anti-HPgV E2 with His tag (8H2) . Antibody concentrations were 10µg . IgG was precipitated with pansorben, and bound E2 detected with 1C4 anti-HPgV E2 antipeptide antibody or anti-His in western blots. Human and mouse IgG1 served as the controls.

Figure 7. HPgV anti-E2 MAb (8H2) precipitates HIV particles and neutralizes HIV *in vitro*. *Panel A*: MAb 8H2 was mixed with HIV particles generated in MT-2 cells were precipitated with Pansorbin as described (3). * = p< 0.01, IC = Isotype control IgG. *Panel B*: MAb 8H2 (10 mg/mL) neutralized > 80% infectivity for 5 different HIV strains representing clades A (#1650), B(#1073, #2969), D (#2521), and Fuzeon resistant isolates (#9488). Data represent the percent reduction compared to neutralization with isotype control antibody. 8H2 did not inhibit mumps or YFV replication.

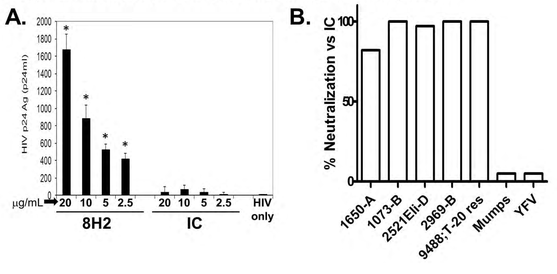
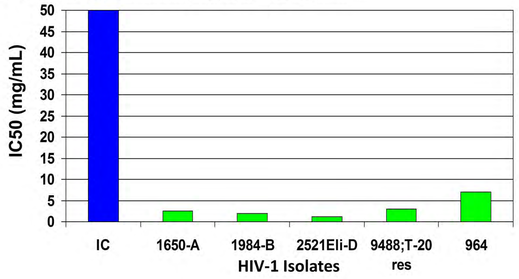


Figure 8. The HIV neutralization inhibitory concentration (IC_{50}) of MAb 8H2 IgG is shown for the 5 HIV-1 isolates in replication competent MT-2 cell culture. The IC_{50} of a previously described murine anti-HPgV-C E2 MAb is shown for comparison (3). The isotype control antibody IC_{50} was > 50 µg/mL.



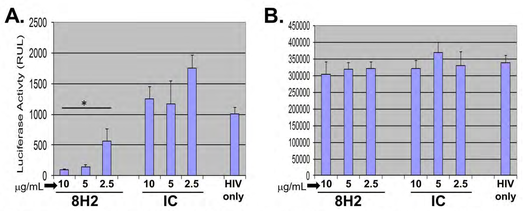


Figure 9. 8H2 MAb inhibits HIVpp transduction. HIV gp160 pseudotyped retrovirus particles (HIVpp; Panel A) and VSVpp particles (Panel B), were added to cells (10ug, p24) with pre-incubation for 2 hrs at 37 C with either anti-HPgV E2 (8H2) or human IgG1 antibodies prior to addition to CD4+ T cells for 4 days. Cells were lysed and luciferase activity analyzed. Since this is single cycle infection it indicates that antibodies prevent HIV-1 binding and/or entry *: p<0.01 (compared with IgG control)

SUMMARY AND CONCLUSION

1. Two new MAbs were raised against HPgV E2
2. These MAbs react with E2 protein in immunoassays.
3. MAb 1C4 is linear, 8H2 is conformational
3. MAb 8H2 precipitates HIV-1 particles and neutralizes multiple HIV-1 isolates (IC_{50} range 2.5 to 7.5 ug/ml)
4. 8H2 MAb reacts with HIV-1 gp41 protein and MPER
5. 8H2 did not neutralize mumps or YFV (not shown)
6. 8H2 inhibits HIV-1 at an entry stage of replication
7. 8H2 further supports testing HPgV E2 as an immunogen to test for HIV vaccine purposes.
8. This further supports HPgV E2 antigen contribution to recent multivalent, HIV-1 protection by vaccination(8)

ACKNOWLEDGEMENTS

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REFERENCES

1. McLinden JH, et al. J Virol 80:12131-40, 2006
2. Jung S, et al., AIDS 21:645-7, 2007
3. Mohr E, et al., J Immunol 185:4496-505, 2010
4. Xiang J, et al., Virology 430:53-62, 2012
5. Eissmann K, et al., PLoS One 8:e54452, 2013
6. Tillmann H, et al., N. Engl. J. Med. 345:715-724, 2001
7. Williams C., et al., N. Engl. J. Med. 350:981-90, 2004
8. Bhattarai N, et al., J Immunol 190:6351-9, 2012
9. Malherbe DC, et al., J Virol 92:e01092-17, 2018