

Session # 45
Poster # 261-T

Protection in vitro among HIV exposed uninfected

Rohan John, Silvio Arango-Jaramillo, and David H. Schwartz.

Department of Molecular Microbiology and Immunology,
The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205.

D. H. Schwartz, MD, PhD
Bloomberg School of Public Health
Dept. of Mol. Micro. & Immunol.
615 North Wolfe Street
Baltimore, MD 21205-2179
Phone: (410) 955-3175
Fax: (410) 955-0105
e mail: dschwartz@jhsph.edu

Introduction

Certain individuals remain uninfected with HIV despite repeated exposures to the virus. A variety of antiviral mechanisms have been shown in some exposed uninfected (EU) individuals. Innate factors include reduced expression of CCR5 and elevated β -chemokine secretion. Acquired MHC Class I restricted immunity and neutralizing antibodies have also been detected. The basis of in vivo resistance is, however, often unknown, and is likely heterogeneous among such a population. Initial innate relative resistance could allow immunizing exposures to progressively enhance specific acquired resistance.

Our published in vitro challenge assay of PBMCs from EUs, showing relative resistance to R5 HIV-1_{BaL} (JID 2000; 181: 897-903), is part of a conflicting literature. This might reflect the fact that in vivo, resistance of EUs is incomplete, and in vitro, depends on the infectivity of the challenge inoculum. PBMC cultures contain both HIV target and antiviral cells, and the outcome of in vitro challenge would depend on their relative susceptibility and antiviral activity.

Specific Aims

Our goal was to generate preliminary data on the magnitude and breadth of antiviral responses in EUs.

We hypothesized that :

- 1) in the absence of the homozygous Δ C32 mutation, increasing R5 viral challenge dose would cause breakthrough infections;
- 2) in vitro resistance would partially cross clades, due to broadly shared epitopes and/or dependence on factors affecting CCR5 (e.g., chemokines).

Methods

Subjects: Individuals with high-risk behavior (for > 2years) including IV drug use, multiple sex partners, and prostitution, were recruited. Those remaining seronegative over 3-4 years of follow-up were studied for in vitro resistance by a challenge assay. Controls consisted of low risk seronegative volunteers whose PBMCs were processed and stored during the same time that the EU samples were collected.

Assay: Cryopreserved PBMCs were thawed, checked for cell viability (>90%), and incubated for approximately 3 and a-half days in RPMI + 10% FCS, with α -CD3 (OKT3, 5 ng/ml) and IL-2 (2 U/ml). Replicate wells (1 x 10⁶ cells per well) were challenged with 3 TCID₅₀ of a local primary R5 clade B isolate (HIV-1_{P27}), and 5 TCID₅₀ of an R5 primary clade E isolate. When cells were available, replicate cultures were further challenged with 50 TCID₅₀ of the clade B isolate and 5 TCID₅₀ of an R5 primary clade C isolate. CD8+ depleted PBMCs (~1% CD8 cells by flow cytometry) were challenged with the low dose of clade B virus. Day 10 supernatants were tested for p24 production by an ELISA (Organon Teknika).

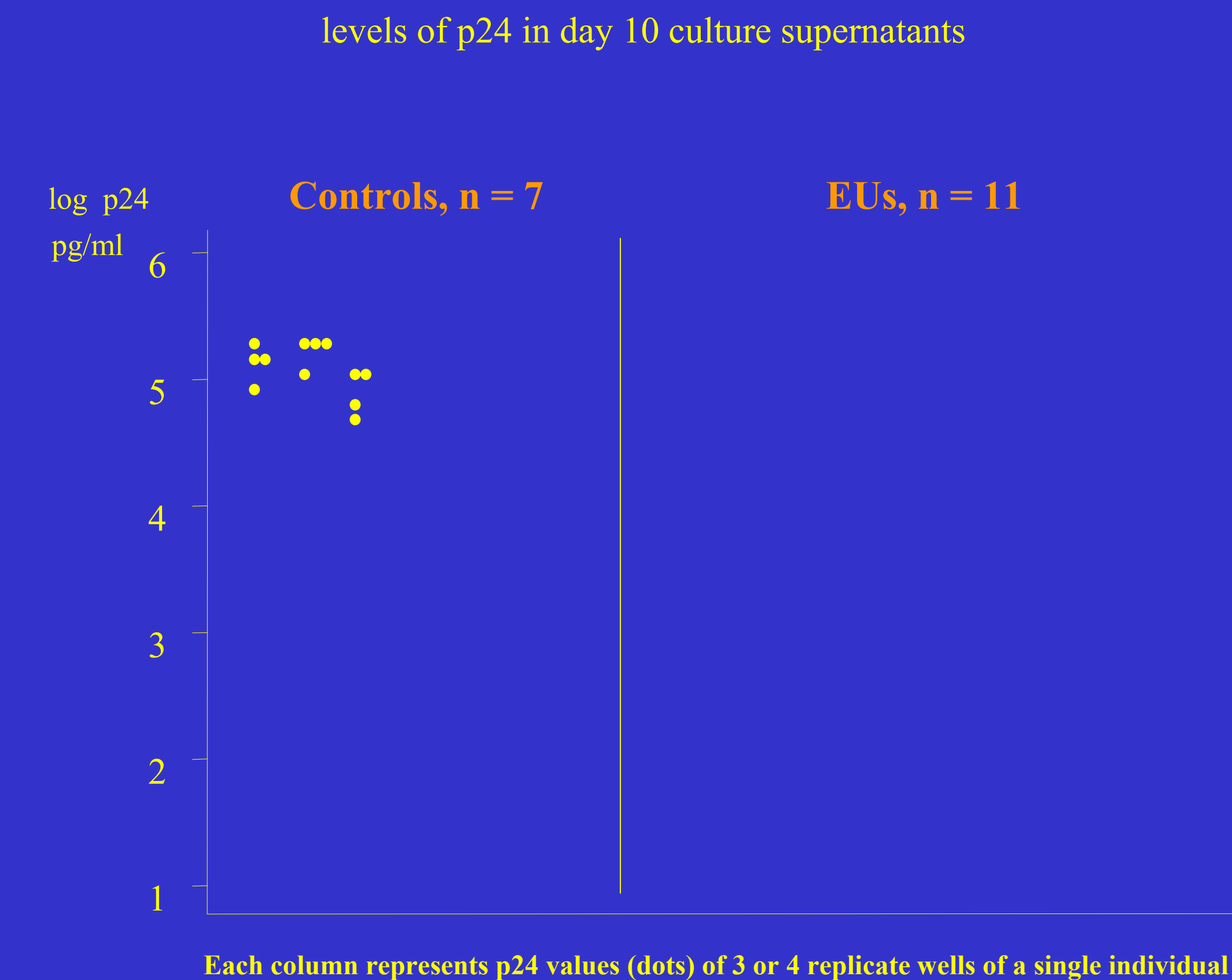
RANTES and MIP-1 β were measured in day of challenge supernatants by an ELISA (R & D Systems).

Cellular activation and proliferation after α -CD3 stimulation was assessed by incorporation of ³[H] thymidine (1 μ Ci/well), added to the wells for the last 6 hours of the 3 and a-half day culture.

Challenge with primary R5 virus from 3 clades – low dose

	susceptible cultures / total no. of cultures		
	Clade B	Clade C	Clade E
Controls <i>n</i> = 20	46 / 76 (60%)	28 / 34 (82%)	54 / 72 (75%)
EUs <i>n</i> = 18	8 / 63 (13%)	14 / 31 (45%)	35 / 63 (56%)
Odds Ratio of Resistance in EUs (95% CI)	8.8 (4.0 - 20.0)	5.7 (1.8 - 18.0)	2.8 (1.3 - 6.0)
<i>p</i> value	< 0.001	0.003	0.007

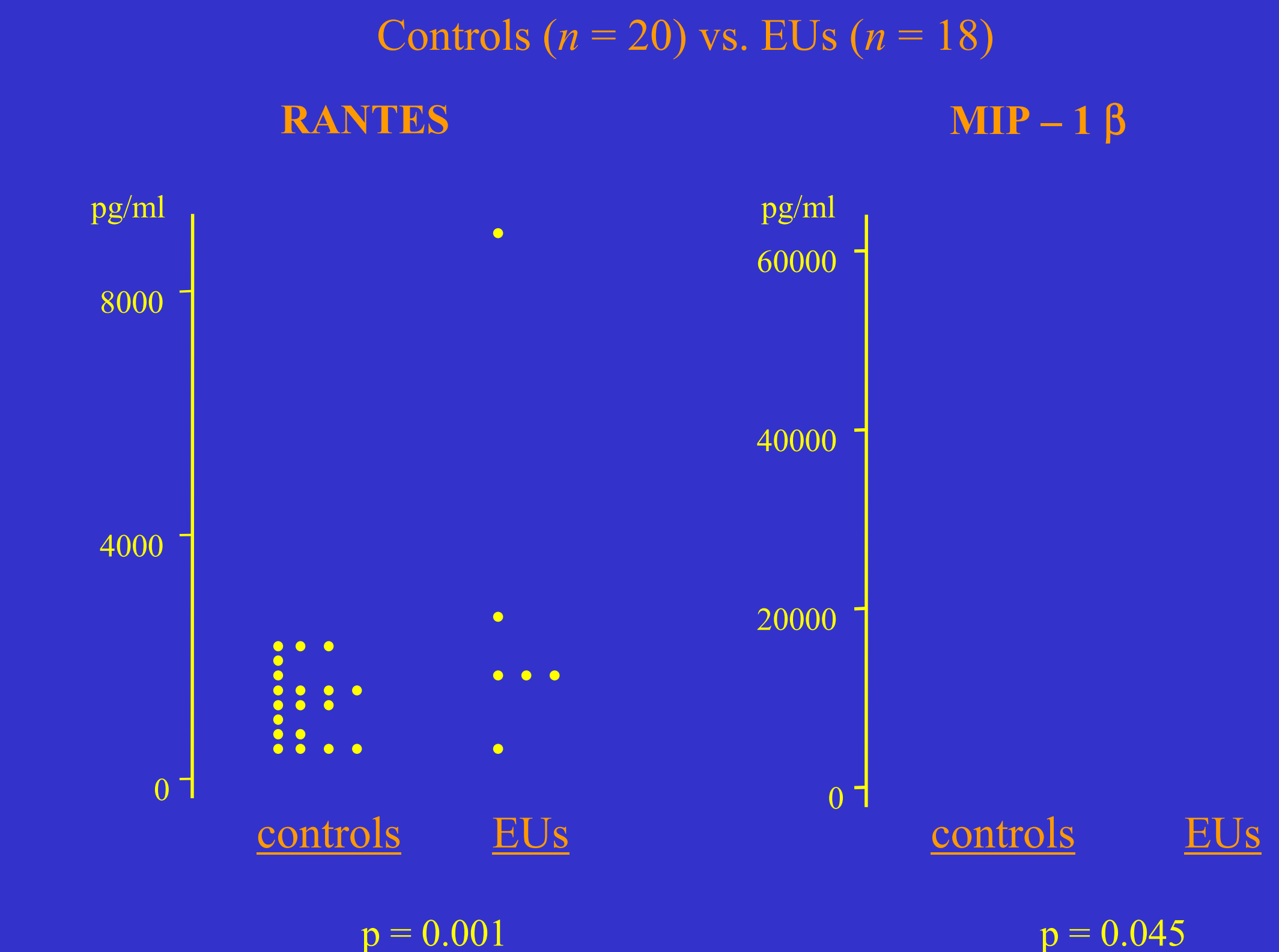
Challenge with primary R5 clade B virus – high dose



Effect of CD8 depletion on susceptibility to clade B in EUs

	susceptible cultures / total no. of cultures	susceptible donors / total no. of donors
Whole PBMCs	8 / 63 (13%)	0 / 18 (0%)
CD8 depleted PBMCs	23 / 42 (55%)	9* / 18 (50%)
* In 9 donors who became susceptible (susceptible cultures / total no. of cultures)		
Whole PBMCs	3 / 31 (10%)	5 / 32 (16%)
CD8 depleted PBMCs	17 / 19 (90%)	6 / 23 (26%)

β -chemokine levels in stimulated PBMC culture supernatants



Results

There was no difference in the level of cellular proliferation after 3 days of α -CD3 stimulation (i.e. day of challenge) between PBMC cultures from EUs and controls (*p*=0.87, *t* test).

Clade B R5 primary isolate virus (HIV-1 P27) at 3 TCID₅₀ infected 46/76 (60%) control cultures, but only 8/63 (13%) EU cultures (*p*<0.001). Estimating from the regression analysis, EUs were approximately 9-fold less susceptible than the low risk controls. At 50 TCID₅₀ of P27 virus, p24 was detected in 28/28 (100%) cultures from 7 controls and 37/42 (90%) cultures from 11 EUs; however, in 6 of these EUs, p24 levels were less than 10% of mean values in the controls.

Challenge with 5 TCID₅₀ of a clade C or E primary R5 isolate gave 28/34 (82%) and 54/72 (75%) positive wells, respectively, in the controls. Among PBMCs from EUs, only 14/31(45%) and 35/63 (56%) cultures were positive for clade C and E (*p* < 0.01 for both). Using the regression analysis, EU PBMCs were approximately 6 and 3-fold less susceptible than control cultures to infection with clade C and E, respectively.

Levels of β -chemokines (RANTES and MIP-1 β) in stimulated PBMC cultures were increased in the EUs as a group vs. controls (*p*<0.05 for both chemokines, Rank Sum test). In 7 of the 18 EUs, amounts of one or both of the chemokines were substantially higher than controls (> mean + 3SD).

CD8 cell depletion abrogated resistance in 9 of 18 EU individuals. Among these nine, culture susceptibility increased from 3 of 31 (10%) wells in the whole PBMCs to 17 of 19 (90%) in the CD8 depleted PBMCs.

Conclusions

- PBMCs from EUs were significantly more resistant (~ 9-fold) than low risk controls to challenge with R5 virus. Decreased cell activation was not responsible for the reduced susceptibility of EU cultures.
- At higher challenge dose of virus, resistance was not absolute.
- CD8 cells were responsible for resistance in half the samples; increased β -chemokines were detected in some EU donors.
- Cross resistance to viruses from clades C and E was observed, but perhaps to a lesser degree than to clade B virus.

EUs exhibit multiple protective mechanisms and a degree of cross-clade resistance. To the extent that cross reactive epitopes are involved, such broadly effective antiviral mechanisms bode well for vaccine efficacy in the setting of low-dose exposures to more than one clade.

Acknowledgements. We thank Eric Zimmerman, Ruval Commendador, Cass Jones, and the other staff of the Johns Hopkins Center for Immunization Research, for assistance with recruitment of study participants, blood collection, and clinical support.