



HIV-1/HCV co-infection: Intraindividual comparison of cellular immune responses against two persistent viruses



Georg M. Lauer^{1,2}, Tam N. Nguyen^{1,2}, Cheryl L. Day^{1,2}, Gregory K. Robbins^{1,2}, Katherine McGowan⁴, Eric S. Rosenberg^{1,2}, Michaela Lucas⁵, Paul Klenerman⁵, Raymond T. Chung³, Bruce D. Walker^{1,2}

¹Partners AIDS Research Center, ²Infectious Disease Division and ³Gastrointestinal Unit, Massachusetts General Hospital and Harvard Medical School, Boston

⁴Infectious Disease Unit, Lemuel Shattuck and Faulkner Hospital, Jamaica Plain, ⁵Nuffield Dept. of Clinical Medicine, John Radcliffe Hospital, Oxford

Georg Lauer
Partners AIDS Research Center
Massachusetts General Hospital East
CNY 149 13th Street
Charlestown MA 02139
glauer@helix.mgh.harvard.edu
(617) 724-7515
(617) 724-9612 fax

Background: Co-infection with HIV and HCV offers the opportunity to compare the human immune response towards 2 different viruses in a single individual. We determined the HLA class I restricted CTL response against HIV and HCV in an ELISPOT assay detecting interferon-gamma secreting cells. The CD4+ T-helper response was assessed using a standard proliferation assay.

Methods: Fresh and/or frozen PBMC from 22 subjects with HIV and HCV co-infection were screened for IFN-gamma secretion by ELISPOT assay. PMBC were incubated with 6 HCV vaccinia constructs covering the whole HCV protein and 4 HIV vaccinia constructs expressing gag, nef, RT, and env genes. Responses were seen as positive if the result for a HIV or HCV specific construct was at least 3-fold the result of cells incubated with a vaccinia construct containing the lac-gene alone. Results are expressed in spot-forming cells (SFC) per 10⁶ PBMC. HCV responses were additionally tested using tetramer analysis. In 17 subjects we also assessed the proliferative response towards 4 HCV proteins and towards HIV p24. Controls consisted of individuals with HIV-1 or HCV mono-infection.

Results: All 22 HIV/HCV co-infected subjects demonstrated responses to HIV vaccinia constructs in the ELISPOT assay, with 12/22 targeting 3 or more. 5/22 subjects demonstrated responses to HCV and all but 1 targeted just 1 of the 6 HCV vaccinia constructs. Cumulative responses to HIV ranged from 140 to > 3000 SFC/10⁶ PBMC with 16/22 having more than 500 SFC/10⁶ PBMC. The 6 subjects recognizing HCV constructs had cumulative responses of maximally 500 SFC/10⁶ PBMC. In the proliferation assay, 9/17 subjects had a significant response to p24 alone, but no response was seen to any of the HCV proteins. In a matched group of HCV mono-infected individuals such a response could be detected in 8/17 individuals (p<0.02).

Conclusions: We describe robust cellular immune responses against HIV-1 in HIV-1/HCV co-infected individuals in different stages and with different courses of HIV-1 infection. In contrast, HCV-specific T-cell responses in the same individuals are absent or at best of low frequency and narrowly directed despite high levels of ongoing HCV replication. These findings suggest that, while both HIV-1 and HCV are in most cases not effectively controlled by the immune system, HCV and HIV-1 might employ distinct mechanisms to evade immune control.

Patient	Sex	Age	CD4 count	ALT	HIV quant	HCV quant	HCV GT	Histology	HIV Tx	HCV Tx
CO-1	M	52	280	121	<50	1334400	1b	ND	HAART	NO
CO-2	M	40	550	126	<50	237440	1a	2 1	HAART	NO
CO-3	M	43	331	68	<400	503820	1a	2-3 1-2	HAART	NO
CO-4	F	45	671	12	<50	26516000	1a	2-3 2	HAART	DC
CO-5	M	41	445	110	<400	467720	1a	3 4	HAART	NO
CO-6	F	35	246	103	9420	118430	2b	2 4	HAART	NO
CO-7	F	56	234	50	<50	POS	3a	ND	HAART	NO
CO-8	M	46	1224	133	88	>1000000	1b	2 1	HAART	NO
CO-9	M	42	140	82	<50	603750	1b	2 1-2	HAART	NO
CO-10	F	31	278	198	251	23000000	1a	ND	HAART	NO
CO-11	M	36	916	121	400	>1000000	3a	2 2	HAART	NO
CO-12	M	43	668	126	<50	779250	1b	2 4	NO	NR
CO-13	M	38	877	186	80	1358300	1a	ND	NO	NO
CO-14	M	43	815	76	<50	793000	1a	2 1	NO	NO
CO-15	F	33	295	162	960	317510	ND	ND	NO	NO
CO-16	M	38	382	72	1910	3059400	1a	ND	NO	NO
CO-17	F	47	318	28	319000	532160	1a	ND	DC	NO
CO-18	F	40	725	39	21300	>1000000	3a	ND	NO	NO
CO-19	M	39	1064	137	94000	POS	ND	ND	NO	NO
CO-20	M	40	308	15	82100	NEG	1a	2 1	DC	ETR
CO-21	F	46	336	17	54	NEG	ND	ND	HAART	ETR
CO-22	M	46	268	50	<50	NEG	ND	ND	HAART	SPONT

Subjects with shaded patient ID were tested in the lympho-proliferative assay

Figure 1a

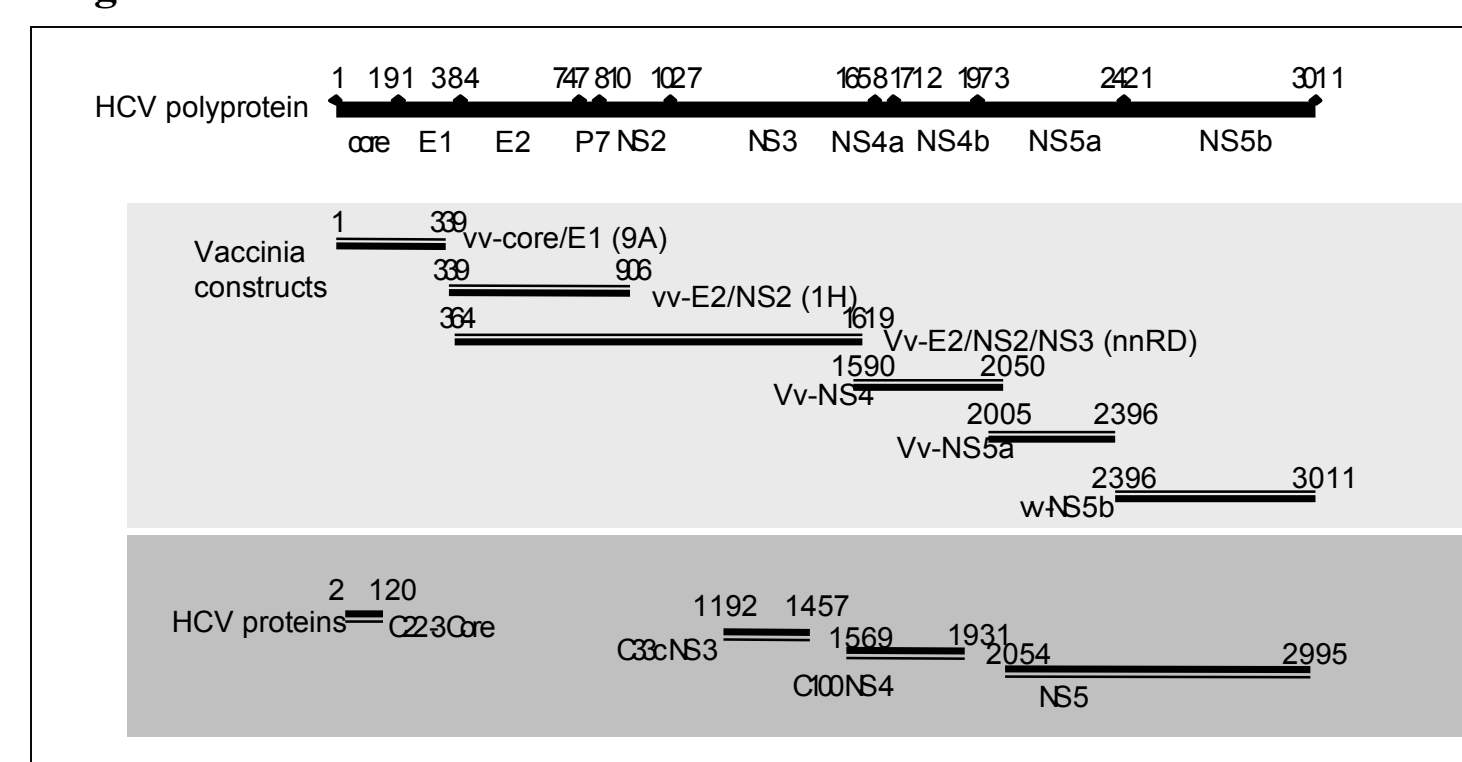
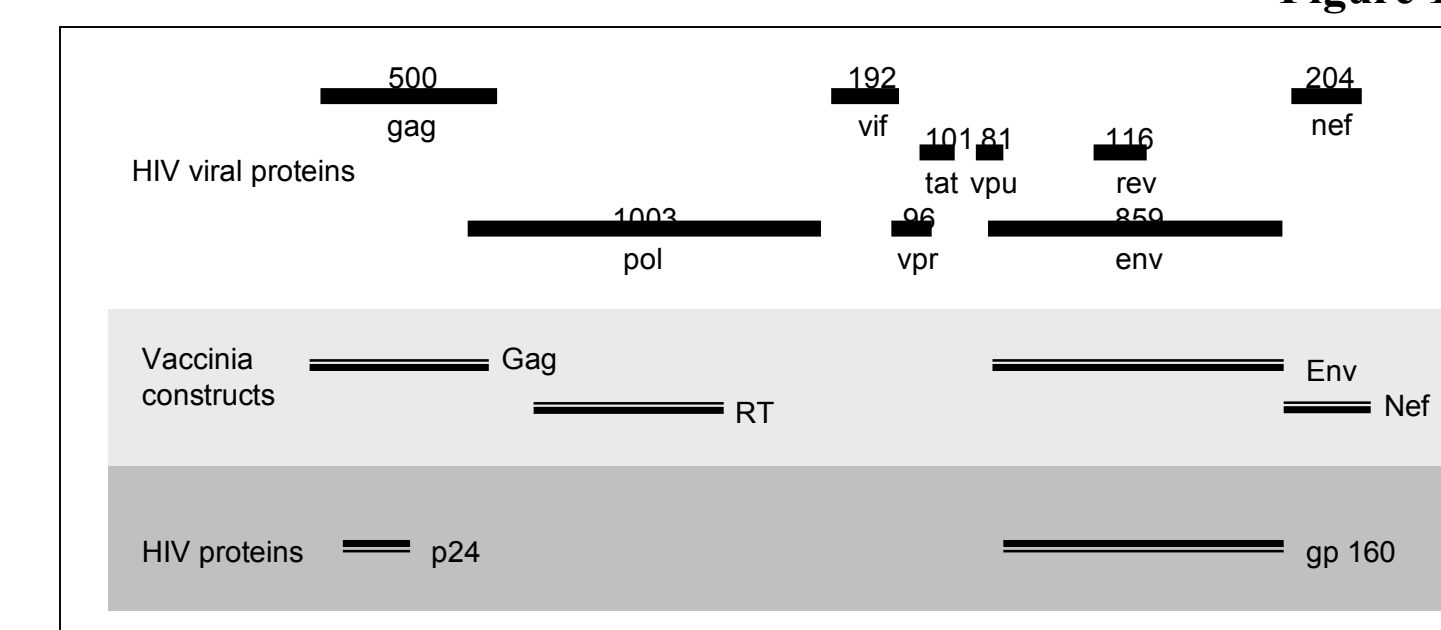


Figure 1b



Introduction:

Co-infection with HIV-1 and HCV is common and of increasing clinical relevance.

Cytotoxic T lymphocytes and CD4+ T helper cells have been shown to play an important role in both HIV-1 and HCV infection:

In HIV-1 infection, the vast majority of persons exhibit detectable CD8+ T lymphocyte responses despite chronic infection. In HCV infection, similar high levels of antigen-specific CD8+ cells can be detected in peripheral blood in some individuals, but especially in chronic infection the overall magnitude and breadth of CD8+ cytotoxic T lymphocyte (CTL) responses is often low.

Persons with chronic HIV-1 infection rarely demonstrate strong HIV-1-specific CD4+ proliferative responses, with the exception of individuals with long-term non-progressive disease. The proliferative response by CD4+ cells upon stimulation with HCV proteins is also weak in individuals with chronic HCV infection, but vigorous CD4+ responses have been described in individuals with spontaneous resolution of infection.

The ability of an individual's immune system to recognize these chronic viral infections simultaneously has not been determined.

Subjects and Methods:

22 individuals with HIV-1/HCV co-infection were studied, with the clinical characteristics outlined in Table 1.

HCV and HIV-1 specific CTL responses were measured in an Elispot assay using recombinant vaccinia viruses expressing all HCV and the major HIV-1 proteins (proteins shown in Figures 1a and 1b). The threshold of significance was established separately for each individual by quadruplicate testing of the response to the control vaccinia vector expressing the *E. coli* beta galactosidase gene product.

HCV CTL responses were also tested using 6 different class I tetramers.

HCV and HIV-1 specific T helper cell responses were measured with a standard proliferation assay using recombinant HCV and HIV-1 proteins (proteins shown in Figures 1a and 1b).

Figure 2

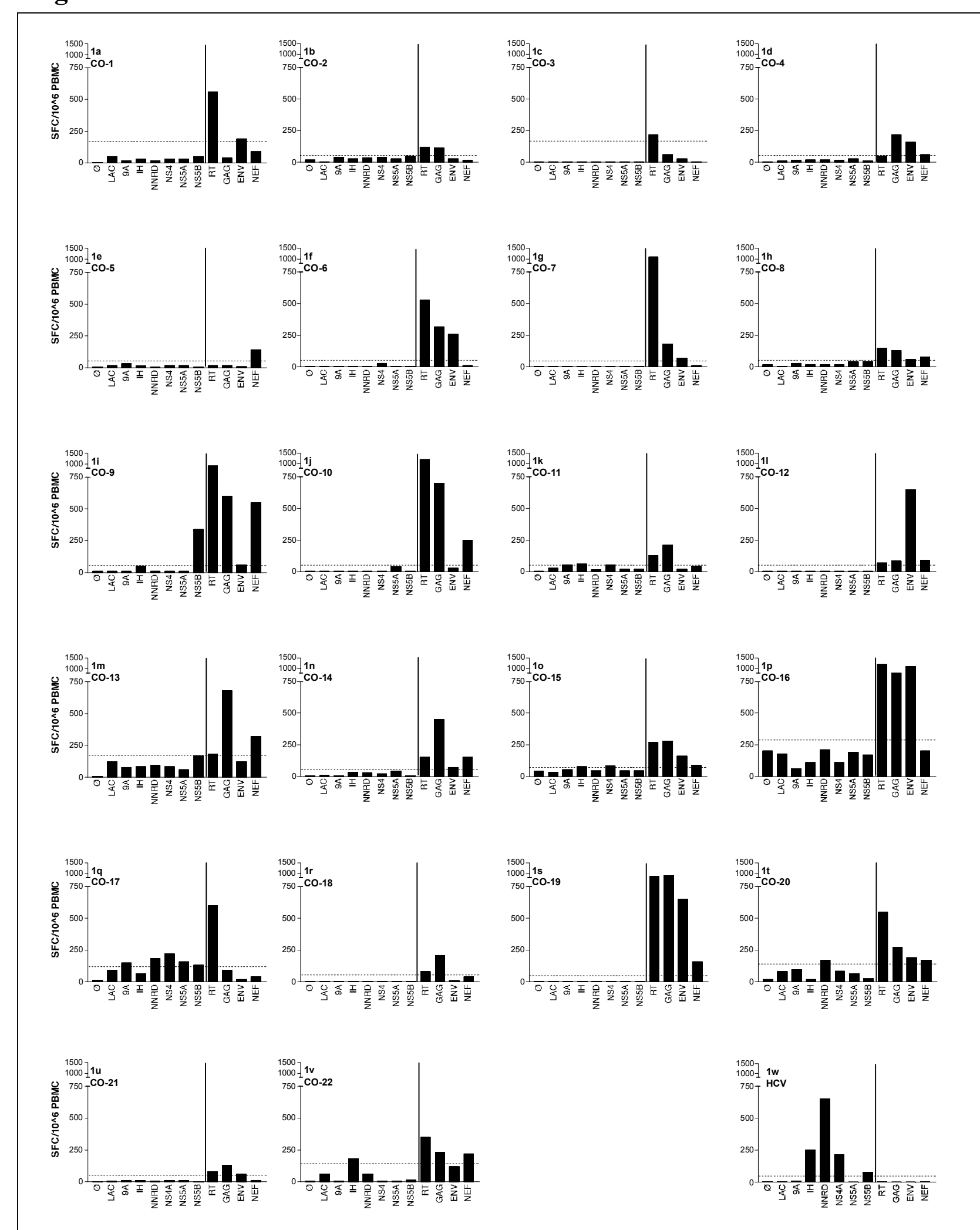


Figure 3

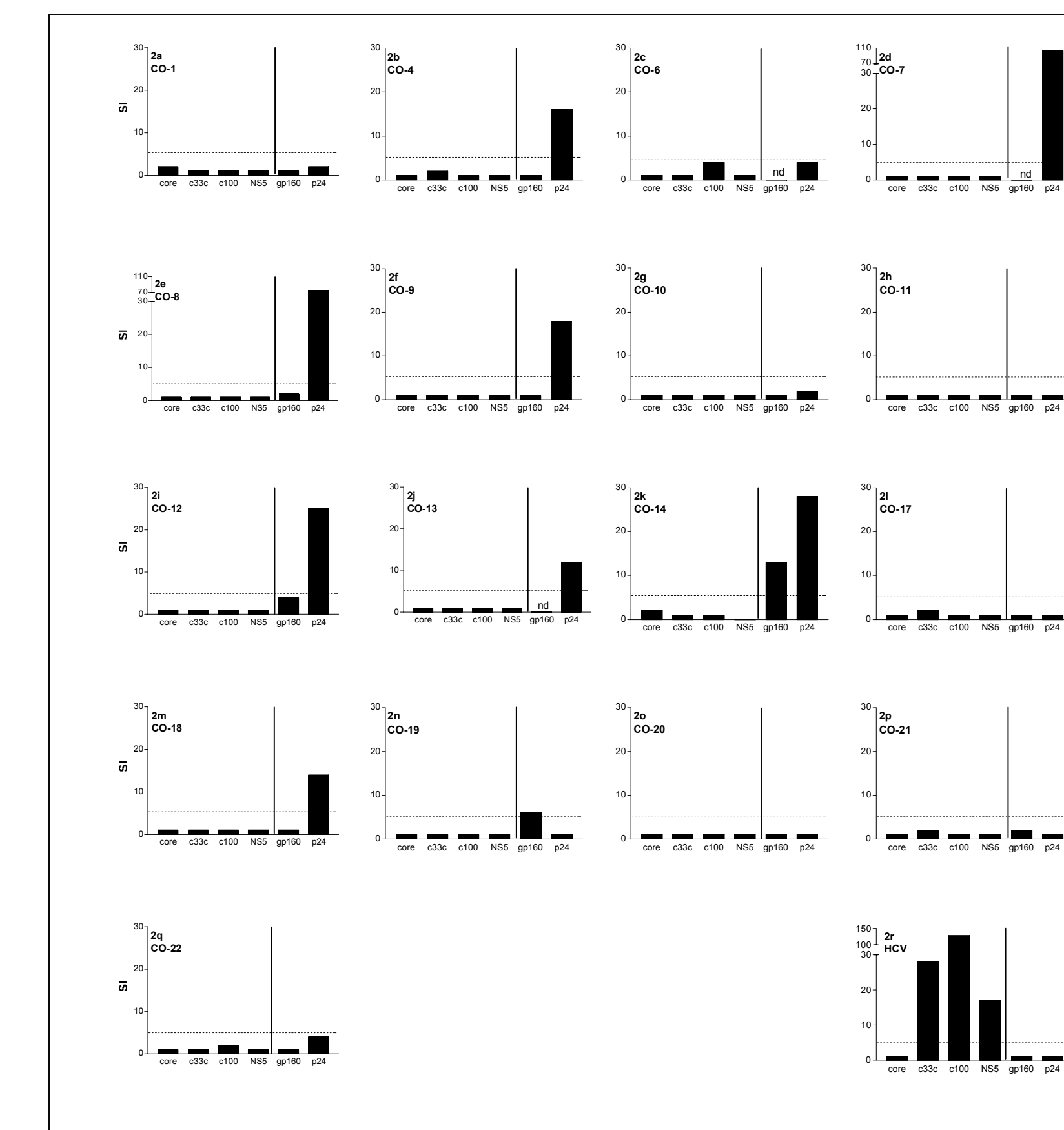


Figure 4

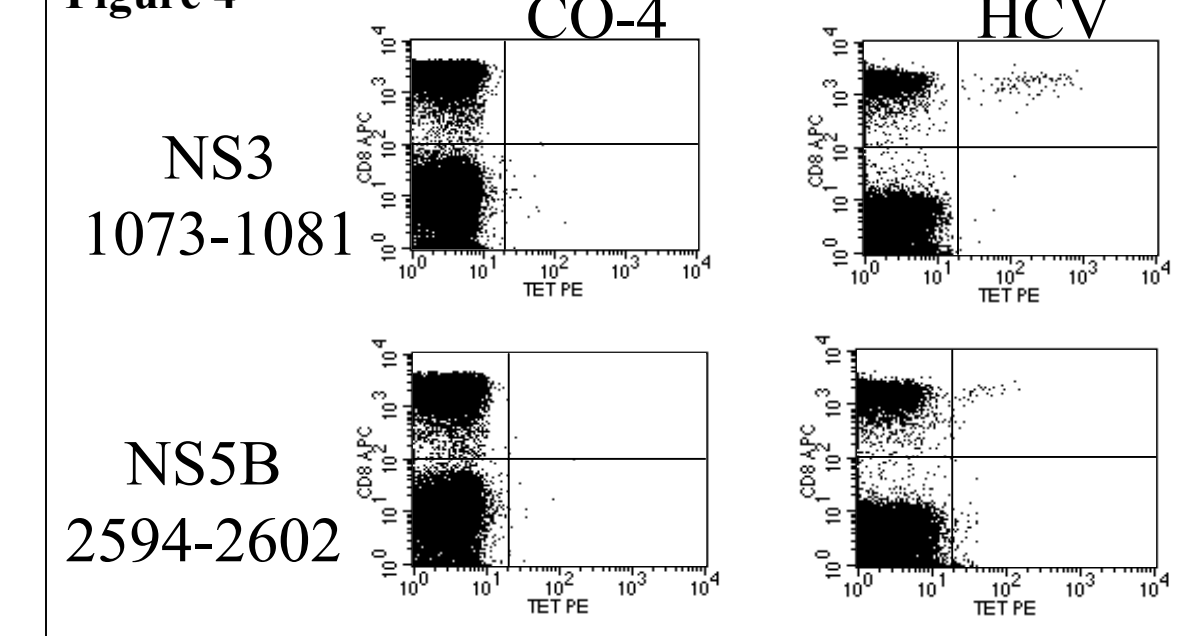


Figure 5

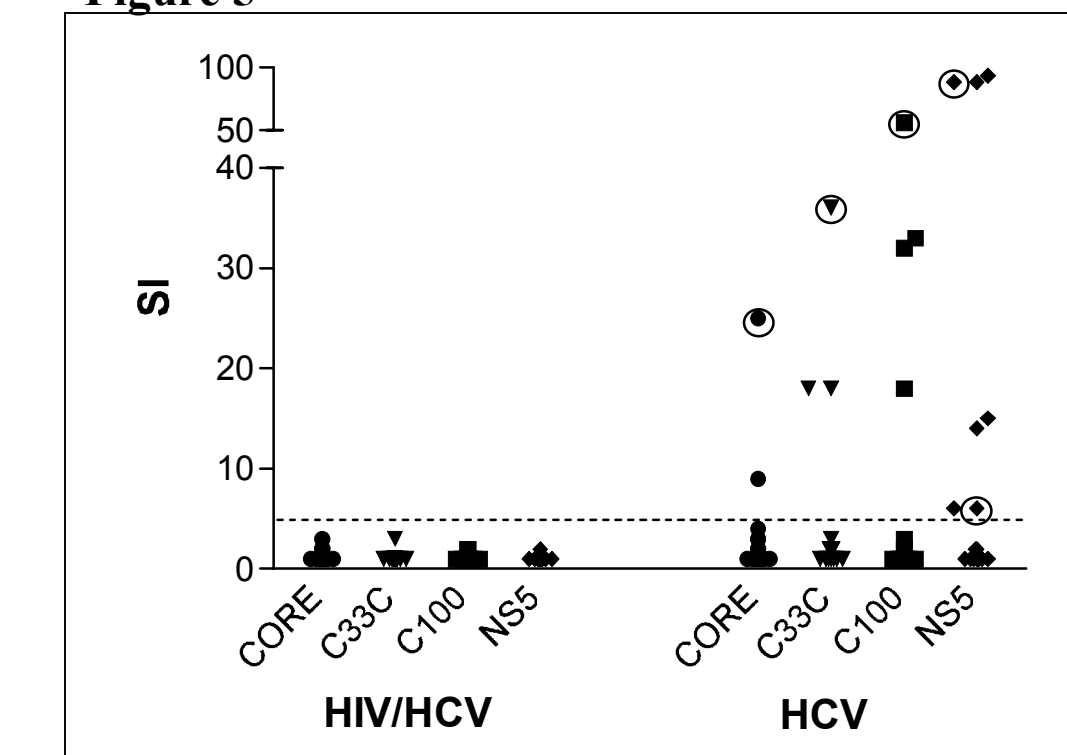


Figure 2:

12 of the 22 persons exhibited IFN gamma production in response to at least three of the four HIV-1 gene products tested, and all 22 were able to target at least one protein.

Responses were detected in persons on and off therapy.

RT was the most commonly recognized antigen (20 of 22) followed by Gag (17 of 22), Env (13 of 22) and Nef (11 of 22).

In contrast, only one of the co-infected persons (subject CO-17) targeted more than one HCV protein above the levels of the negative controls, and HCV-specific CD8 T-cell responses were completely absent in 17 of the 22 persons.

Tetramer analysis did not reveal HCV-specific responses which had not been detected by the Elispot assay (examples in Figure 4)

Figure 3:

The analysis of the CD4+ proliferative responses was restricted to the 17 individuals from whom freshly isolated cells were available.

Nine of 17 subjects had a detectable HIV-1 p24-specific proliferative response to HIV-1.

All 3 subjects tested with spontaneous control of HIV-1 replication (CO-12, CO-13 and CO-14) had detectable proliferative responses to p24 protein.

Surprisingly we also found strong proliferative responses to HIV-1 p24 in 4 out of 8 individuals who were successfully treated for HIV-1 infection with HAART (CO-4, CO-7 to CO-9).

In contrast, none of these HCV/HIV co-infected persons had proliferative responses to HCV.

Figure 5:

As the HCV proliferative responses were so surprisingly low, the HCV antigens were also used to test a matched cohort of HCV mono-infected persons.

In contrast to the results in the co-infected individuals, we were able to detect an HCV specific proliferative response in 8 of 17 persons (47%) with HCV mono-infection (p<0.002).

Summary:

HIV-1 and HCV are in most cases not effectively controlled by the immune system.

This is true for HIV-1 despite a strong and broad specific CTL response.

In contrast, the same host usually has not a similar CTL response against HCV.

HCV and HIV-1 might employ distinct mechanisms to evade immune control.

HIV-1/HCV coinfection seems to alter the helper cell responses against the two viruses:

HCV specific proliferative responses were significantly lower compared to HCV mono-infection

There was a trend towards stronger HIV-1 specific responses, especially in individuals on HAART.