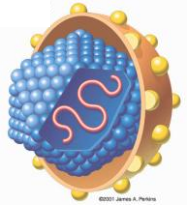

HCV superinfection and reinfection

(and dual infection and recombination too!)

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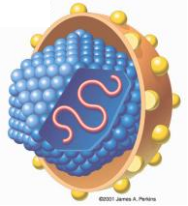
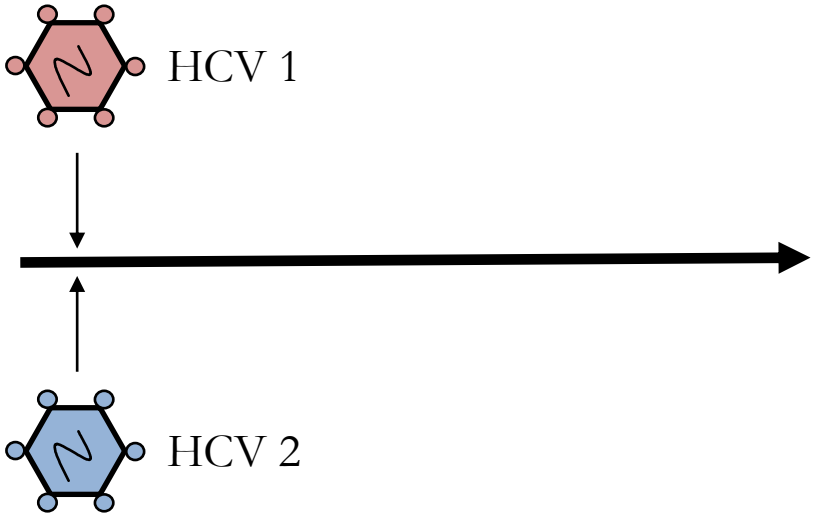


HCV dual/co-infection

- ***Dual infection*** occurs when an individual is infected with HCV derived from ≥2 individuals (e.g., hemophiliacs given blood products from multiple individuals). Dual infections can further be divided into co-infections and superinfections.
- ***Co-infection*** is defined as infection with ≥2 heterologous HCVs simultaneously or within a very narrow window period before infection with the first HCV has resulted in an immunologic response to that virus.



Blackard, 2007. *J Inf Dis* 195:519

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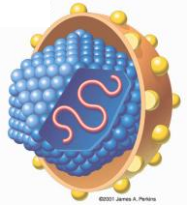
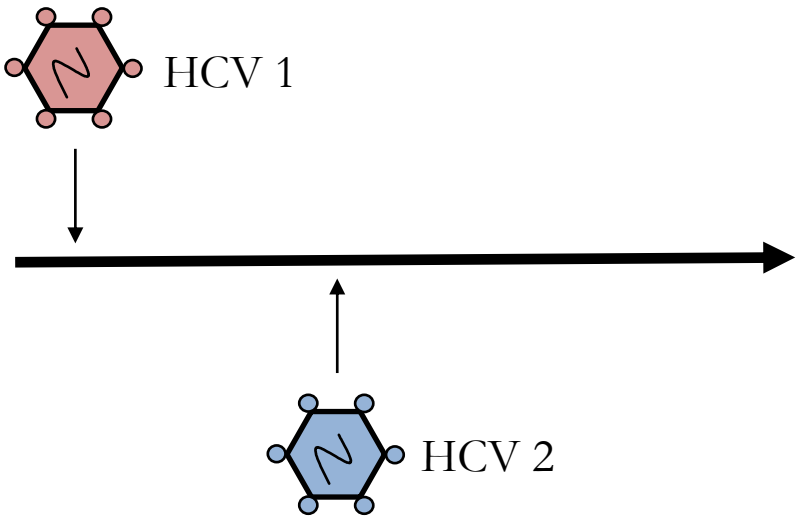


HCV superinfection

- ***Superinfection*** is defined as infection with a second HCV after the establishment of persistent HCV infection and development of an immunologic response to the first virus.



Blackard, 2007. *J Inf Dis* 195:519

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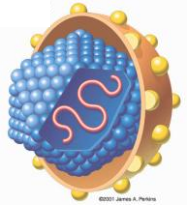
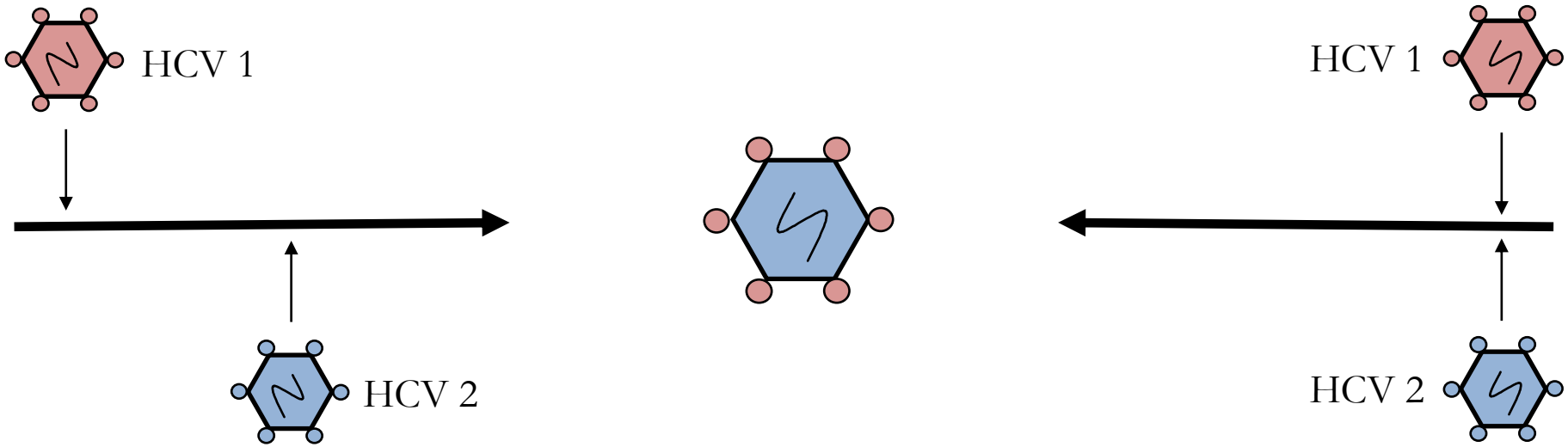
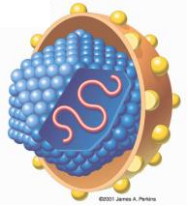


HCV recombination

- ***Recombination*** involves the exchange of genetic material between two related viruses during co-infection of a host cell.

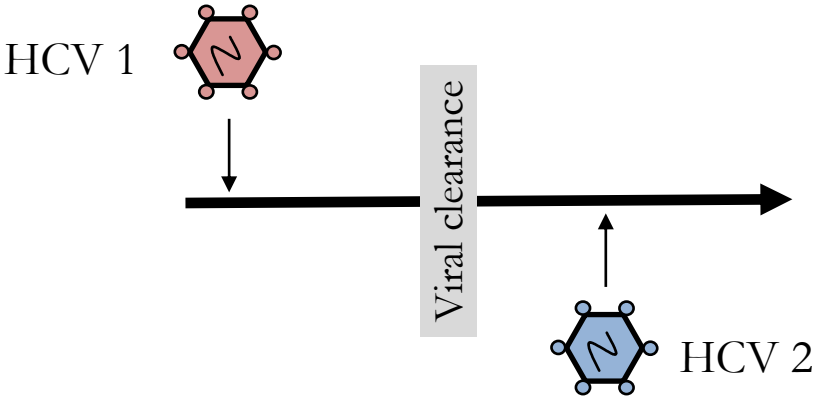


Blackard, 2007. *J Inf Dis* 195:519



HCV reinfection

- ***Reinfection*** describes a primary infection that is completely cleared virologically prior to a subsequent, secondary infection with either a homologous or a heterologous HCV.



Blackard, 2007. *J Inf Dis* 195:519

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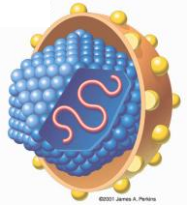

HCV dual/co-infection is relatively common

- Dual infection in >10% of study population
  - Viazov, 2000. *Infection* 28:21; Toyoda, 1998. *J Inf Dis* 26:440; Qian, 2000. *J Viral Hepat* 7:153; Matsubara, 1996. *J Ped Gastro & Nutr* 22:1; Giannini, 1999. *J Lab Clin Med* 134:68; Pham, 2010. *Hepat* 52:1564
- Dual infection in 5-10% of study population
  - Schneeberger, 1998. *J Clin Micro* 36:1711
- Dual infection in <5% of study population
  - Schroter, 2003. *J Clin Virol* 27:200; Schijman, 2004. *Clin Diag Lab Imm* 11:433; Blatt, 2000. *J Viral Hepat* 7:196

Investigation of Putative Multisubtype Hepatitis C Virus Infections In Vivo by Heteroduplex Mobility Analysis of Core/Envelope Subgenomes[▽]

Hui Li,[1] Lisa V. Thomassen,[2] Ayaz Majid,[5] Brian J. McMahon,[6] Dana Bruden,[6] Susan McArdle,[1] Nazneen Bano,[1] Minjun Chung,[1] Robert L. Carithers,[3] James D. Perkins,[4] Daniel G. Sullivan,[1] and David R. Gretch[1,3]*

Departments of Laboratory Medicine,[1] Pathology,[2] Medicine,[3] and Surgery,[4] University of Washington Medical Center, Seattle, Washington; Department of Medicine, University of Miami, Miami, Florida[5]; and Arctic Investigators Unit, Centers for Disease Control and Prevention, Anchorage, Alaska[6]

TABLE 2. Evidence of mixed or switched genotypes detected by RFLP and confirmed by HMA based on risk group

Risk group (*n*)	No. (%) of mixed/switched genotypes:	
	Detected by RFLP	Confirmed by HMA
IDU (226)	16/226 (7.1)	3/16 (18.8)
Blood transfusion (52)	3/52 (5.8)	0/3 (0)
Other (96)[a]	9/96 (9.4)	0/9 (0)
Total (374)	28/374 (7.5)	3/28 (10.7)

[a] Other risk factors include greater than 10 lifetime sexual partners, sexual partners with history of IDU, tattooing, piercing, intranasal cocaine use, household contact with HCV, and unknown.

TABLE 3. Resolution of multiple genotype HCV test results using HMA

Route of infection and patient	Result by:		Interpretation
	RFLP	HMA	
IDU			
005[a]	1b-to-2a switch	1b	False 2a by RFLP
015	2a-to-1a switch	2a to 1a switch	Confirmed switched genotypes
021	2a-to-3a switch	3a	False 2a by RFLP
030	1a-2a mix	1a	False 2a by RFLP
	1a-1b-2a mix	1a	False 1b-2a mix by RFLP
062	1a-1b mix	1a-1b mix	Confirmed mixed genotypes
068	1a-1b mix	1b	False 1a by RFLP
146	1a-2a mix	1a	False 2a by RFLP
177	1a-2a mix	1a	False 2a by RFLP
192	1a-1b mix	1a	False 1b by RFLP
198	1a-1b mix	1b	False 1a by RFLP
201	1a-1b mix	1a	False 1b by RFLP
267	2b-3a mix	3a	False 2b by RFLP
281	1a-2a mix	1a	False 2a by RFLP
292[a]	1a-1b mix	1a	False 1b by RFLP
332	1a-to-2b switch	1a-to-2b switch	Confirmed switched genotypes
349	1a-1b mix	1b	False 1a by RFLP
Blood transfusion			
050	1b-to2b-to-1b switch	Negative; 1b	Not confirmed
086	1a-1b mix	1a	False 1b by RFLP
	1b	1a	False 1b by RFLP
310	1a-1b mix	1b	False 1a by RFLP
Other[b]			
020	1b-to-3a switch	3a	False 1b by RFLP
043	1a-1b mix	1a	False 1b by RFLP
105	1a-to-1b switch	1a	False 1b by RFLP
112	1a-1b mix	1a	False 1b by RFLP
113	1a-2a mix	1a	False 2a by RFLP
140	1a-1b mix	1a	False 1b by RFLP
154	1a-1b mix	1b	False 1a by RFLP
187	1a-1b mix	1b	False 1a by RFLP
243	1a-1b mix	1a	False 1b by RFLP

Li, 2008. *J Virol* 82:7524

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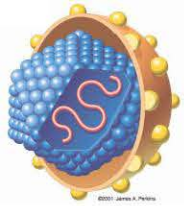

Case reports of HCV superinfection

Risk category, HCV genotype (region; method)
Injection-drug use
3A→ 1A
1A→ 3A
1B→ 1A
1A→ 1A[a] (n = 2) (HVR1 or NS5B; genotype- and strain-specific PCR and cloning)
Transplantation
2B→ 3A (n = 2) (core; genotype-specific PCR)
Chimpanzee
1B→ 2A→ 1A
1B→ 1A (n = 2) (core; genotype-specific PCR)
Transfusion
1B→ 1B[a] (n = 2) (HVR1; standard PCR)
1B→ 1B[a] (HVR1; genotype-specific PCR and cloning)
Type II→ type III→ type II (E1 and NS3; standard PCR)
2B→ 2B[a] + 1A
2A→ 2A[a] + 1B
1B → 1B[a] (NS5B and E2; strain-specific PCR and cloning)
Colonoscopy
1B→ 4 (region not reported; restriction-fragment length polymorphism)

NOTE. HVR1, hypervariable region 1; PCR, polymerase chain reaction.

[a] Superinfecting HCV was a highly divergent strain of the same genotype.

Blackard, 2007. *J Inf Dis* 195:519; Laskus, 1996. *Virol* 220:171; Ramirez, 2010. *J Gen Virol* 91:1183; Halfon, 1999. *J Hepat* 30:970; van der Laar, 2009. *J Hepat* 51:667

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Population studies of HCV superinfection

- Despite ongoing IDU, 0 of 8 patients with persistent HCV switched HCV genotype
  - Thus, "patients with ongoing or prior HCV infection appear to develop immunity that protects against further infection with HCV despite repeated exposure."
- Among 37 who recently developed chronic HCV, superinfection was observed in 6
  - Reinfection/superinfection occurred more often in participants with ongoing IDU

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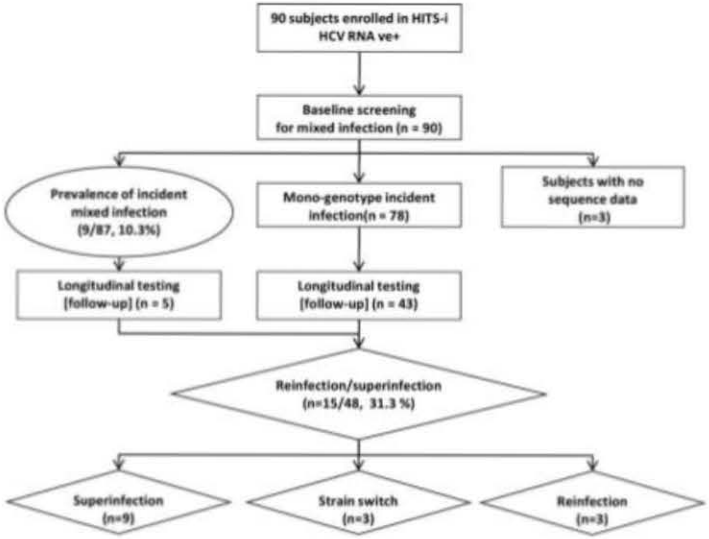
Grebely, 2011. *Hepat* in press; Dove, 2005. *J Viral Hepat* 12:574

Population studies of HCV superinfection

- Among 87 IDUs with incident HCV infections, 9 had mixed infections at baseline, while 15 had superinfections or reinfections at follow-up



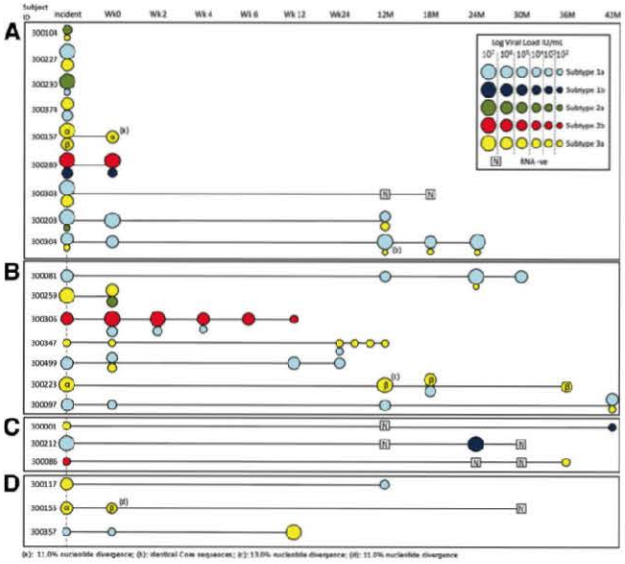


Fig. 3. Twenty-two subjects with multiple infection. All individuals are shown relative to a time scale that comprised the time of incidence of HCV infection to 42 months (42M). HCV RNA-negative subjects enrolled in HITS-p (n = 87) who became RNA-positive were screened for multiple infection with two or more HCVs. Subtypes are color-coded as shown in the key. Circle sizes correlate to log of viral load (IU/mL) of each HCV population at each time point. Boxes indicate RNA (negative) time points. The 22 cases of multiple infection are grouped into (A) incident mixed infection (n = 9), (B) superinfection with no incident mixed infection (n = 7), (C) reinfection (n = 3), and (D) strain switch (n = 3).

Pham, 2010. *Hepat* 52:1564

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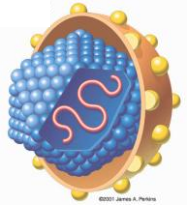
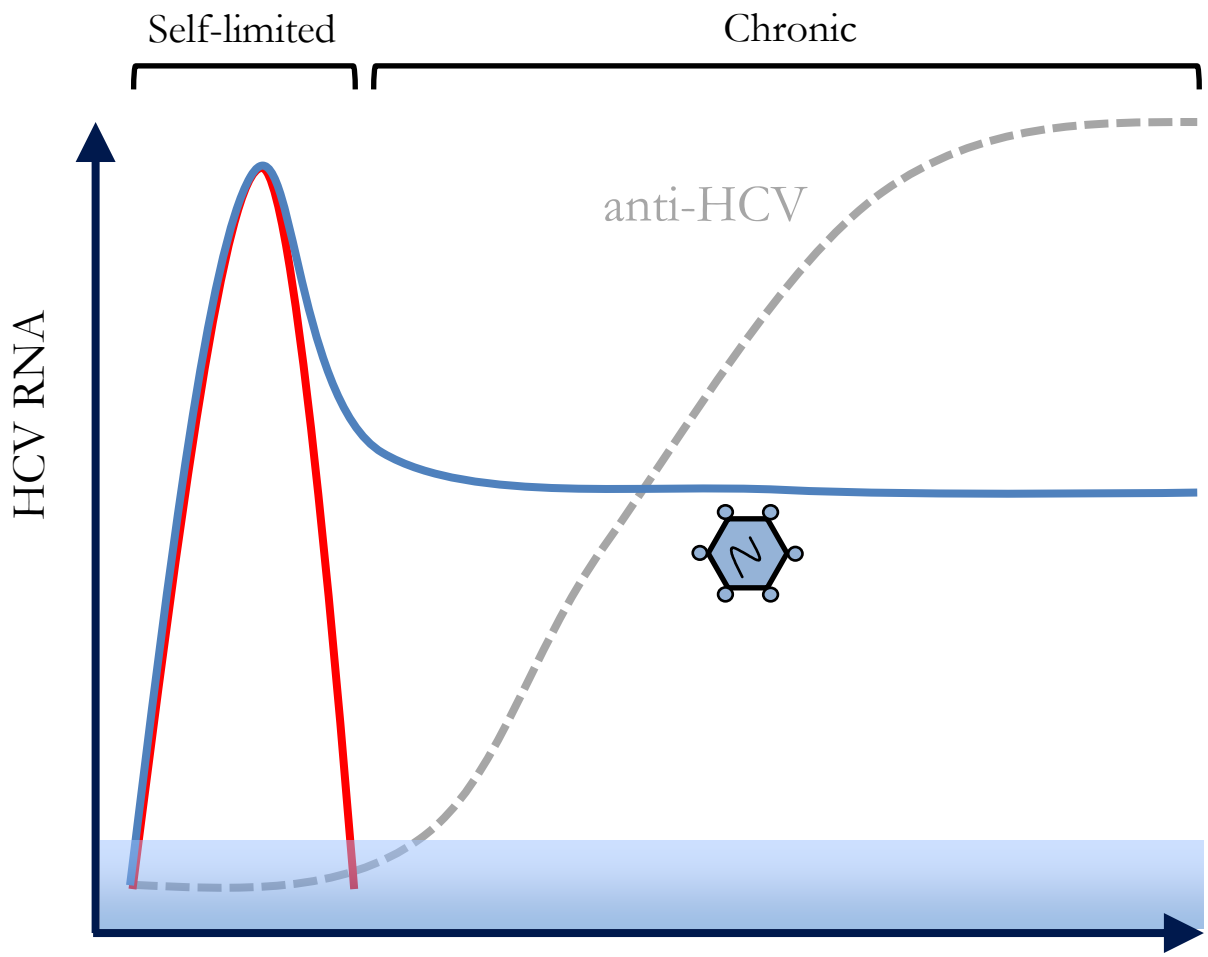
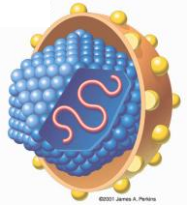
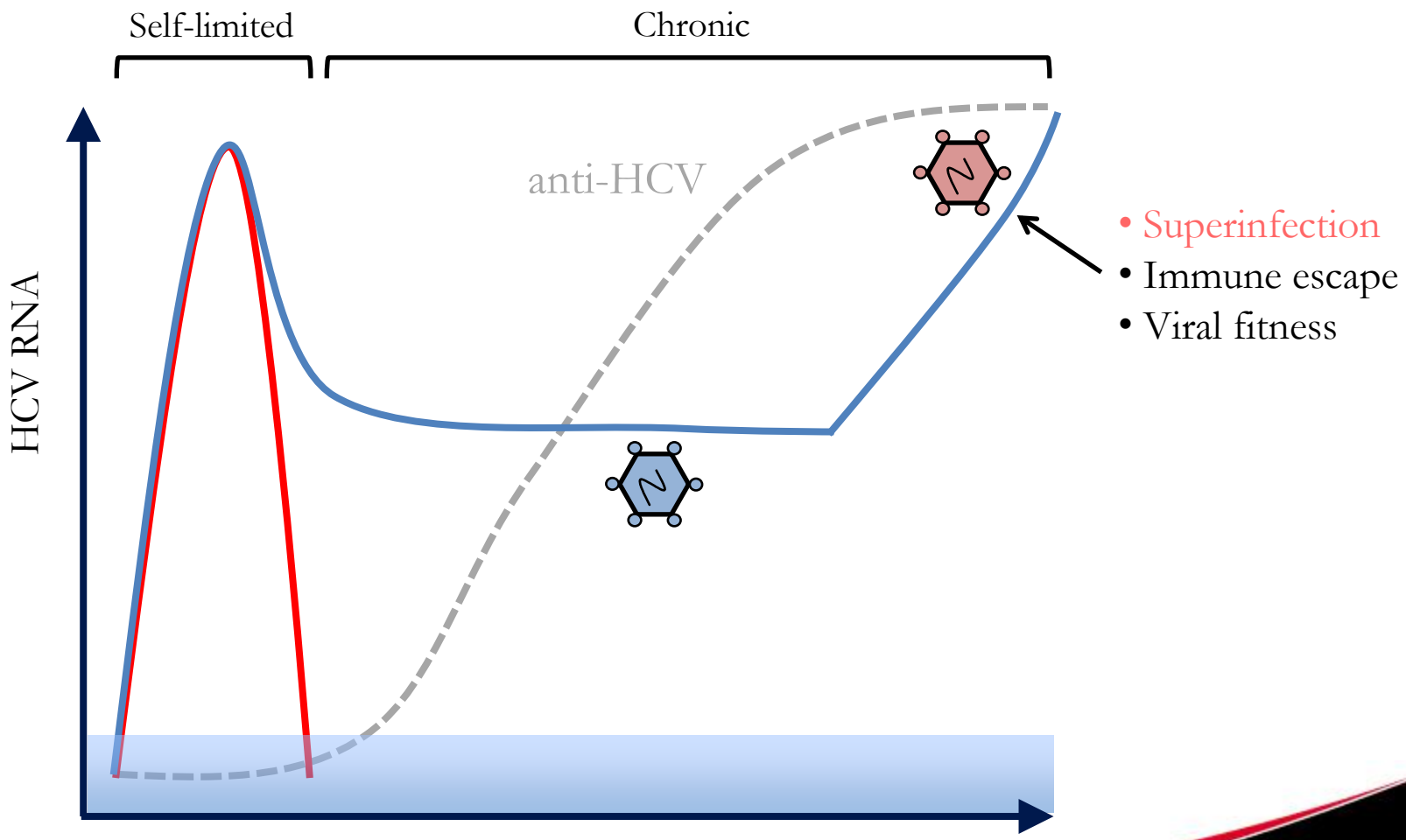


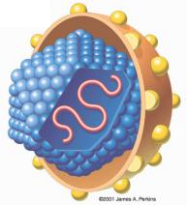
Viremia during HCV infection





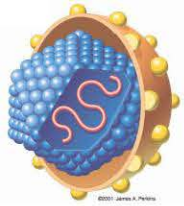
Viremia during HCV infection





What is the impact of HCV superinfection ?

- Overall HCV viral loads
- Disease progression
- Innate and adaptive immune responses
- Spontaneous clearance
- Drug resistance and treatment response rates
- Transmission
- Biological phenotype



HCV recombination – case reports

Strain	Origin	Recombination sites*	Reference
Inter-genotypic recombinants			
RFl_2k/1b	Russia	Within NS2, inside the residue V/I-931	AY587845 [9]
D3, **2i/6**	Vietnam	NS2/NS3 junction, between residues 1022 and 1042	DQ155560 [17]
SE-03-07-1689, **RF3_2b/1b**	Philippines	Beginning of NS3, between G-1038 and L-1039	DQ364460 [18]
Rl, **2/5**	France	Beginning of NS3, between residues 1027 and 1033	AM408911 [19]
D177, **RF_2b6w**	Taiwan	Beginning of NS3, at the residue 1030	EU643835 [20]
Intra-genotypic recombinants			
PE22, **RF2_1b/1a**	Peru	Within NS5B, inside the residue Y-2660	AJ582127, AJ582131, AJ582134 [21]
HC-J1, **1a/1c**	Japan	2 sites within E1–E2 at residues 356 and 570	D10749 [22]
Khajal, **1a/1c**	India	5 sites in the core to NS3 regions at residues 154, 307, 614, 900, 1147	AY651061 [23]
H23, **1b/1a**	Uruguay	Within the core, at the residue 16	EU934902–07 [24]
Intra-subtype recombinants			
One strain (1b)	Spain	Within NS5B, at the residue 2243	AY378694 [25]
Several strains (1a, 1b, 3a)	Spain	1 or 2 sites within E1–E2 or 1 or 2 sites within NS5A	[26]

1a/1b recombinant	Experimentally infected chimpanzees	Gao, 2007; *J Gen Virol* 88:143
2k/1b recombinant	Georgia/France	Morel, 2010. *J Clin Virol* 47:382
2b/1b recombinant	Japan	Yokoyama, 2011. *Arch Virol* 156:1591
2b/1a recombinant	**United States**	**Bhattacharya, 2011. *Virol J* 8:458**

Morel, 2010. *J Viral Hepat* 18:77

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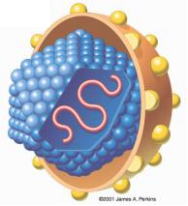
HCV recombination in population studies

- Concordant genotypes detected in core-E1 vs. NS5B for 222 patients in China (Zhou, 2010. *J Clin Virol* 49:272)
- Discordant genotypes detected in the 5'UTR vs. NS5B from 2 of 82 IDUs in China (Xia, 2008. *J Med Virol* 80:1142)
- Discordant genotypes detected in core vs. NS5B for 0 of 118 German IDUs and 2 of 47 Russian IDUs (Viazov, 2010. *J Med Virol* 82:232)
- Discordant genotypes detected in E1 vs. NS5B for 1 of 124 IDUs in Portugal (Calado, 2011. *J Med Virol* 83:608)
- Analysis of 17,712 sequences from from 111 patients revealed recombination events in 18.0% (Sentandreu, 2008. *PLoS One* 9:e3239)
- Analysis of NS5A sequences revealed recombination in 1 of 6 patients undergoing HCV therapy (Moreno, 2006. *Virol J* 3:87)



Does superinfection lead to recombination?

- 1a + 1a superinfection (n = 2)
  - no recombination at later timepoint
- 3a + 1a, 1a + 3a, and 1b + 1a superinfection
  - no recombination at later timepoint

Bernardin, 2006. *J Viral Hepat* 13:532

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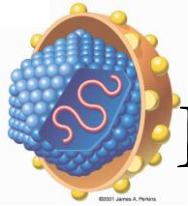

HCV superinfection/recombination *in vitro*

- Huh7.5 cells acutely infected with HCV were resistant to infection with cell culture-produced HCV
  - Superinfection exclusion occurred post-entry and downstream of primary translation
  - Replicon-containing cells are permissive to re-infection after treatment with HCV protease inhibitor
- Simultaneous infection of Huh7 cells resulted in replication of both viral genomes in the same cell; however, when infections were performed sequentially, the secondary infection was severely impaired
  - Superinfection exclusion was not due to reduction of surface expression of CD81 or scavenger receptor BI, nor due to functional block at virus entry, but rather was mediated by interference at RNA translation
- Tight junction proteins CLDN1 and OCLN are downregulated during primary infection and prevent superinfection
- Based on experimental system with selectable HCV replicons, recombination is rare; "however, due to the rapid virus turnover and the large number of infected liver cells *in vivo*, it is expected that recombination will be of biological importance when strong selection pressures are operative."

Tscherne, 2007. *J Virol* 81:3693; Schaller, 2007. *J Virol* 81:4591; Liu, 2009. *J Virol* 83:2011; Reiter, 2011. *J Hepat* in press

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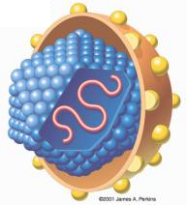

HCV reinfection – animal studies

- In contrast to humans, 50-60% of chimpanzees clear HCV infection
- Farci *et al.* reported that all chimpanzees that were rechallenged with HCV had reappearance of viremia
- Others have also rechallenged chimpanzees that had previously cleared HCV infection and reported more complex findings . . .
  - No T-cell proliferative response in all animals with <u>chronic</u> HCV (T cell anergy?)
  - Detectable T-cell response in 1 of 2 <u>acutely</u> infected animals
  - However, in a different study, all 9 animals rechallenged with <u>homologous</u> genotype developed self-limited infections, while 4 of 11 animals challenged with <u>heterologous</u> genotypes developed chronic infections

Farci, 1992. *Science* 258:135; Bassett, 1998. *J Virol* 72:2589; Bassett, 2001. *Hepat* 33:1479; Prince, 2005. *J Inf Dis* 192:1701

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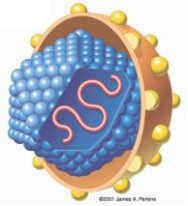

HCV reinfection – human studies

Reference	Study population	Median age (years)	Percent active IDU	Median years follow-up	New infections	HCV incidence	HCV RNA testing interval
Mehta, 2002 *Lancet* 359:1478 #@	Uninfected (n = 164) Previously infected (n = 98)	32 41	79% 65%	2.4 2.1	21% 12%	8.6/100 pyo 5.4/100 pyo	6.3 months
Grebely, 2006 *Hepat* 44:1139 #	Uninfected (n = 926) Previously infected (n = 152)	41 44	48% 26%	2.8 5.2	19% 9%	8.1/100 pyo 1.8/100 pyo	15.6 months
Micallef, 2007 *J Viral Hepat* 14:413	Uninfected (n = 423) Previously infected (n = 18)	23 23	100% 100%	1.0 1.2	27% 72%	17/100 pyo 31-42/100 pyo	
Aitken, 2008 *Hepat* 48:1746	Uninfected (n = 55) Previously infected (n = 50)	25 25	54% 74%	~2.5 ~2.0	16% 47%	15.5/100 pyo 46.8/100 pyo	3 months
Osburn, 2010 *Gastro* 138:315	Uninfected (not included) Previously infected (n = 22)	--- 25	--- 100%	---	--- 50%	--- 30.1/100 pyo	32-33 days
Currie, 2008 *Drug Alc Dep* 93:148 #	Uninfected (not included) Previously infected (n = 38)	--- 46	--- 42-45%	--- 6.3	--- 3%	--- 0.47/100 pyo	6 months
Backmund, 2004 *Clin Inf Dis* 39:1540	Uninfected (not included) Previously infected (n = 18) *	--- 32.1	---	--- 2.8	--- 2 of 17 (12%)	--- 0-4.1/100 pyo	1 year
Grebely, 2010 *J Gastr Hepat* 25:1281 #	Uninfected (not included) Previously infected (n = 18) *	--- 44	--- 66%	--- 2	--- 2 of 35 (6%)	--- 3.2-5.3/100 pyo	1 year

* Enrolled after sustained virologic response
# HIV-positive persons included
@ HCV RNA in liver also evaluated

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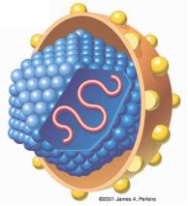

HCV reinfection and treatment

- Several case reports of new infections after spontaneous or IFN-mediated clearance of previous HCV infection
- 18 hemodialysis patients treated with IFN and re-evaluated for RNA after 6 and 18 months
  - 5 of 15 (33%) non-responders changed HCV genotype at 18 months implying re-infection
- 52 HIV-positive MSM previously treated for acute HCV
  - 11 (21%) later tested HCV RNA positive
  - Reinfection rate of 15.2 per 100 person-years
  - Cumulative incidence for 2 year period was 33%

Asselah, 2003. *Gut* 52:900; den Hollander, 2005. *AIDS* 19:640; Cotte, 2009. *Gastro Clin Bio* 33:977; Proubst, 2000. *J Clin Micro* 38:3125; Lambers, 2011. *AIDS* 25:F21; Arrais, 2008. *J Med Virol* 80:80

HCV reinfection – human studies

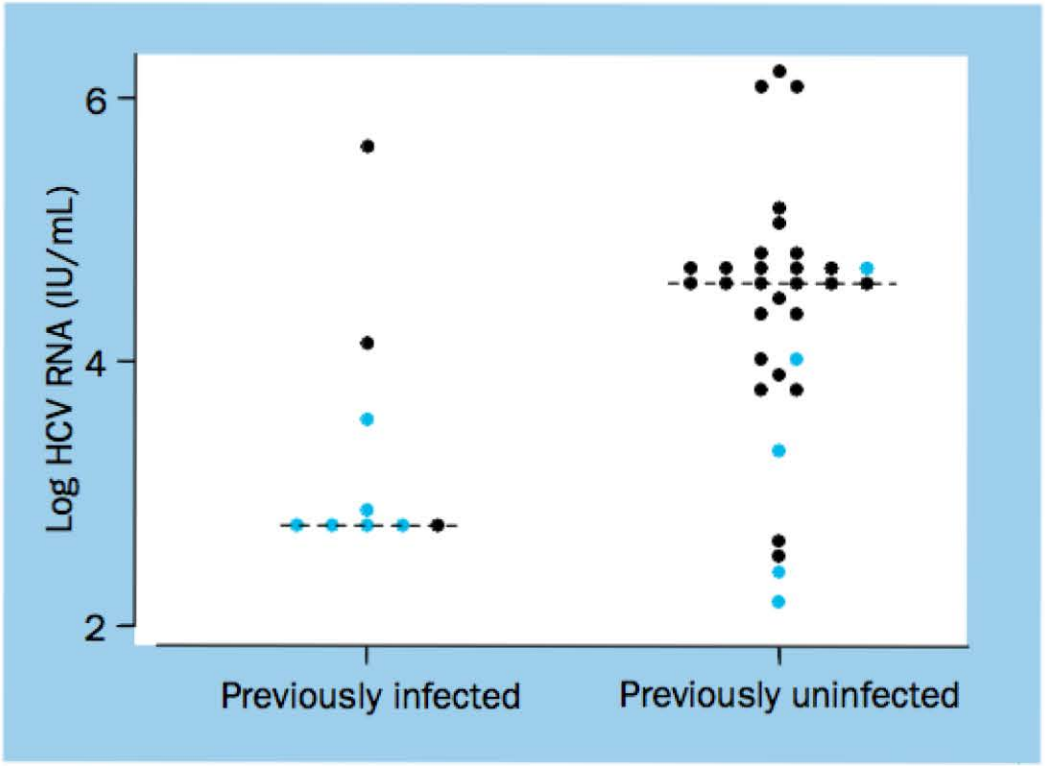

Figure 3: **Peak concentrations of HCV viraemia among previously infected and initially uninfected individuals**
Black circles represent people who developed persistent infection and blue circles represent people who cleared. Dashed lines represent medians.

Mehta, 2002. *Lancet* 359:1478

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HCV reinfection – human studies

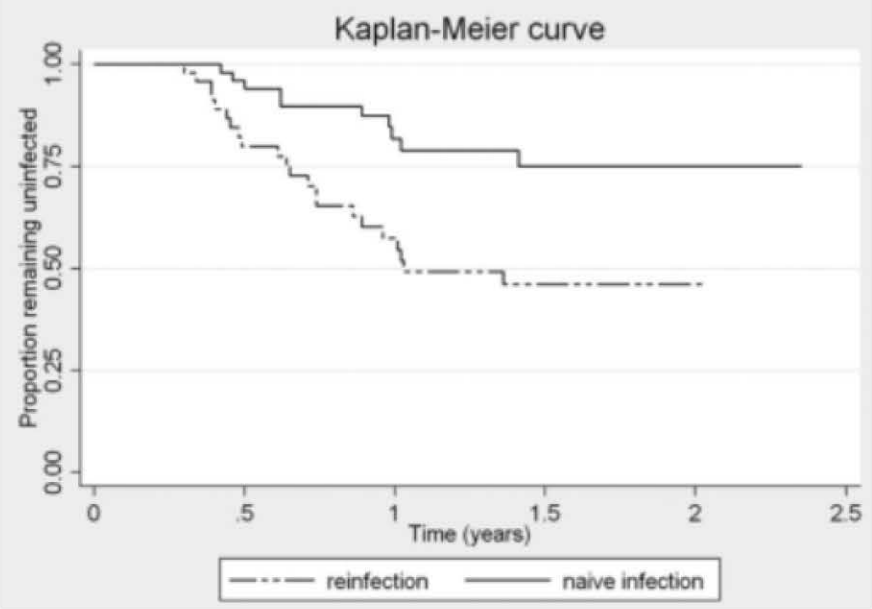

Table 2. Incidences of HCV Infection

Incidence Type	Cases	Susceptibles	PYs	Incidence Per 100 PYs	95% CI
Naive infection	10	45	54.6	15.5	8.3-28.8
Reinfection	23	27	39.2	46.8	31.1-70.3

Aitken, 2008. *Hepat* 48:1746

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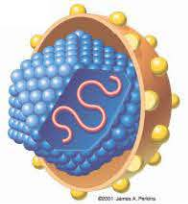

HCV reinfection – human studies

Table 2. Phylogenetic Analysis of Viral Sequences and Plasma ALT Levels During Primary Infection and Reinfection Within the Same Subject

		Primary infection		Reinfection A		Reinfection B			
Subject ID	ALT[a]	Genotype	ALT[b]	Genotype	ALT[b]	Genotype	ALT[b]	Divergence[c]	Outcome[d]
18	13	1a	74	1a	55			0.109	P
19	16	1a	411	1a	577			0.071	P
27	30	3a	559	1a	553			0.562	C
48	16	3a/1a	258	1a	63	3a	29	0.592, 0.595	C, C
57	10	1a	211	1a	20			0.103	C
133	17	1a	351	1a	42			0.116	C
152	17	1a	535	1b	347	1a	NA[e]	0.375, 0.383	C, C
170	6	1a	38	3a	61			0.628	NA
172	16	1b	387	1b	64			0.163	C
176	22	1a	785	3a	62			0.625	C
180	18	3a/1a	475	1a	84			0.102	C

[a]Baseline alanine aminotransferase (ALT) value prior to infection; all subjects returned to baseline prior to reinfection.
[b]Maximum ALT value during infection.
[c]First value represents divergence between primary infection and reinfection A. Second value represents divergence between reinfection A and reinfection B.
[d]C, cleared; P, persistent; NA, excluded from analysis of outcome. The first and second letters indicate the outcome of the first and second reinfections, respectively.
[e]NA, subject never returned to baseline following reinfection A.

Osburn, 2010. *Gastro* 138:315

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HCV reinfection – human studies

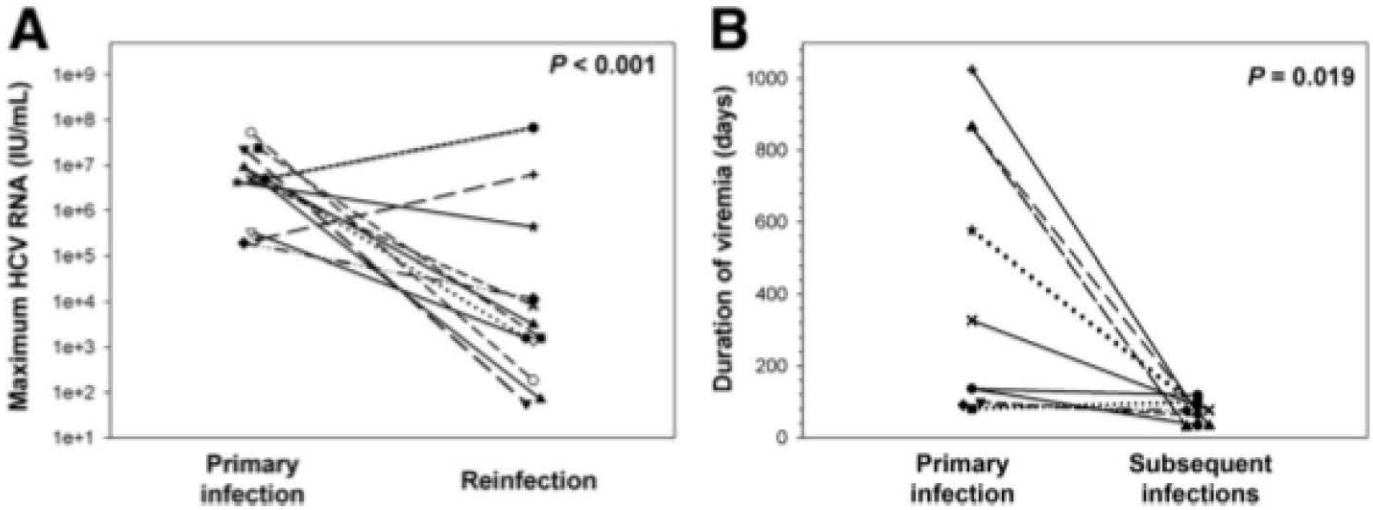

Figure 3. Clearance of a primary infection attenuates the infection kinetics of subsequent infections. (*A*) Maximum hepatitis C virus (HCV) RNA concentrations (IU/mL) detected in serum samples obtained during primary and subsequent infections in subjects with sufficient follow-up following the detection of a reinfection. Triangles represent maximum viremia detected in reinfection-persistent subjects. The maximum viremia in each subject during initial infection and reinfection is connected by a line. Median maximum HCV RNA concentration of reinfections was significantly lower than primary infections ($P < .001$). (*B*) Duration of viremia (days) during primary infections and subsequent infections in reinfection-cleared subjects. The duration of initial and reinfection viremia in each subject is connected by a line. The duration of viremia during reinfection was significantly lower than in primary infection ($P = .019$).

Osburn, 2010. *Gastro* 138:315

HCV reinfection – human studies

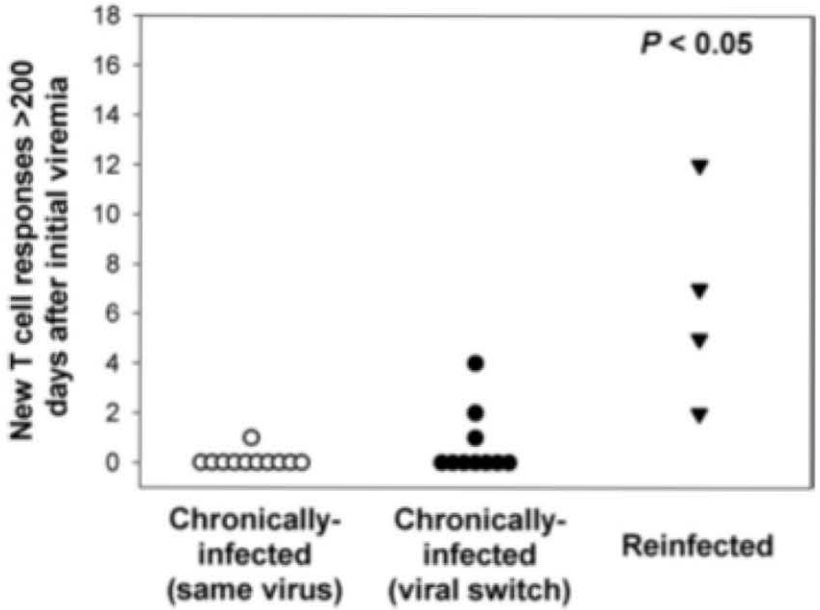

Figure 4. Significantly greater numbers of new T-cell responses are detected during reinfection than during chronic infection. Values represent new T-cell responses in chronically infected subjects in whom the same virus is detected during chronic infection, chronically infected subjects in whom more than 1 genetically distinct virus is detected during chronic infection, and reinfected subjects. A new T-cell response was defined as a peptide that was recognized, in an interferon-γ ELISpot, by peripheral blood mononuclear (PBMC) obtained >200 days following the first detection of viremia, but not by PBMCs collected at earlier time points in reinfected subjects and chronically infected individuals. $P > .05$ compared to both chronically infected groups.

Osburn, 2010. *Gastro* 138:315

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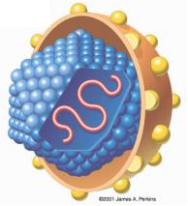

HCV reinfection – what does it tell us?

- Immune responses associated with HCV clearance are not 100% protective against subsequent re-exposures; however, other factors are also important
  - Innate immune responses (ISGs and IL28B)
  - Peripheral versus intrahepatic responses
  - Route of transmission and frequency of exposure
  - Size of inoculum
  - Viral diversity: genotype, % homology, quasispecies diversity, viral escape
  - Time elapsed since previous exposure
  - Other co-infections
  - Genetic correlates of viral clearance
  - Demographic (age, gender, race) and behavioral differences
  - Study design issues: definition of *reinfection* or *superinfection*, prospective versus convenience sampling strategy, testing interval, lower limit of detection of HCV RNA, specificity of assay, compartment in which RNA or genotype is assessed, behavioral/risk modifications and/or treatment-related optimism

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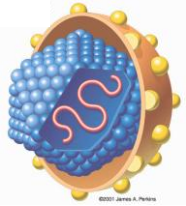
Barth, 2011. *Hepat* 54:1135; Wyatt, 1998. *J Virol* 72:1725



HCV genotypes switches can occur

- 6 of 14 hemodialysis patients experienced HCV genotype change after 13-21 months of follow-up
  - Genotype switches "may be due to either genotype-specific intermittent viremic status or viral interference"
- HCV genotype change observed in 19 of 32 hemophiliacs and were more common in HIV-positive individuals
  - Genotype switches could be false hybridizations during line probe assay or changes in predominant strains during mixed infection
- HCV genotype change in 4 of 15 IDUs (23.3 per 100 person-years of observation

Devereux, 1996. *J Viral Hepat* 3:43; Pujol, 1998. *Arch Virol* 143:823; Eyster, 1999. *J Inf Dis* 179:1062; Aitken, 2004. *J Med Virol* 74:543; Jarvis, 1994. *J Inf Dis* 170:1018

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Failure of Hepatitis C Therapy in HIV-Coinfected Drug Users Is Not Due to a Shift in Hepatitis C Virus Genotype

Soriano, 2005. *J Inf Dis* 192:1245; Soriano, 2003. *J AIDS* 32:465

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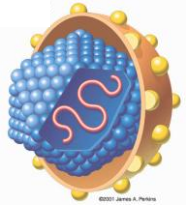
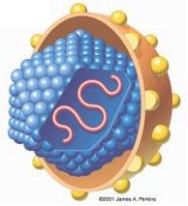


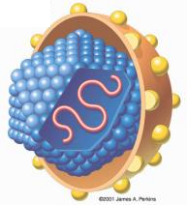
What are we still missing?

- Effectiveness of primary immune response on protection against secondary infection
- Biological consequences for viral fitness: drug resistance, virulence, transmission, disease progression
- Highly sensitive techniques to assess superinfection and recombination
- Relevance to future vaccine design and development
- Studies of factors such as treatment optimism that may impact on-going risk behaviors
- Careful monitoring of transmission of drug resistance virus



All things considered . . .

- Superinfection, recombination, and reinfection strongly suggest that a primary infection does not generate sterilizing immunity against subsequent exposures
- Yet, careful examination of the multiple immunologic, virologic, and genetic factors associated with viral clearance – or limited reinfection – will greatly facilitate vaccine design and development, as well as suggest more effective therapeutic strategies
- Effective HCV vaccine should evoke protective immunity against multiple HCV subtypes / genotypes



HCV superinfection and reinfection

(and dual infection and recombination too!)

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