Primary, post-primary and non-specific IgM responses

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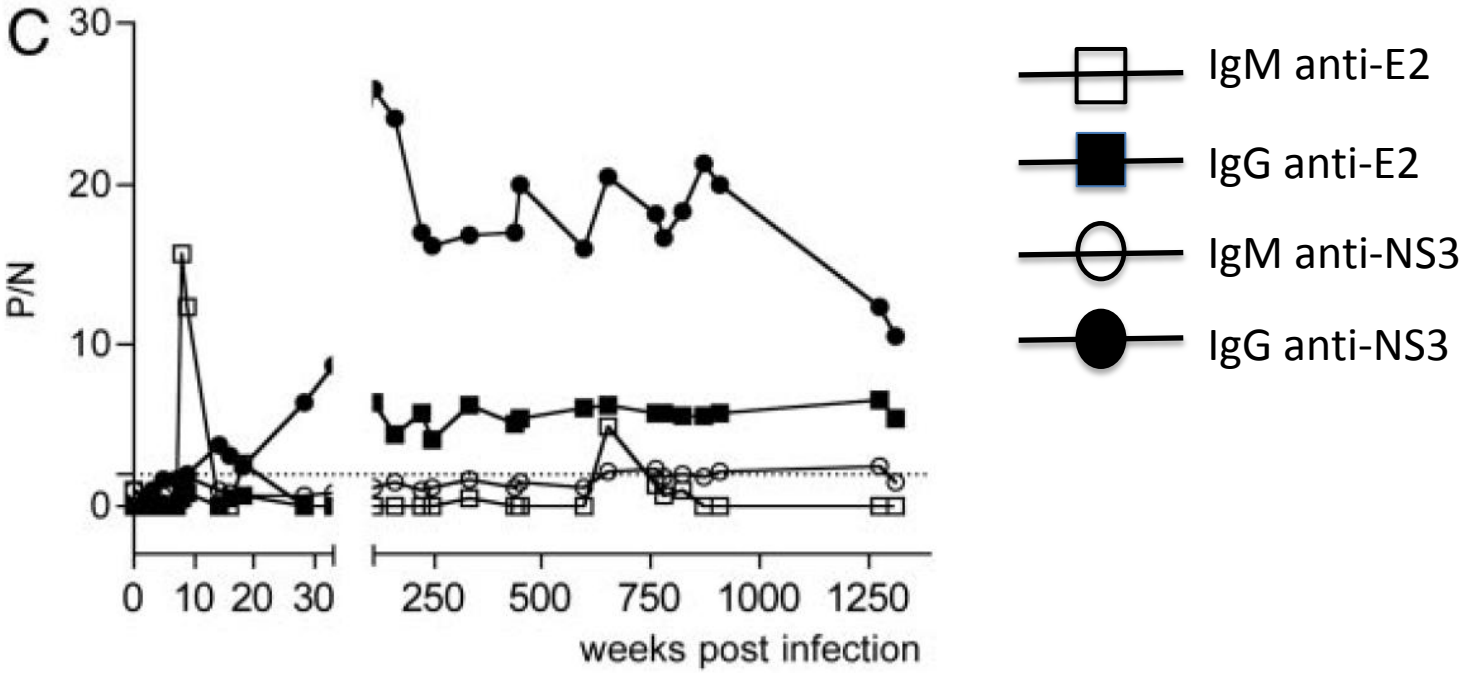
HCV-specific IgM responses during acute HCV infection

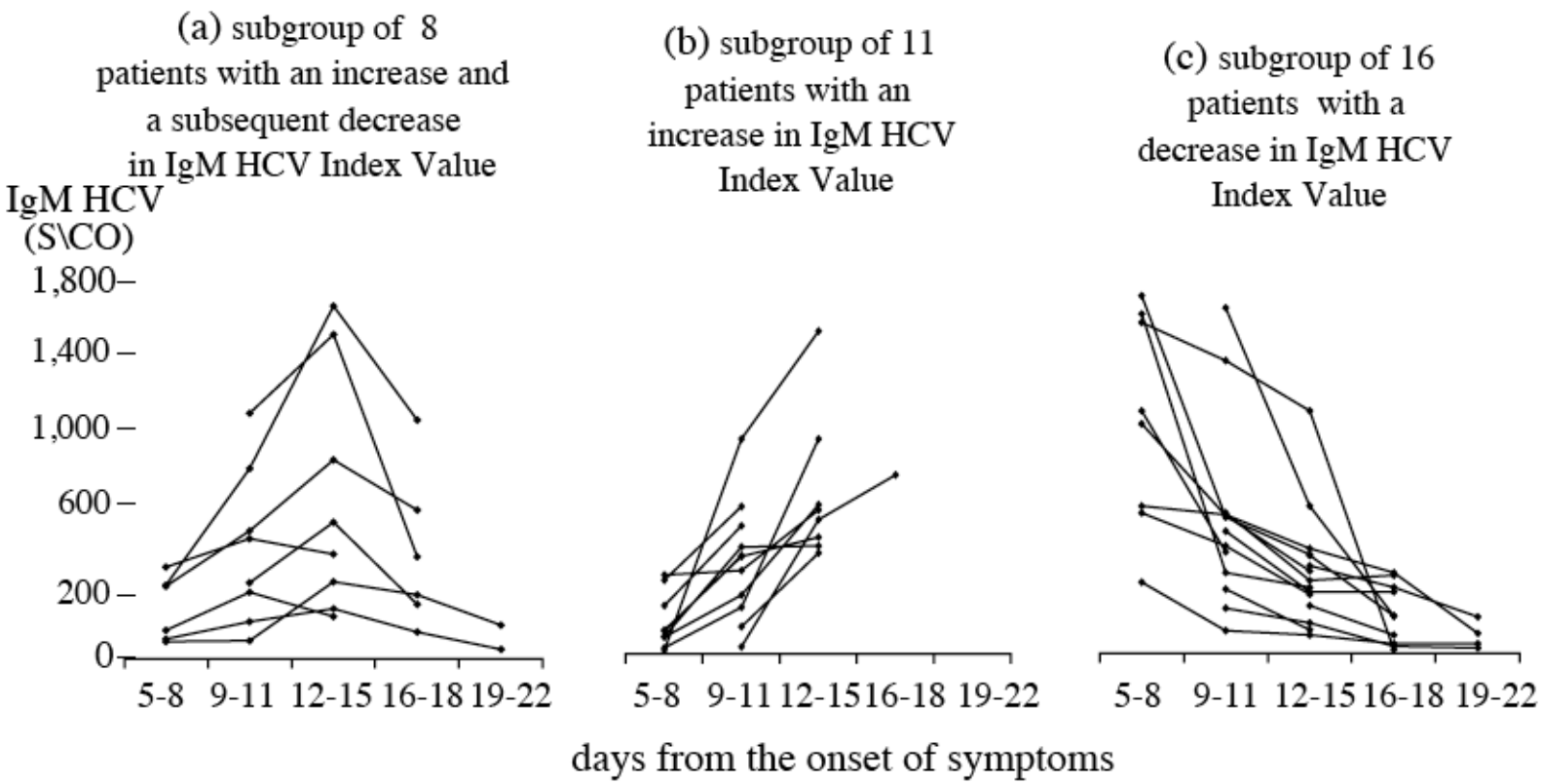
- Delayed
- Variable
- Targets: core, E2, NS3, NS4A/B
- May arise at nearly the same time as IgG responses
  - IgG avidity increases during persistent infection
- No clear correlation with outcome of primary infection

Transient IgM response during acute HCV infection in patient H
(infected by transfusion, 1977)



Logvinoff et al, 2004 PNAS

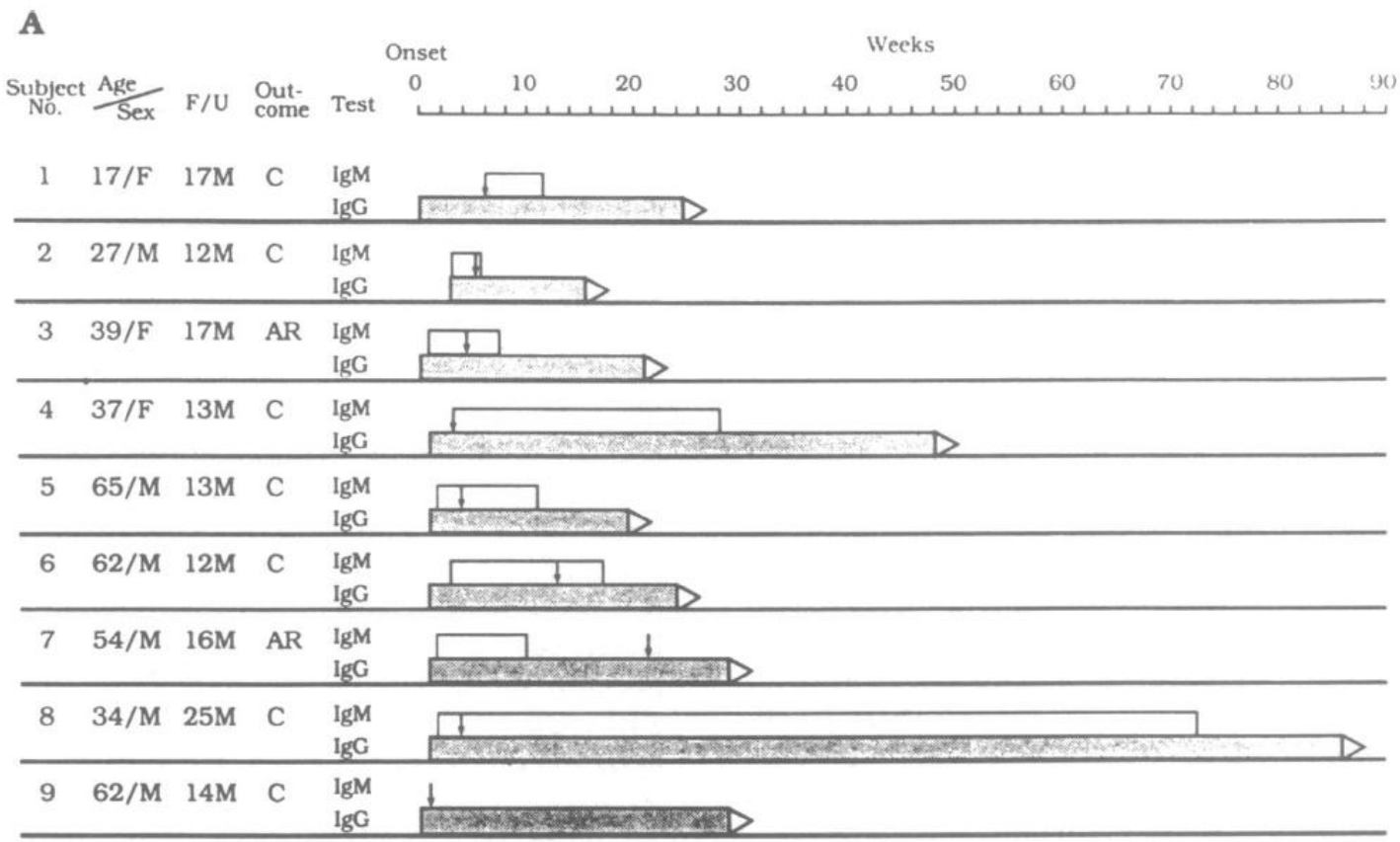
Variable patterns of HCV-specific IgM reactivity in acute infection



E. Sagnelli et al. / Journal of Hepatology 42 (2005) 646–651

IgM can overlap with IgG detection and may persist

Medical Sciences: Chen *et al.* ***Proc. Natl. Acad. Sci. USA 89 (1992)***



IgM-associated pathology in chronic HCV infection

- Mixed cryoglobulinemia (MC)
  - IgM rheumatoid factor
  - Immune complex disease
  - Vasculitis
  - Neuropathy
  - Glomerulonephritis
  - Increased risk of B cell Non-Hodgkin lymphoma (NHL)

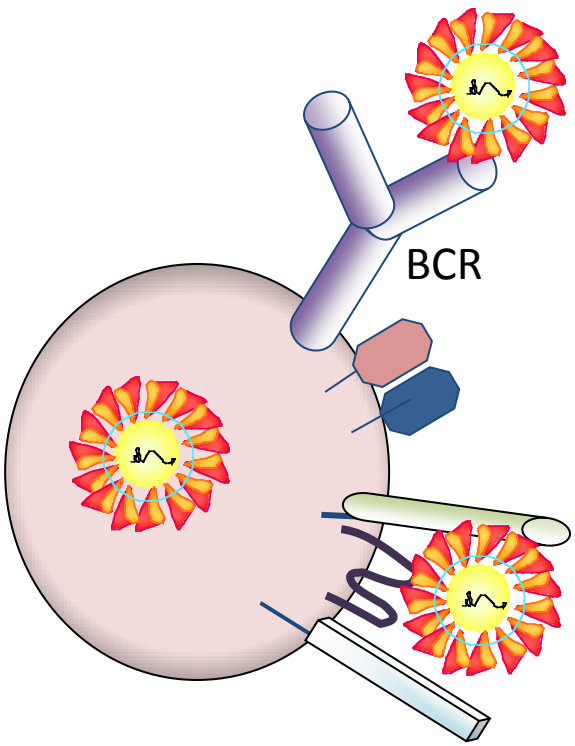
- Almost all patients with symptomatic MC have HCV infection

- MC may be a precursor of NHL
- NHL often regresses with successful antiviral therapy

Pathogenesis of MC and NHL in HCV infection: models

Chronic antigen stimulation?
(What is the antigen?)

Marukian et al
Hepatology 2008



Infection of B cells leading to altered function and proliferation?

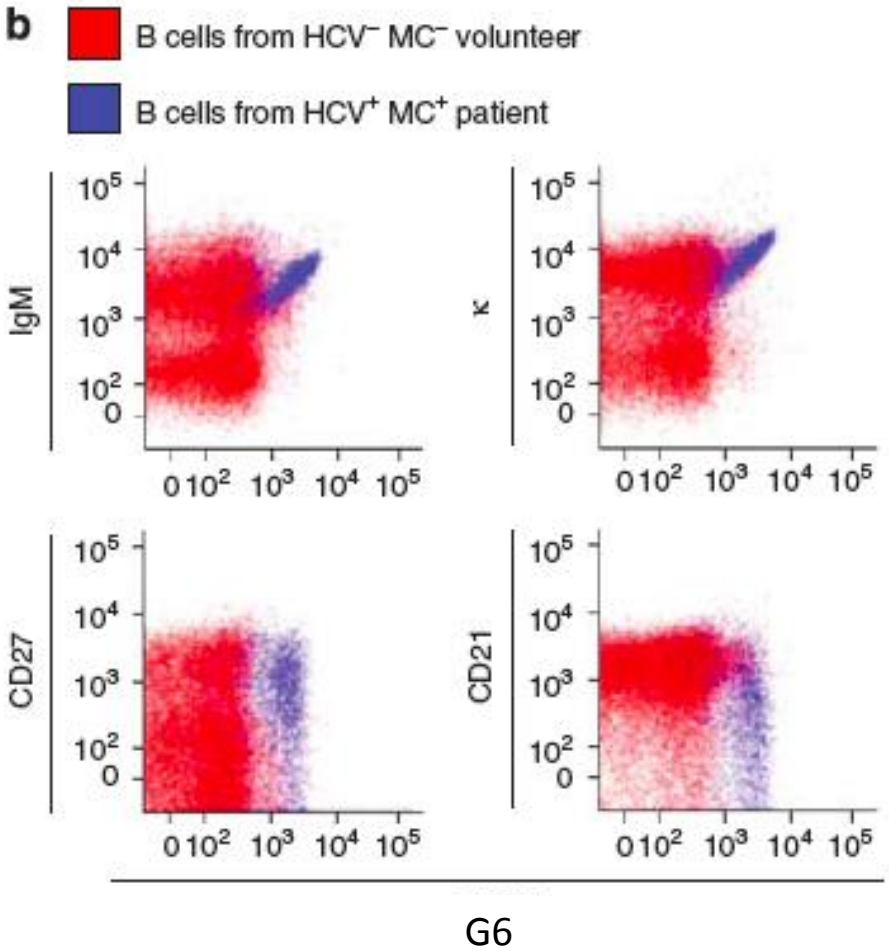
In HCV patients with MC, activated B cells express a very limited set of antigen receptors

Polyclonal stimulation?

Charles et al
Blood 2008
Ni et al.
J. Immunol. 2003

Co-receptor
(CD19, CD81, CD21)

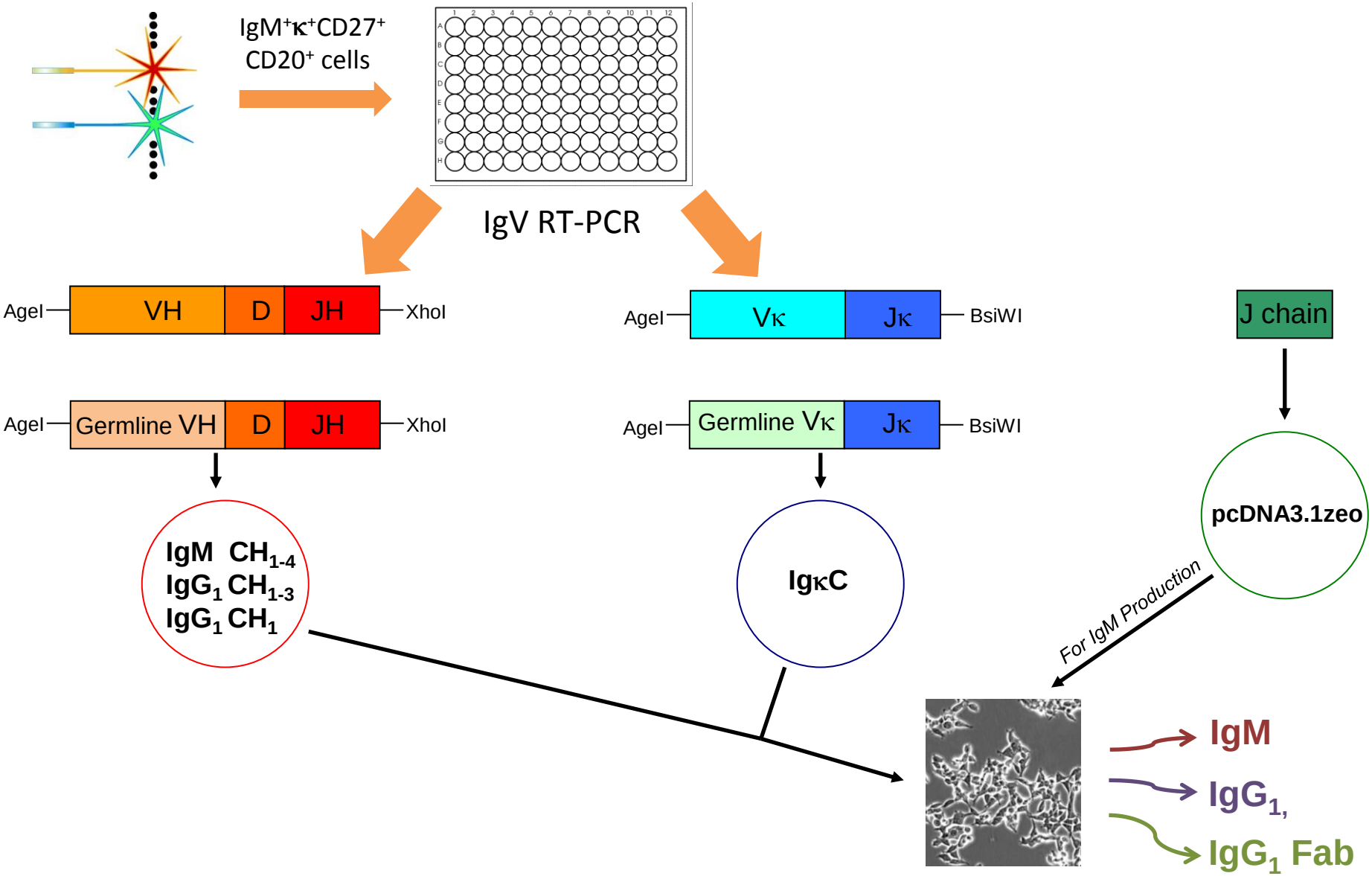
G6 mAb* identifies clonally expanded B cells in HCV MC patients



*G6 recognizes V_H1-69

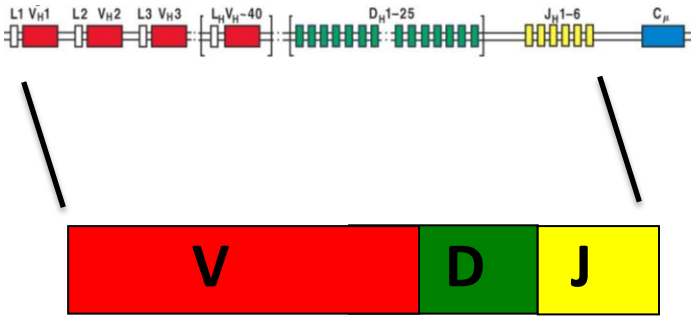
Charles and Dustin, Kidney International 2009

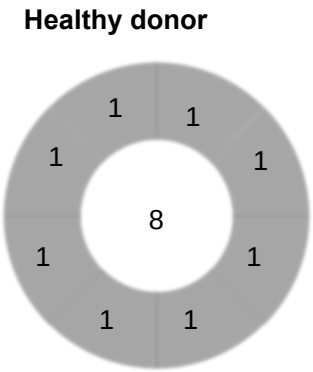
Single B Cell Ig Cloning And Expression Strategy



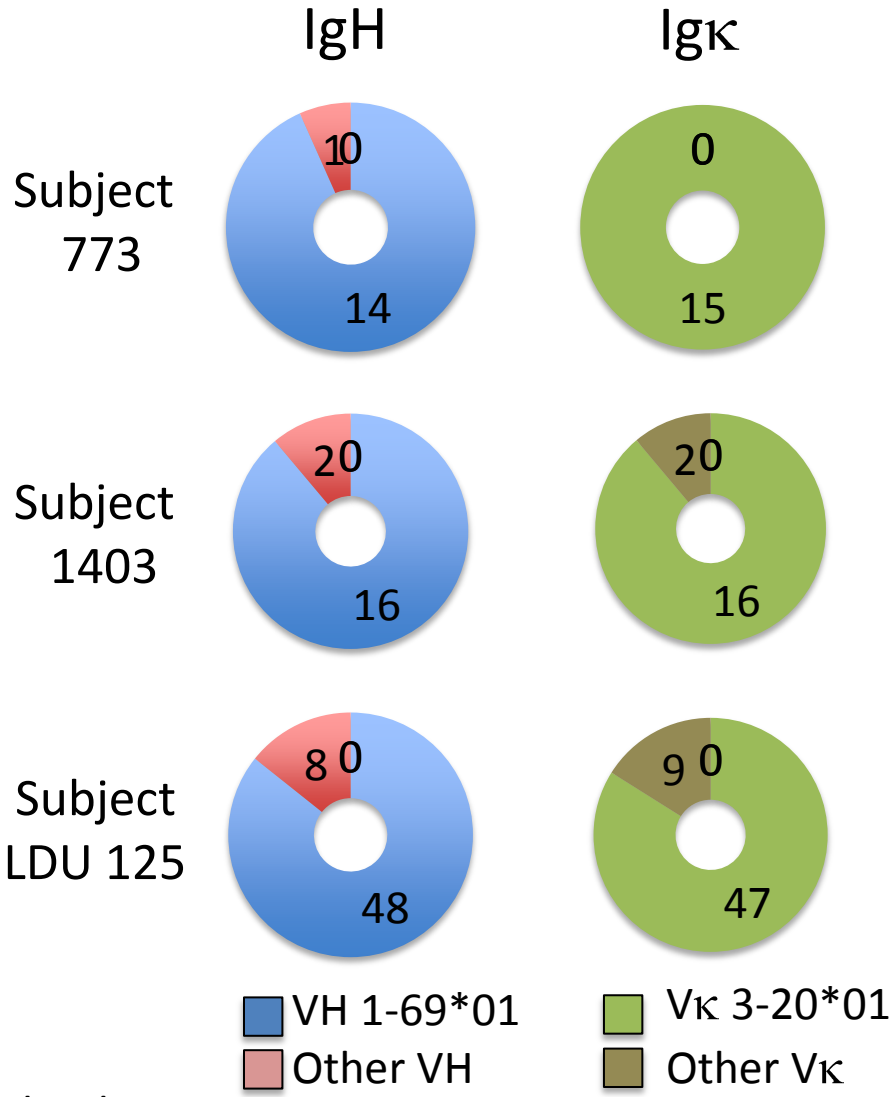
Charles *et al*, J. Immunological Methods 2011

IgM$^+$κ$^+$ Memory B Cells from HCV MC Patients Are Biased Towards V_H1-69 and Vκ 3-20 Gene Segments





V3-74*01/
D3-16*02/J4*02
V1-46*01/
D3-16*02/J4*02
V3-15*07/
D1R1*01R/J2*01
V3-30*03/
D6-06*01/J6*02
V3-07*01/
D3-16*01/J4*02
V3-74*01/
D7-27*01/J3*02
V3-74*01/
D1-20*01/J4*02
V4-04*02/
D4-17*01/J4*02



Charles *et al*, Blood 2008

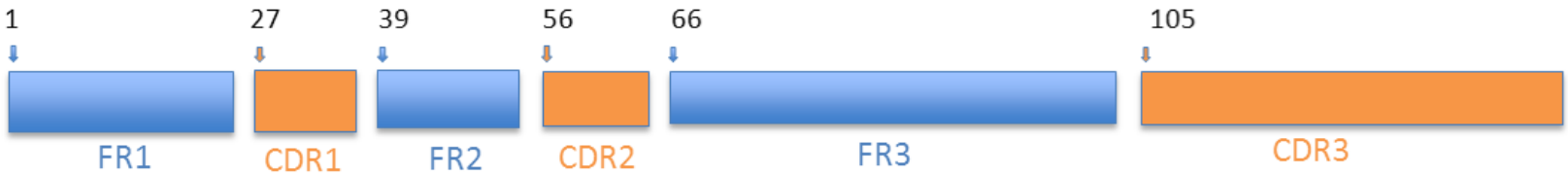
IgM$^+\kappa^+$ Memory B Cells From HCV$^+$MC$^+$ Patients Bear Restricted Immunoglobulins

Subject	Status	# Clones	V_H	D_H	J_H	VD (P,N)	DJ (P,N)	Heavy Chain CDR3 Sequence
LDU 077	Healthy	1/8	3-74*01	3-16*02	4*02	0,13	1,2	CVR**ASWYYDR**WGQG
		1/8	1-46*01	3-16*02	4*02	1,6	1,3	CAR**EHGGGYDY**WGQG
		1/8	3-15*07	IR1*01R	2*01	0,30	1,0	CAT**GGRHSDYLLRGYFDL**WGRG
		1/8	3-30*03	6-06*01	6*02	0,0	0,11	CAK**YSSTSNYYYGMDV**WGQG
		1/8	3-07*01	3-16*01	4*02	0,3	0,2	CAS**GLRYFDY**WGQG
		1/8	3-74*01	7-27*01	3*02	0,24	0,18	CAR**ARGWAVETGSSEMIWAFDI**WGQG
		1/8	3-74*01	1-20*01	4*02	0,14	1,4	CVK**DYNWNAGDY**WGQG
		1/8	4-04*02	4-17*01	4*02	0,3	0,0	CAR**GNGDYYFDY**WGQG
LDU 095	Healthy	1/2	5-a	3-10*01	4*02	1,4	0,10	CAR**HGYGSGSYYDY**WGQG
		1/2	4-34	2-0*01R	6*02	0,34	0,0	CAR**VITRASPGTDGRTNMDV**WGQG
1446	HCV$^+$MC$^-$	2/20	3-33*01	3-22*01	4*02	0,5	0,6	CAR**EEDYYDSRVSDY**WGQG
		1/20	3-30*01	2-15*01	3*02	0,15	1,0	CAR**ENPRMDIVVVVAATYAFDI**WGQG
		1/20	3-48*02	1R*01R	4*03	0,4	1,14	CAS**WVGGAGGRNDY**WGQG
		1/20	3-33*01	4-17*01	3*02	0,14	0,8	CAR**PRGGVTVTSRGDAFDI**WGQG
		1/20	5-a*01	2-02*01	6*03	0,17	0,8	WAR**HGGKGQVVPAAIGNYYYYMDV**WGKG
		1/20	4-b*01	6-19*01	4*02	0,6	0,14	CVR**DSSGWVNGGSDY**WGQG
		1/20	3-15*01	4-17*01	1*01	1,6	1,5	CTT**ETPTTVTTHR**WGQG
		1/20	3-23*01	6-06*01	4*02	1,7	1,7	CAK**DELHSSSSPGPFDY**WGQG
		1/20	3-33*01	4-23*01	3*02	2,6	3,0	CAR**DPFSTVVTPDAFDI**WGQG
		1/20	3-15*01	2-08*02	5*02	3,3	1,7	CTT**DGPVVYAIRGGWFDP**WGQG
		1/20	1-03*01	2-21*02	4*02	2,7	2,1	CAR**GVIYCGGDCYSGDY**WGQG
		1/20	3-23*01	2-15*01	6*02	0,2	0,11	CAK**GGYAEGAYYYYGMDV**WGQG
		1/20	3-15*01	1-07*01	4*02	4,5	2,2	CTT**DLFLLELRCDY**WGQG
		1/20	3-30*03	3-22*01	4*02	0,30	0,1	CAK**GGDTAMVLSNYYDSSGVDY**WGQG
		1/20	5-51*01	3-10*01	4*02	0,10	0,21	CAR**RKSDYYGSGRAGFSDY**WGQG
		1/20	4-34*01	3-10*01	4*02	1,5	0,5	CAR**APSITMVRGVINEFDY**WGQG
		1/20	3-21*01	4-17*01	4*02	0,5	0,3	CAR**DRPTVTTVFDY**WGQG
		1/20	3-15*01	3-10*01	6*03	2,2	0,0	CTT**DRYYGSGSYYYGMDV**WGQG
		1/20	1-03*01	6-25*01	6*03	3,1	0,2	CAR**ASGHYYYYMDV**WGKG
		1/20	3-15*01	6-19*01	4*02	0,7	0,13	CTT**VGVIAVAGGFRGDY**WGQG
773	HCV$^+$MC$^+$	7/15	1-69*01	6-13*01	4*02	0,0	0,8	CAR**EGIAAAVNPFDY**WGQG
		3/15	1-69*01	3-03*01	4*02	0,16	0,2	CAR**DYGERESGYYFY**WGQG
		4/15	1-69*06	2-15*01	4*02	1,4	1,14	CAR**EGRSGNVNPYDY**WGQG
		1/15	1-69*06	3-16*01	2*02	2,8	0,13	CAR**EGEAGGVGRNWAFDI**WGQG
1403	HCV$^+$MC$^+$	9/18	1-69*01	3-22*01R	4*02	1,5	0,19	CAR**AYGNNPTEVYWVY**WGQG
		5/18	1-18*01	2-15*01	6*02	0,9	0,34	CAR**DGAVCSGSSCRGNYYYYGLDV**WGQG
		1/18	1-69*01	4-17*01	4*02	1,2	2,9	CAR**EGYGDYGRPYDY**WGQG
		1/18	3-20*01	1-26*01	6*02	2,1	0,0	CAR**AGGSYYYYYGMDV**WGQG
		1/18	3-21*01	3-22*01	4*02	3,11	1,6	CTR**DGPYYDSSGFEY**WGQG
		1/18	3-23*01	2-15*01	6*02	1,13	1,8	CAK**TLRSNVVVVATTHYYGMDV**WGQG
1308	HCV$^+$MC$^+$	15/15	3-7*01	1-26*02	3*02	0,0	0,12	CAR**DGDLGSGSRSMGAFDI**WGQG
1432	HCV$^+$MC$^+$	22/22	1-69*01	3-03*01	4*02	0,16	0,2	CAR**DYGERESGYYFY**WGQG
1716	HCV$^+$MC$^+$	16/20	1-69*01	5-05*01	4*02	1,9	0,4	CAR**EGFPDTAMVNPFDY**WGQG

V_H1-69 variable region is associated with broadly cross-neutralizing antibodies

- HIV
  - C.-c. Huang et al., PNAS 2004
- H1N1 influenza
  - J. Wrammert et al., J Exp Med 2011
- Respiratory syncitial virus
  - J. V. Williams et al., Molecular Immunology 2009
- HCV
  - Chan et al., Blood 2001
  - Keck et al., J Virol 2008

Different HCV MC Patients Express Highly Similar Clonal IgVH




```
                 FR1                      CDR1       FR2                 CDR2        FR3                                  CDR3
VH1-69*01        QVQLVQSGAEVKKPGSSVKVSCKAS GGTFSSYA  ISWVRQAPGQGLEWMGG IIPIFGTA  NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYC AREGIAAAVNPFDY&

773_D7           ------------------------- -----N-- ----------------- -------G  K-----------------------------------  AREGIAAAVNPFDY
773_D10          ------------------------- -----T-- ----------------- ---M----  ------K--------------------------------  AREGIAAAVNPFDY
773_E3           ------------------------- -------- ----------------- -------- -------------------------------------  AREGIAAAVNPFDY
773_E5           ------------------------- -----R-- ----------------- -------P  ---------------D----------N------------  AREGIAATINPFDY
773_F8           ------------------------- -------- ----------------- -------- ---------------T--------------------F--  AREGRSGNLNPYDY
773_G1           ------------------------- -----R-- ----------------- -------- K---------------------------F--------  AREGIAAAVNPFDY
773_G7           -----------------------S- -------- ----------------- ---L---G  ----R----L--------N-V--------F--------  AREGRSGNLNPYDY

1403_D9          ------------------------- -------- ----------------- -------- -------------------------------------  ARVGNNPTEVY
1403_F2          ------------------------- --S--R-T ----------------- ---M---- ------------------T------N---YD-----F-  AREGYGDYGRPYDY

1432_D2          ------------------------- --S----- ----------------- V--L---- ---E---------------------T------M-----  ARDYGERESGYYFY
1432_E2          -----------------------T- --S----- ----------------- -------- ---E-----------------------------------  ARDYGERESGYYFY
1432_E3          ------------------------- --S----- ---M-------------- -------- ---E------------------------G---------  ARDYGERESGYYFY
1432_E11         ------------------------- -------- ----------------- -------- ---E-----------------------------------  ARDYGERESGYYFY

125_A4           ------------------------- -------- ----------------- -------- -------------------------------------  ARGPDSSGYYYFY
125_B2           ------------------------- -D---T-- ----------------- -------P  ----R-------------R-S---V-G-T-----I---  AREFNFDTTGYYFLY
125_B10          ------------------------- -----NS- ----------------- -------- ----R----------T--------M-------------  ARGPDSSGYYYFY
125_D9           ------------------------- ------S- ----------------- -------- ----R-----------------K-M-------------  ARGPDSSAYY
125_D12          ------------------------- -----N-- ----------------- -M-----P  -----------------RR-V---V-G-T-----I---  AREFNYDSSGYYY
125_E3           ------------------------- -----N-- ------V---------- -T------  -------------------------------------  ARGPDSSGYYYFY
125_E5           ------------------------- -------- ----------------- -------- ------------------------D--------------  ARGPESSGYYYFY
125_H5           ------------------------- -------- ----------------- -------- -------------------------------------  ARGPETSGYYYFY
```

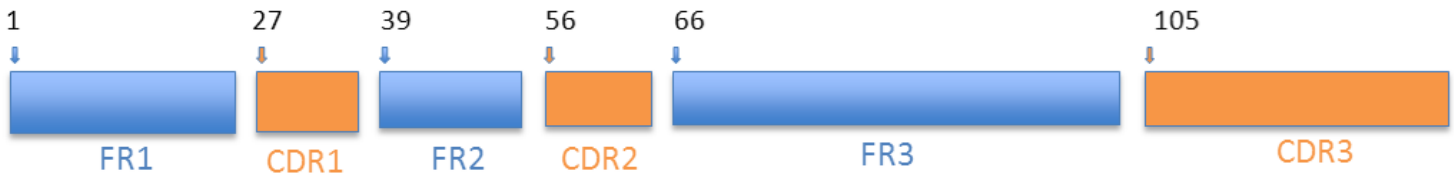
VH1-69*06

Two broadly-neutralizing anti-HCV mAbs

```
CBH5             ------------------------T --K---NF -------V--------- VV-V--GG  S-P------L-----D-R--S-----G---D-------  ARPHGDSSGIYRRPLDY
(Chan et al, 2001)

HC11             ------------------------- -A----FI F---------------- ---M----  K--PRL-----------------D-----F--------  AMEVPGFCRGGSCSSGYMDV
(Keck et al, 2008)
```

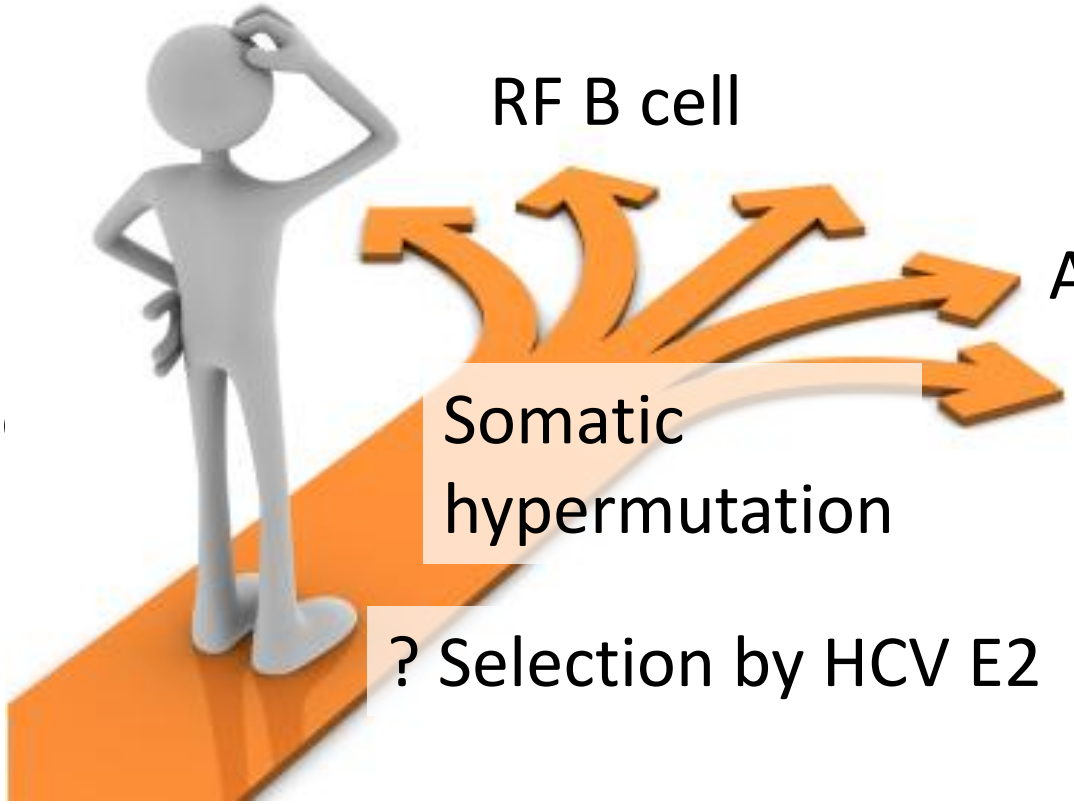
FR: Framework Region *CDR: Complementary Determining Region*

Different HCV MC Patients Express Highly Similar Clonal IgVκ




```
Vk3-20*01      EIVLTQSPGTLSLSPGERATLSCRAS  QSVSSSY  LAWYQQKPGQAPRLLIY  GAS  SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC  &

773_D7         --------------------------  -----N-  V----------------  ---  T-----------------------------------  QQYGSSPQT
773_D10        --------------------------  -----T-  ------------------  ---  T-----------G-----------------------  QQYGSSPQT
773_E3         --------------------------  -----T-  ------------------  ---  T-----------------------------------  QQYGSSPQT
773_E5         --------------------------  -----T-  -----H-----------  -S-  T-----------------------------------  QQYGSSPQT
773_F8         --------------------------  -----T-  ------------------  ---  T-----------------------------------  QQYGSSPQT
773_G1         --------------------------  -----T-  ------------------  ---  T-----------------------------------  QQYGSSPQT
773_G7         --------------------------  -------  -----H-----------  ---  ----------Y--------------------------  QQYGSSPQT

1403_D9        --------------------------  -------  ----------V------  ---  ----------R----A--------------------  QQYGSSPT
1403_F2        --------------------------  -I-----  ------------------  ---  ---------------------S------------S-  QQYGSSPYT

1432_D2        ------------------------S--  -------  ------------------  ---  --------------------S----------------  QQYGSSPRT
1432_E2        --------------------------  -------  -------L---------  -S-  ------------------------------------  QQYGSSPRT
1432_E3        ------------------------S--  -------  ------------------  ---  --------------------S----------------  QQYGSSPRT
1432_E11       ------------------------S--  -------  ------------------  ---  ------------------------------------  QQYGSSPRT

125_A4         --------------------------  LTI--N-  -------R---------  ---  N--A---------------------------------  QQYASSRT
125_B2         ----------------Q---------  LTL--N-  V----------------  ---  ---A-------------------------------  QQYASSRT
125_B10        --------------------------  -------  ------------------  ---  ---------------A------V-------------  QQYGSSPYT
125_D9         --------------------------  -----T-  ------------------  ---  ---------------A------V-------------  QQYGSSPYT
125_D12        --------------------------  -----T-  ------------------  -T-  ------------------------------------  QQYGSSPST
125_E3         --------------------------  -----T-  ------------------  -TF  ------------------------------------  QQYGSSPYT
125_E5         --------------------------  -----T-  ------------------  -T-  ------------------------------------  QQYGSSPYT
125_H5         --------------------------  -----T-  ------------------  -T-  ------------------------------------  QQYGSSPYT

HC11           --------------------------  H-----N  ---------------M-  ---  R-----------------------------------  QQYGSSPIT
(Keck et al, 2008)

VK1-5          DIQMTQSPSTLSASVGDRVTITCRAS  QSISSW  LAWYQQKPGKAPKLLIY  DAS  SLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYC  &

CBH5 (VK1-5)   -------------------A------  ------  ------------------  ---  NVQD-------------------N------------  QYYNFSGT
```

Rheumatoid factor: a good antibody that took a wrong turn?



RF B cell

Anti-HCV B Cell

Germlin

$V_H1\text{-}69^+$

Somatic hypermutation

? Selection by HCV E2

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