Role of Basic / Translational Research in Viral Myocarditis

성균관의대

삼성서울병원 순환기내과

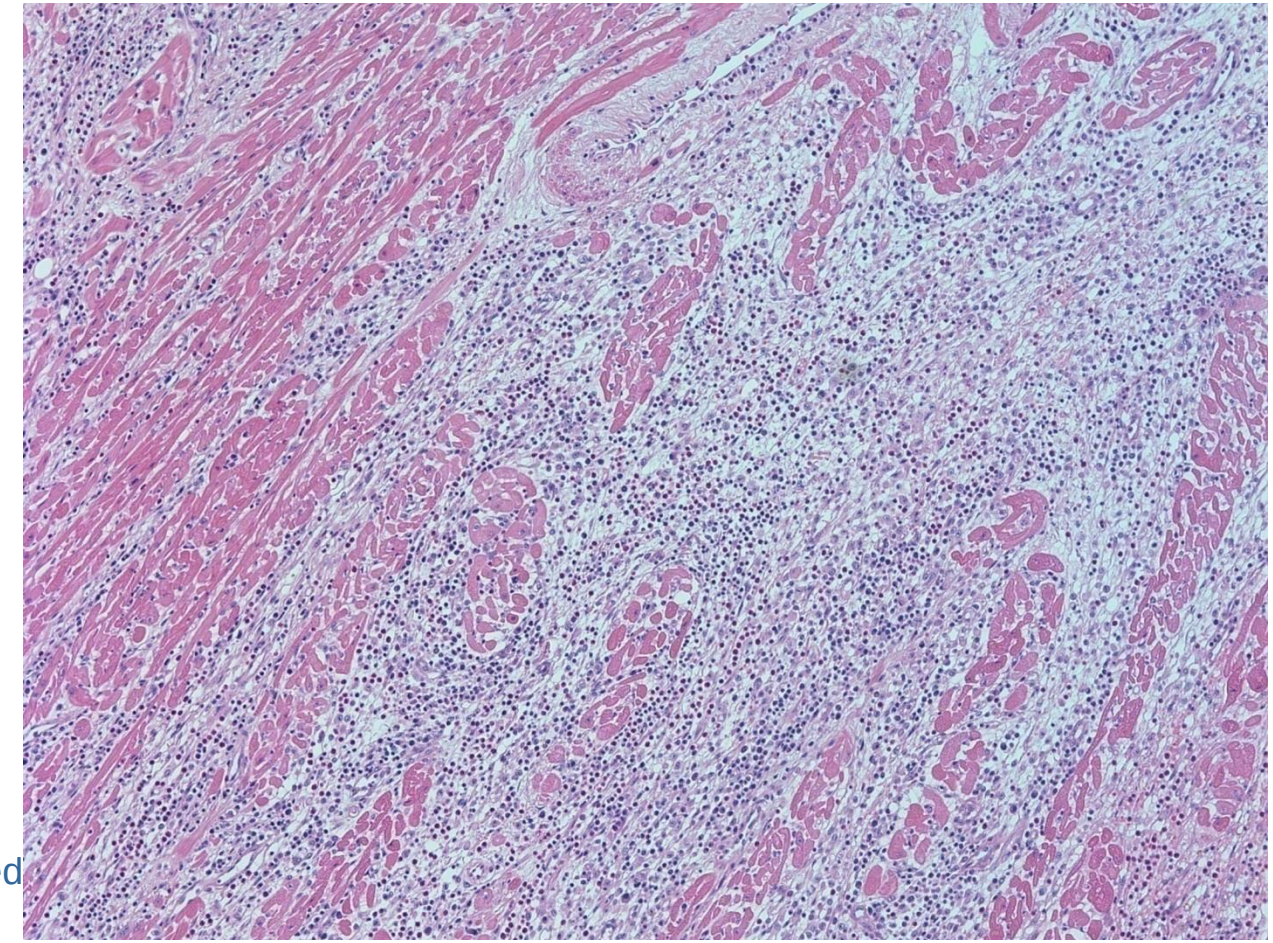
전은석

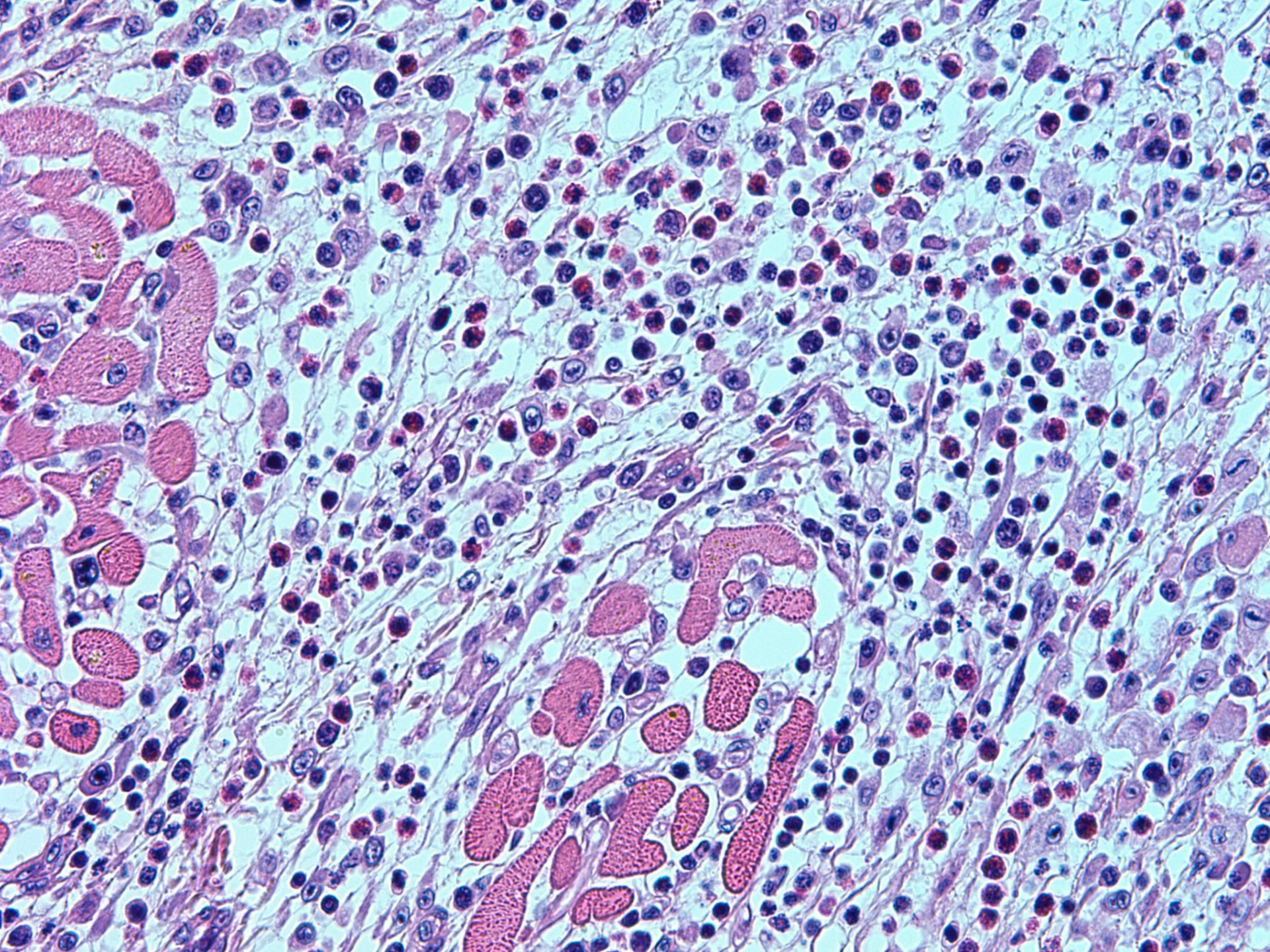
Talk about ....

1. Myocarditis…. Definition / Etiology
2. Pathogenesis: Viral
3. Diagnosis :
   - Development of ELISA
4. Clinical course
   - Outcome of Fulminant myocardtis and prognostic factors
5. Treatment
   - Developing antiviral agents

Definition of Myocarditis

- an INFLAMMATION INFILTRATE and by INJURY to the adjacent myocardial cell that is *not typical of* INFARCTION



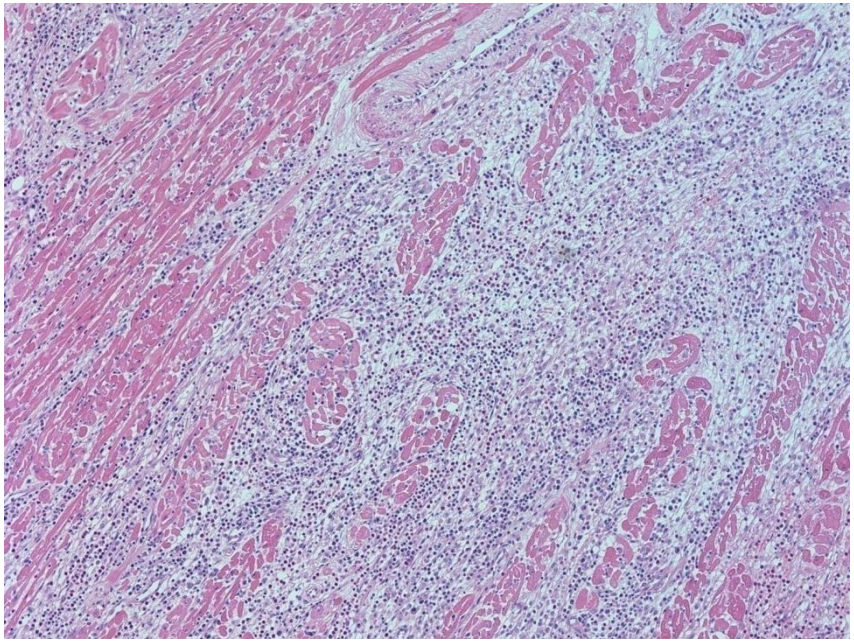


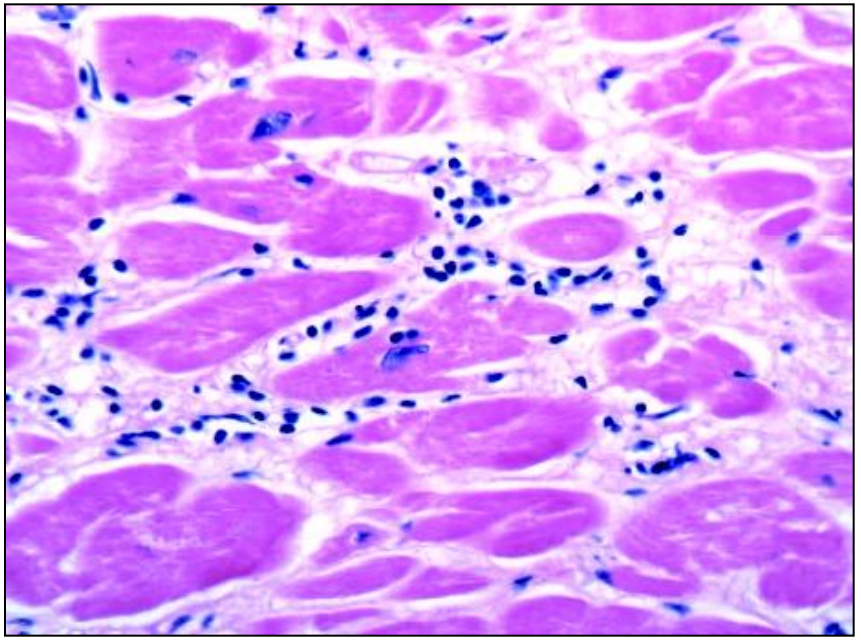
Dallas Criteria

- Inflammations
  - Active vs borderline
  - inflammation cell infiltration with or w/o myocardial necrosis or injury
- Inflammatory infiltrates;
  - lymphocytic, eosinophillic, granulomatous
- Amount of inflammation
  - Mild, moderate, severe
- Distribution of inflammation
  - Focal, confluent, diffuse

Dallas Criteria

- Inflammations
  - Active vs borderline
  - inflammation cell infiltration with or w/o myocardial necrosis or injury



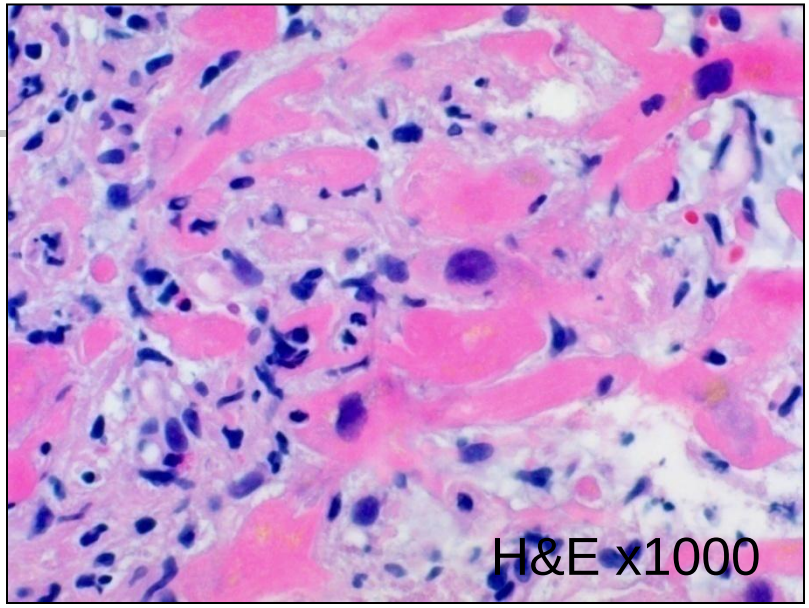


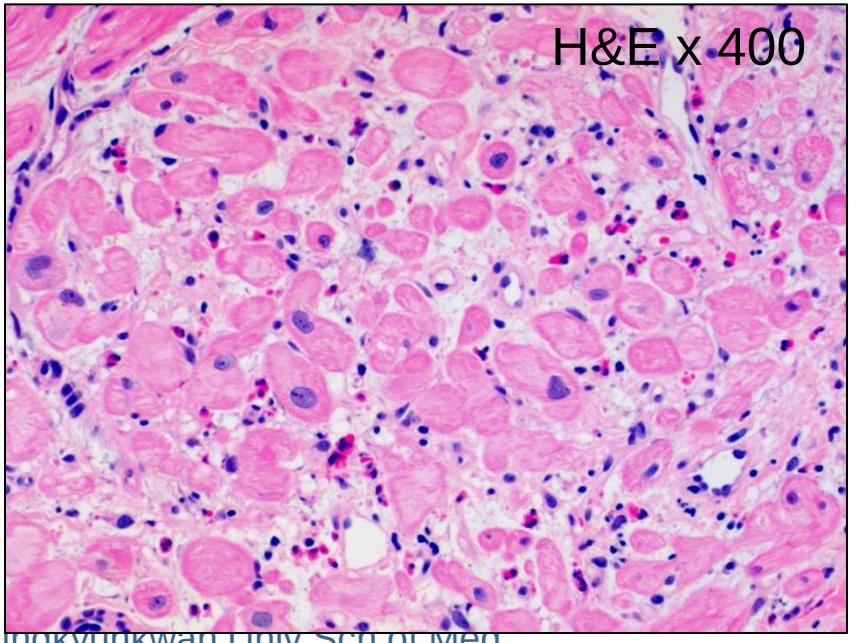
Circulation 2006;113:876

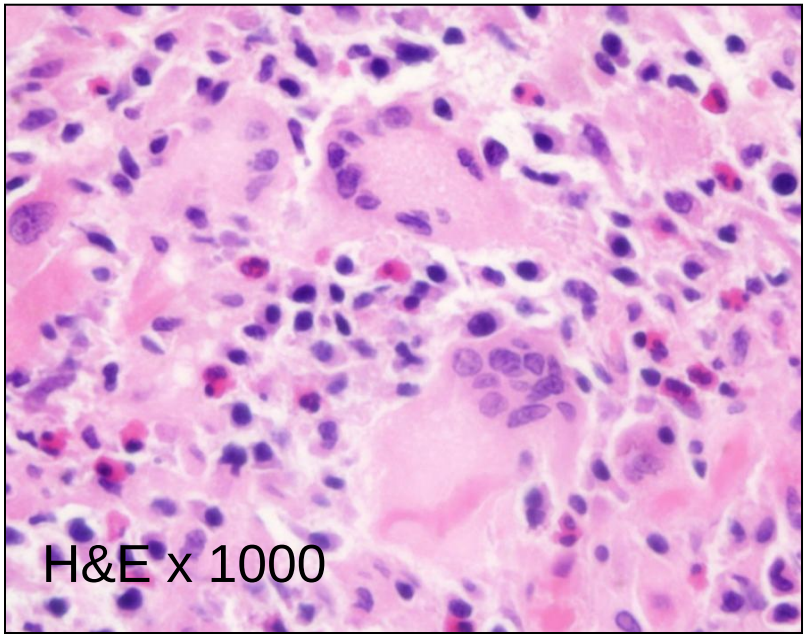
Dallas Criteria

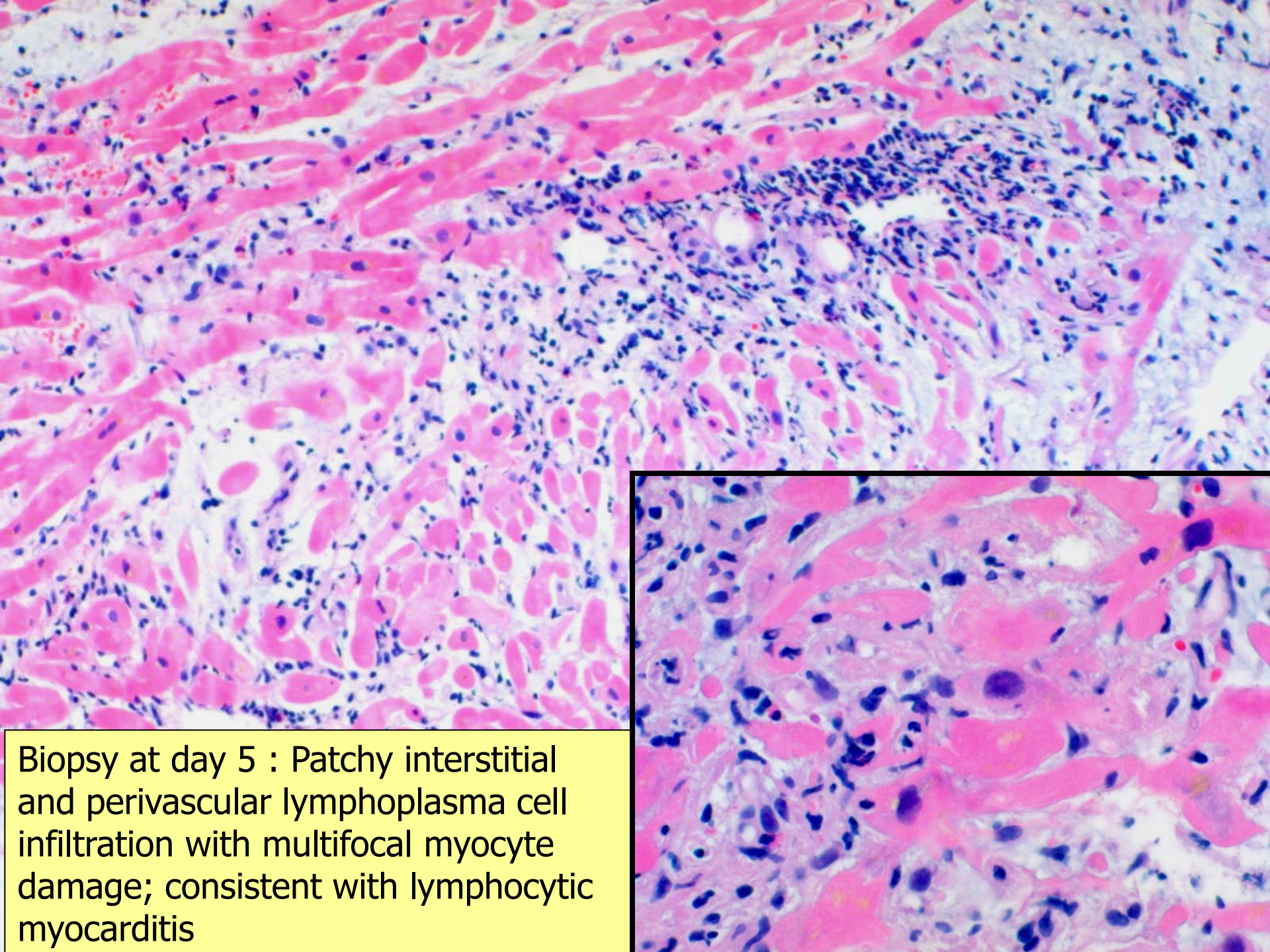
Inflammatory infiltrates

- lymphocytic
- eosinophillic
- granulomatous

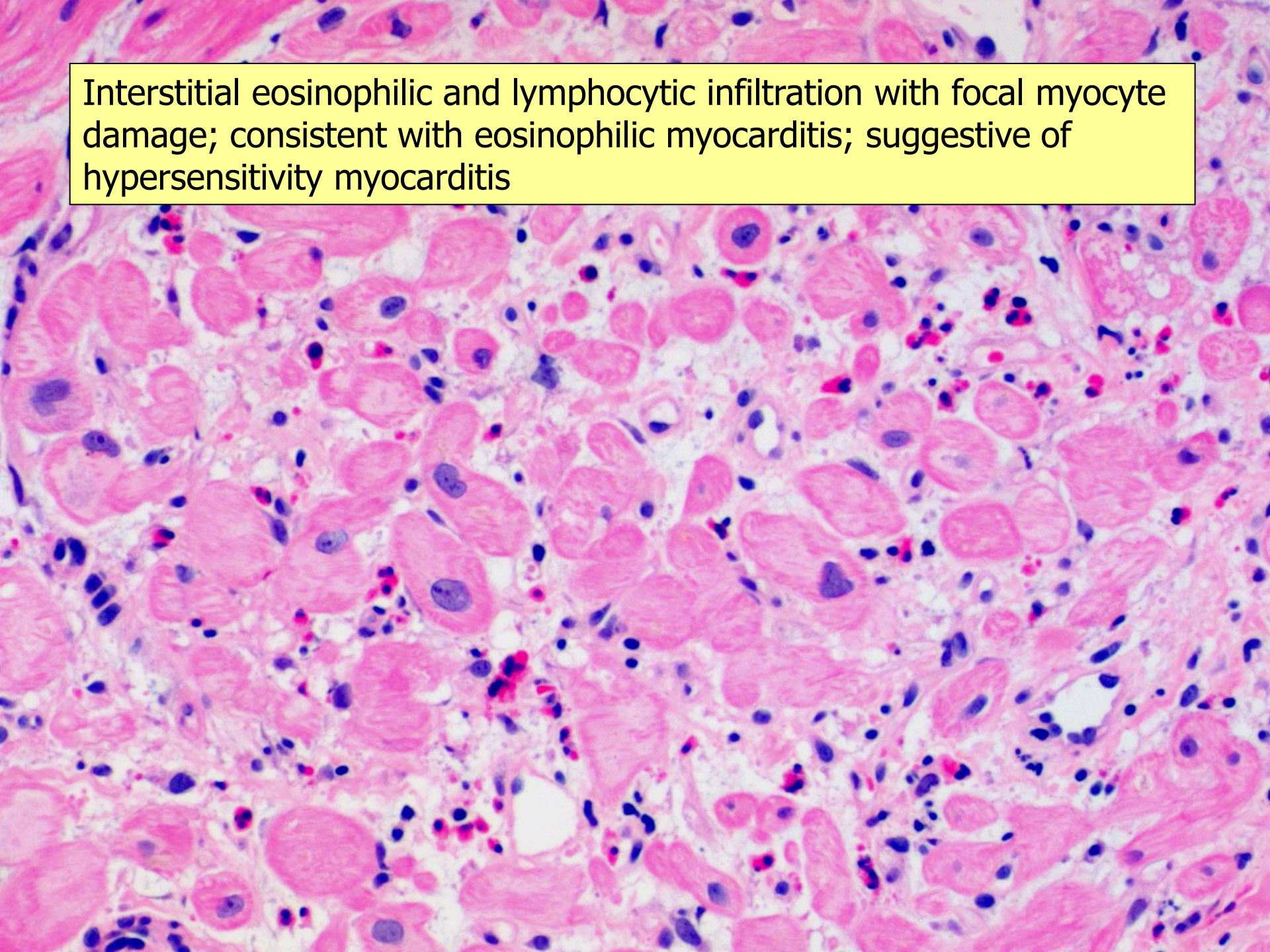


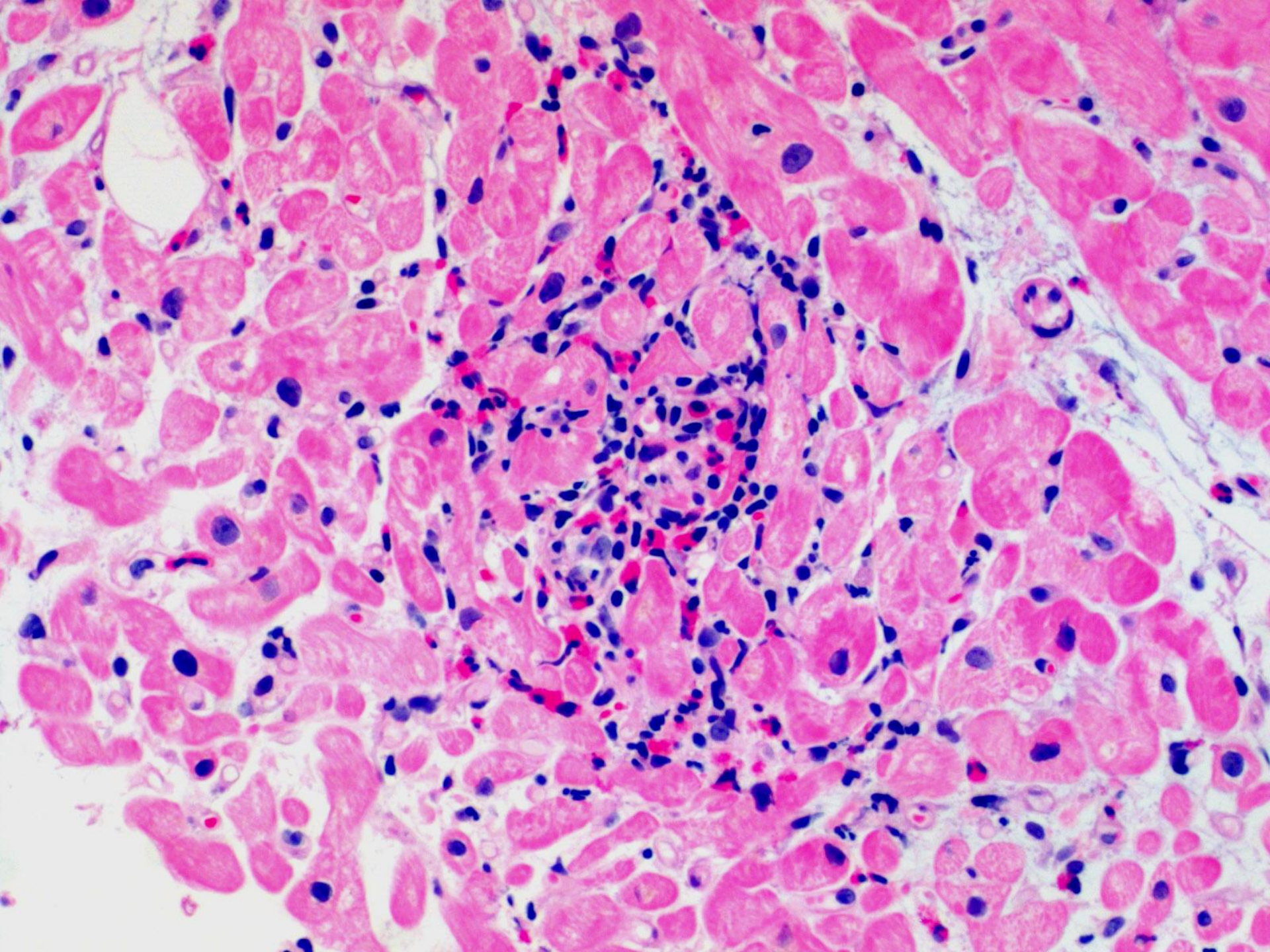


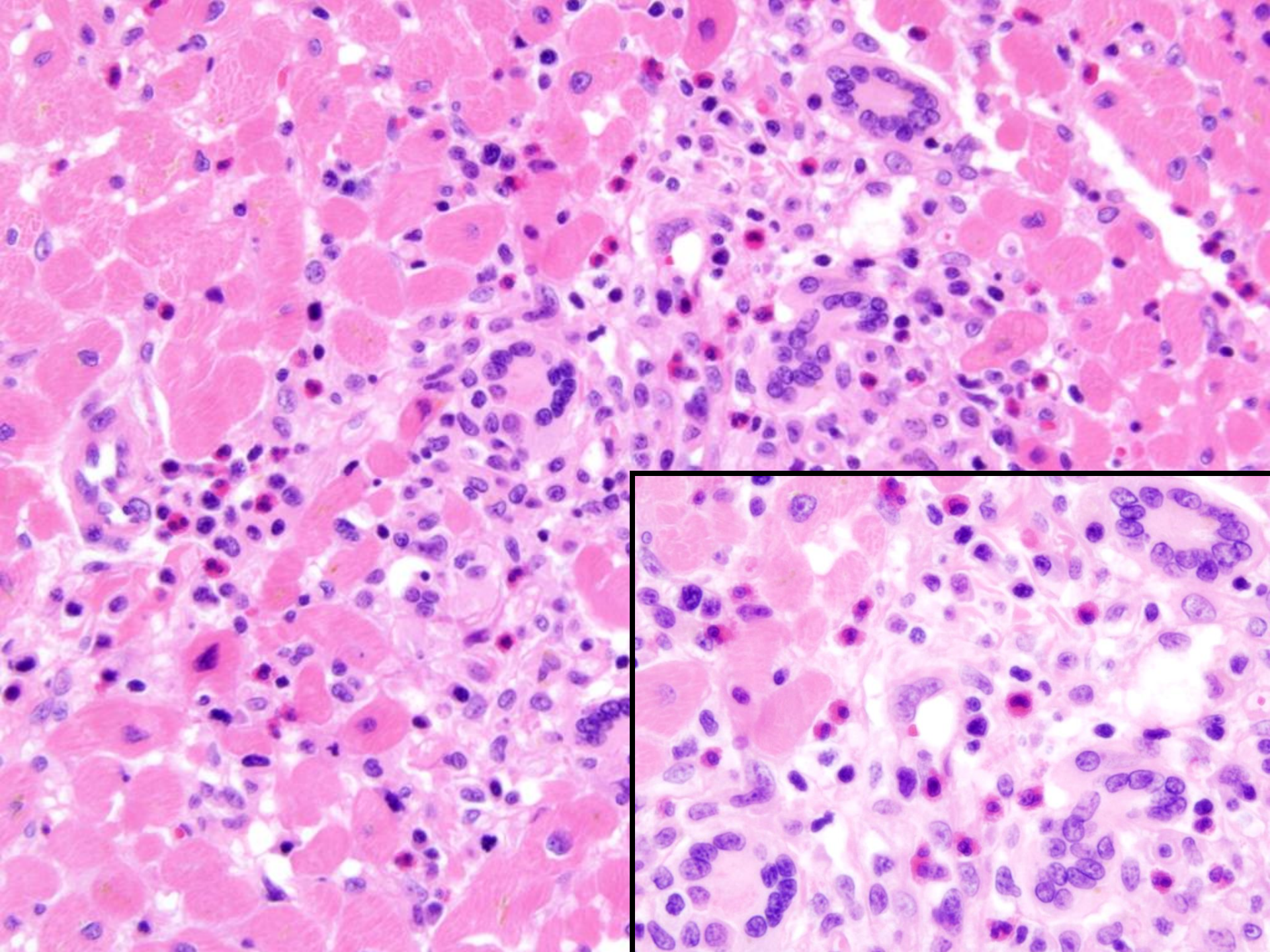




Biopsy at day 5 : Patchy interstitial and perivascular lymphoplasma cell infiltration with multifocal myocyte damage; consistent with lymphocytic myocarditis


Interstitial eosinophilic and lymphocytic infiltration with focal myocyte damage; consistent with eosinophilic myocarditis; suggestive of hypersensitivity myocarditis





Etiologic Agents of Myocarditis

Infectious Agents

Virus

- **Coxsackievirus(A,B)**
- **Adenovirus**
- **HIV**
- **HCV**

Bacteria :

- mycobacterial / streptococcal mycoplasma pneumoniae
- Treponema pallidum

Metazoa : Trichinosis, Echinococcosis

Protozoa : **Trypanozoma cruzi**

Fungus : Aspergillosis, Cadidiasis ....

Toxic Agents

- **Anthracyclines**
- **Catecholamines**
- Interleukin-2
- Coccaine

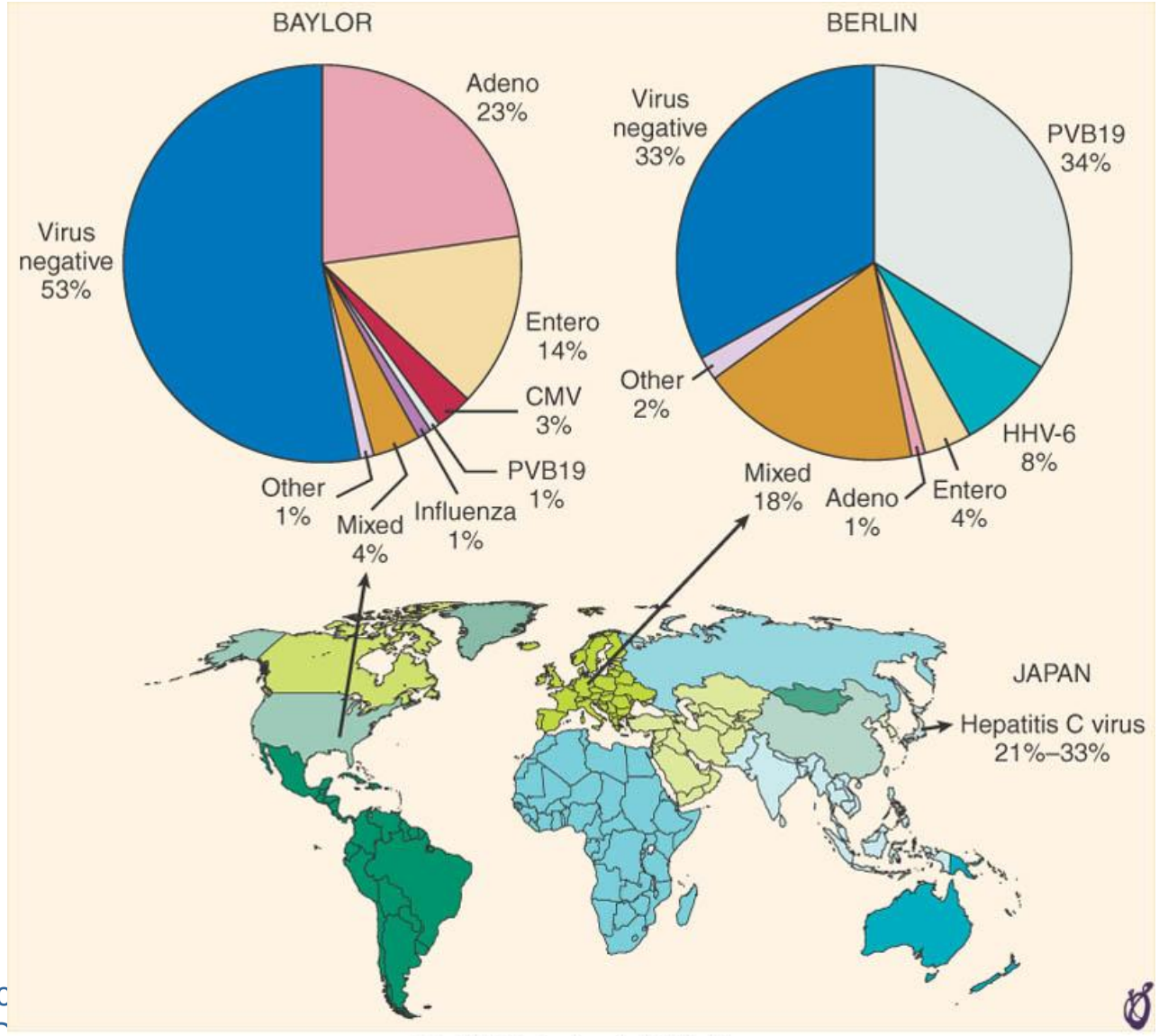
Hypersensitivity

- sulfonamide
- cephalosporin
- **diuretics/digoxin**
- **TCA**
- **dobutamine**

Immunological Syndromes

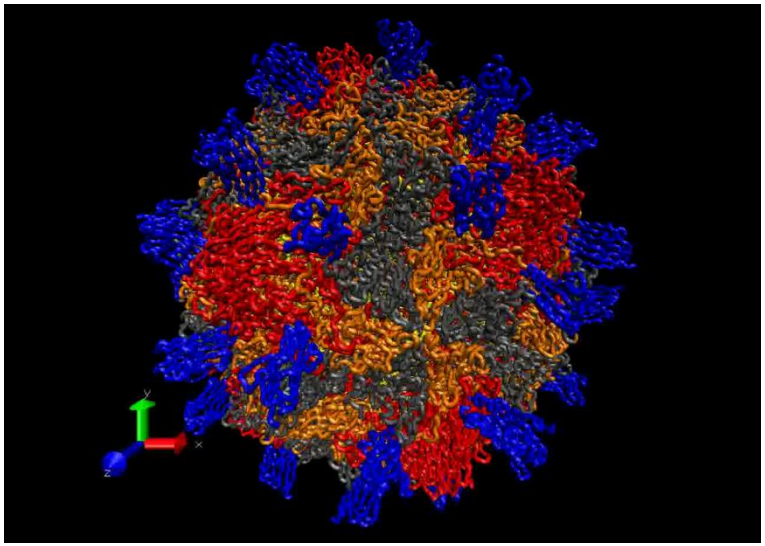
- Chug-Strauss
- infl bowel dis
- Sarcoidosis
- diabetes
- Sarcoidosis
- SLE
- Takayasus'
- Thyrotoxicosis
- Wegener's
- PSS / MCTD

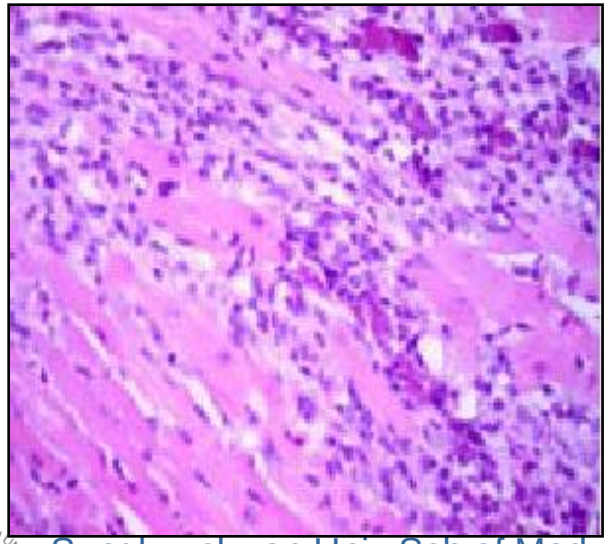
Incidence of Viral Genomes in Myocardium

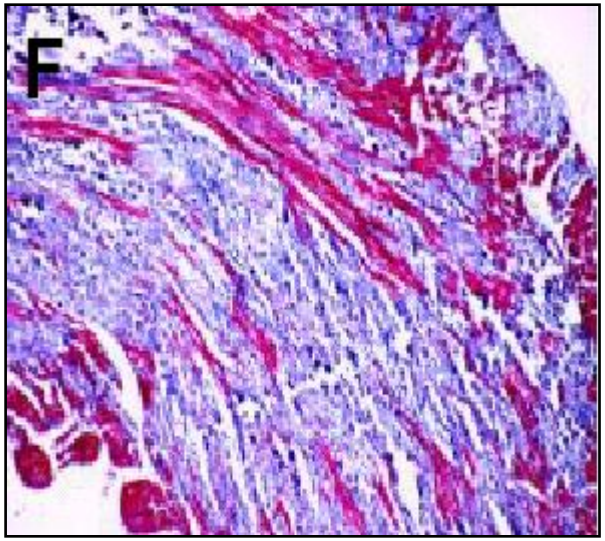


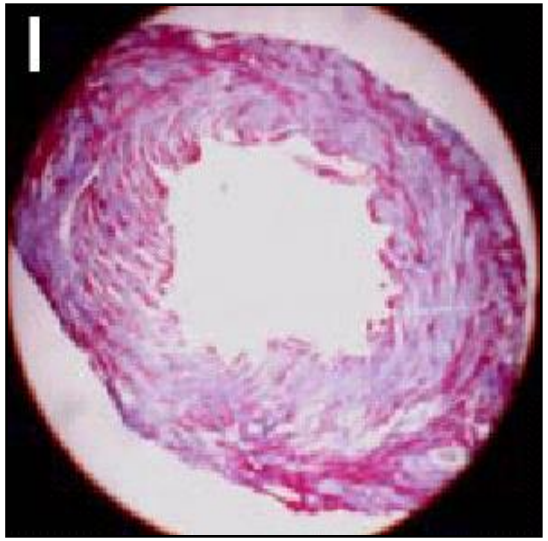
Coxsackievirus Type B3 (CVB3)

- 7.4 Kb ssRNA picornavirus
- highly cardiotropic
- induces myocarditis
- progress to cardiomyopathy









Diagnostic Criteria

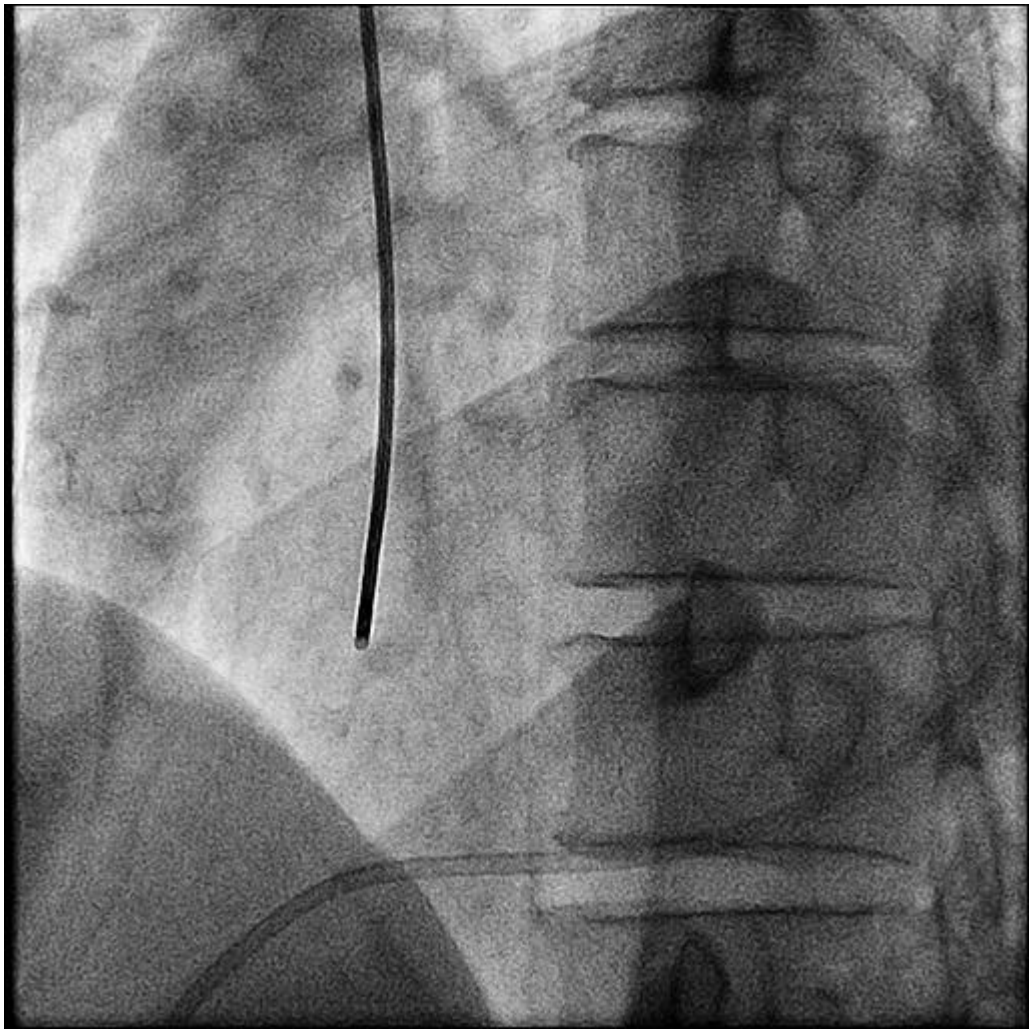
1. Clinical Symptoms
2. Myocardial Injury
3. Cardiac MRI
4. Biopsy

TABLE 66–2	Expanded Criteria for Diagnosis of Myocarditis
Suspicious for myocarditis = 2 positive categories	
Compatible with myocarditis = 3 positive categories	
High probability of being myocarditis = all 4 categories positive (Any matching feature in category = positive for category)	
Category I: Clinical Symptoms	
Clinical heart failure	
Fever	
Viral prodrome	
Fatigue	
Dyspnea on exertion	
Chest pain	
Palpitations	
Presyncope or syncope	
Category II: Evidence of Cardiac Structural/Functional Perturbation *in the Absence* of Regional Coronary Ischemia	
Echocardiography evidence	
Regional wall motion abnormalities	
Cardiac dilation	
Regional cardiac hypertrophy	
Troponin release	
High sensitivity (>0.1 ng/ml)	
Positive indium-111 antimyosin scintigraphy	
and	
Normal coronary angiography *or*	
Absence of reversible ischemia by coronary distribution on perfusion scan	
Category III: Cardiac Magnetic Resonance Imaging	
Increased myocardial T_2 signal on inversion recovery sequence	
Delayed contrast enhancement following gadolinium-DTPA infusion	
Category IV: Myocardial Biopsy—Pathological or Molecular Analysis	
Pathology findings compatible with Dallas criteria	
Presence of viral genome by polymerase chain reaction or in situ hybridization	

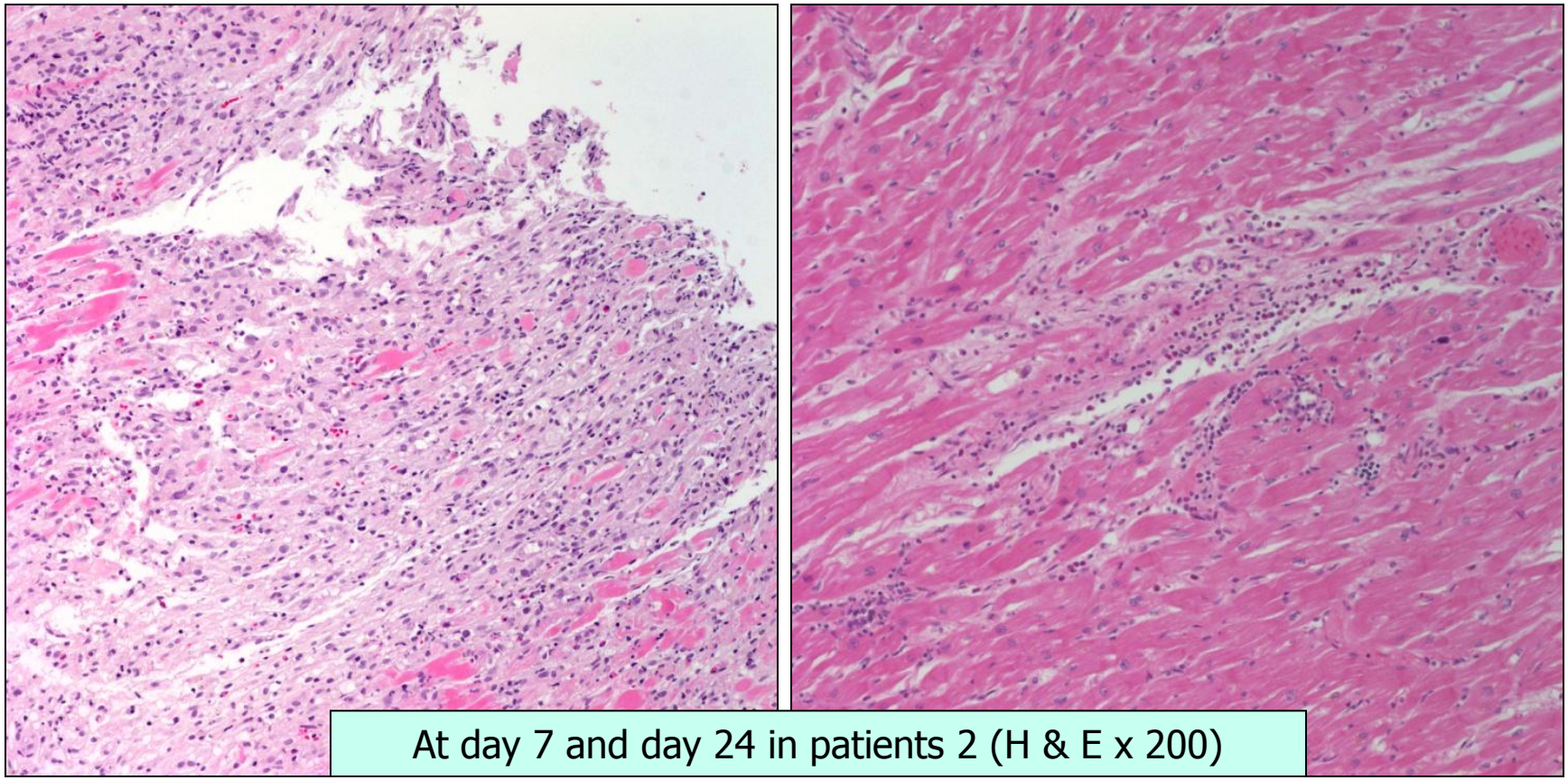
Diagnostic evaluation

1. Endomyocardial biopsy
2. Cardiac biomarkers
   - Troponin I; sensitivity 34%, specificity 89%
   - CK, CK-MB;low predictive value
3. Immunologic Approaches
   - Immunohistochemical staining for Lc
   - MHC expression – chronic for of myocarditis
4. Myocardial imaging

심근조직검사 Myocardial Biopsy



Pathologic changes in serial biopsy

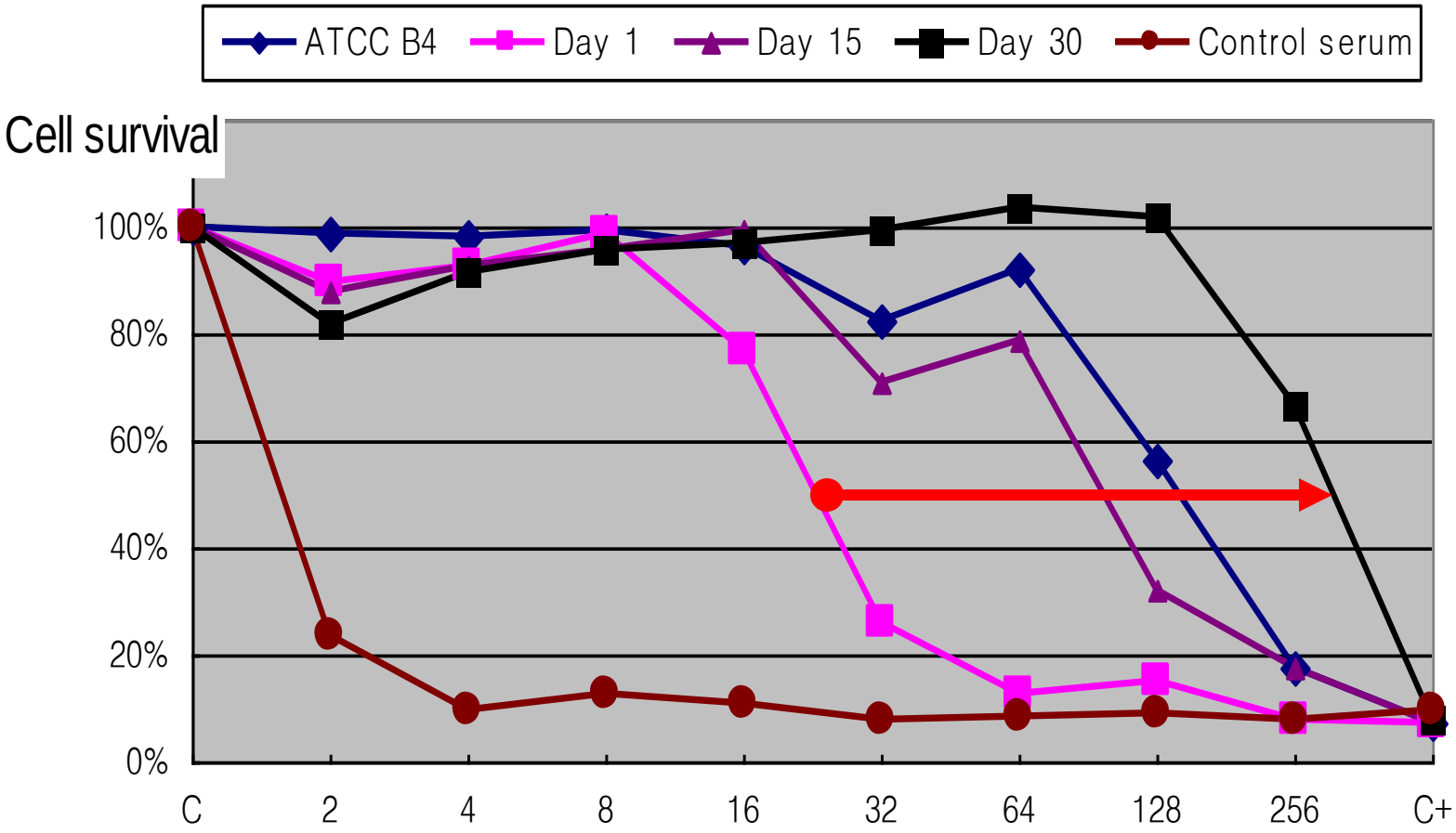


At day 7 and day 24 in patients 2 (H & E x 200)

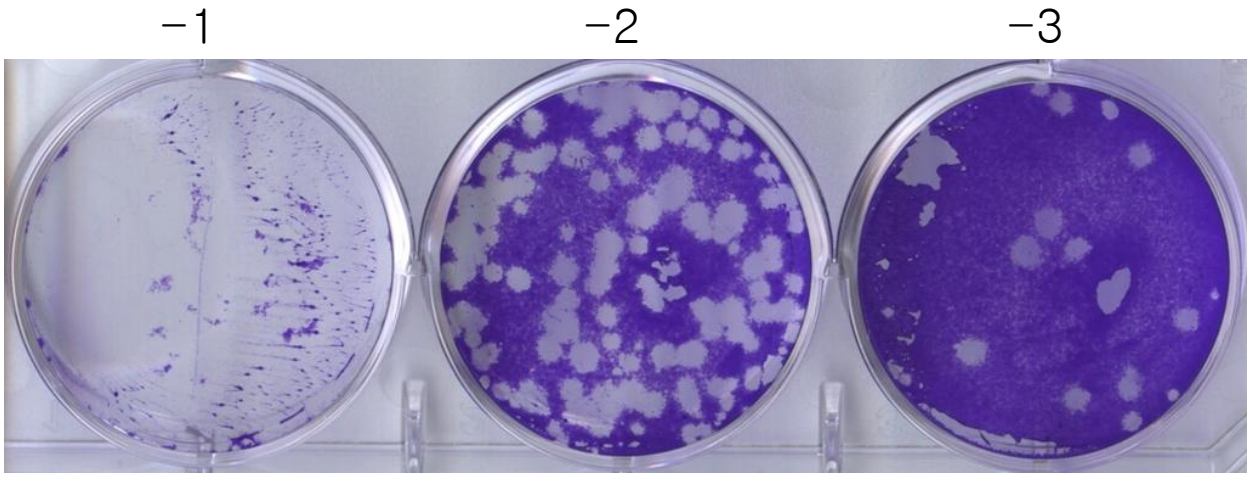
Diagnosis of Viral Myocarditis

- Serology
  - 4-fold increase of Neutralization Ab titer
- Virus culture in tissue
- Viral genomes in tissue
  - by PCR, Hybridization
- Viral proteins in heart

Neutralization test with serial sera

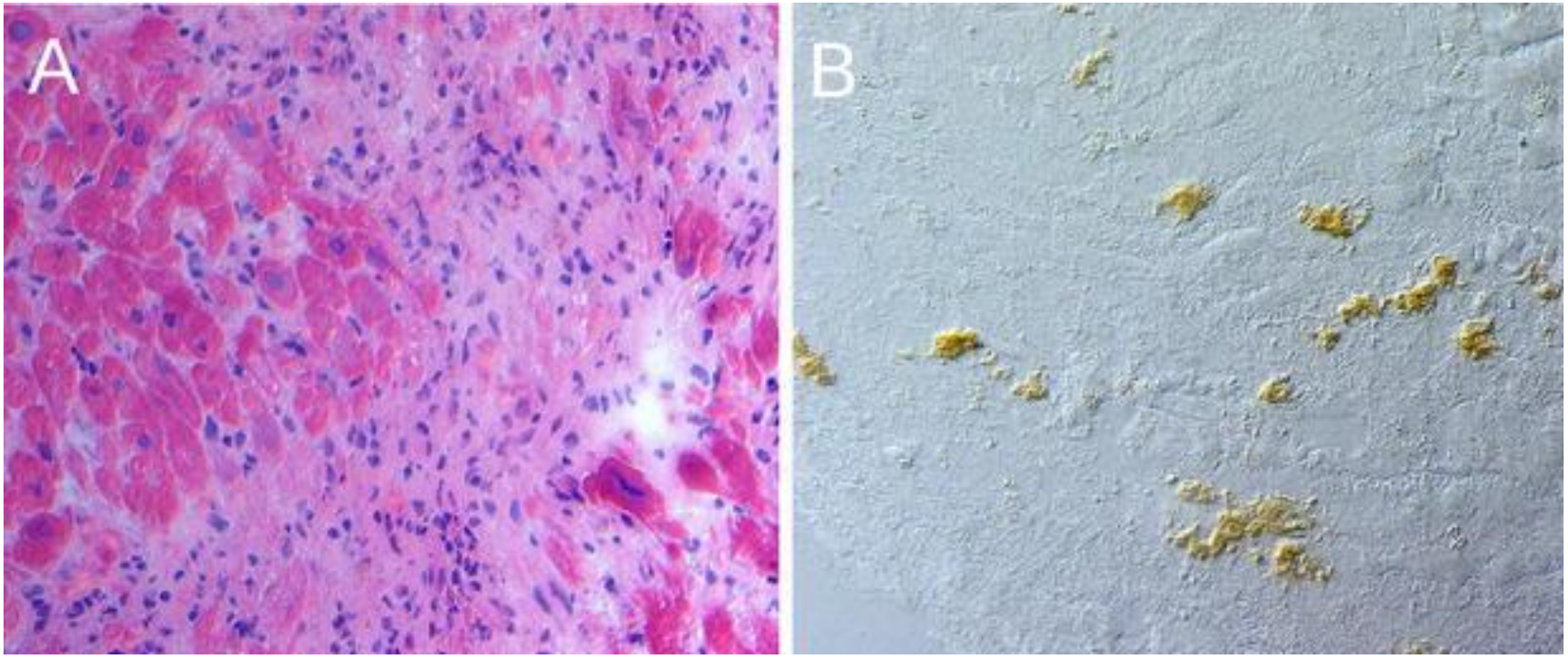


PFU assay from tissue with HeLa



Viable virus was isolated from frozen left atrial tissue at day 1.
The final concentration of virus was 1.5 x 10^4 PFU/ml

Detection of enteroviral Genome by *in situ Hybridization* in CVB2 myocarditis



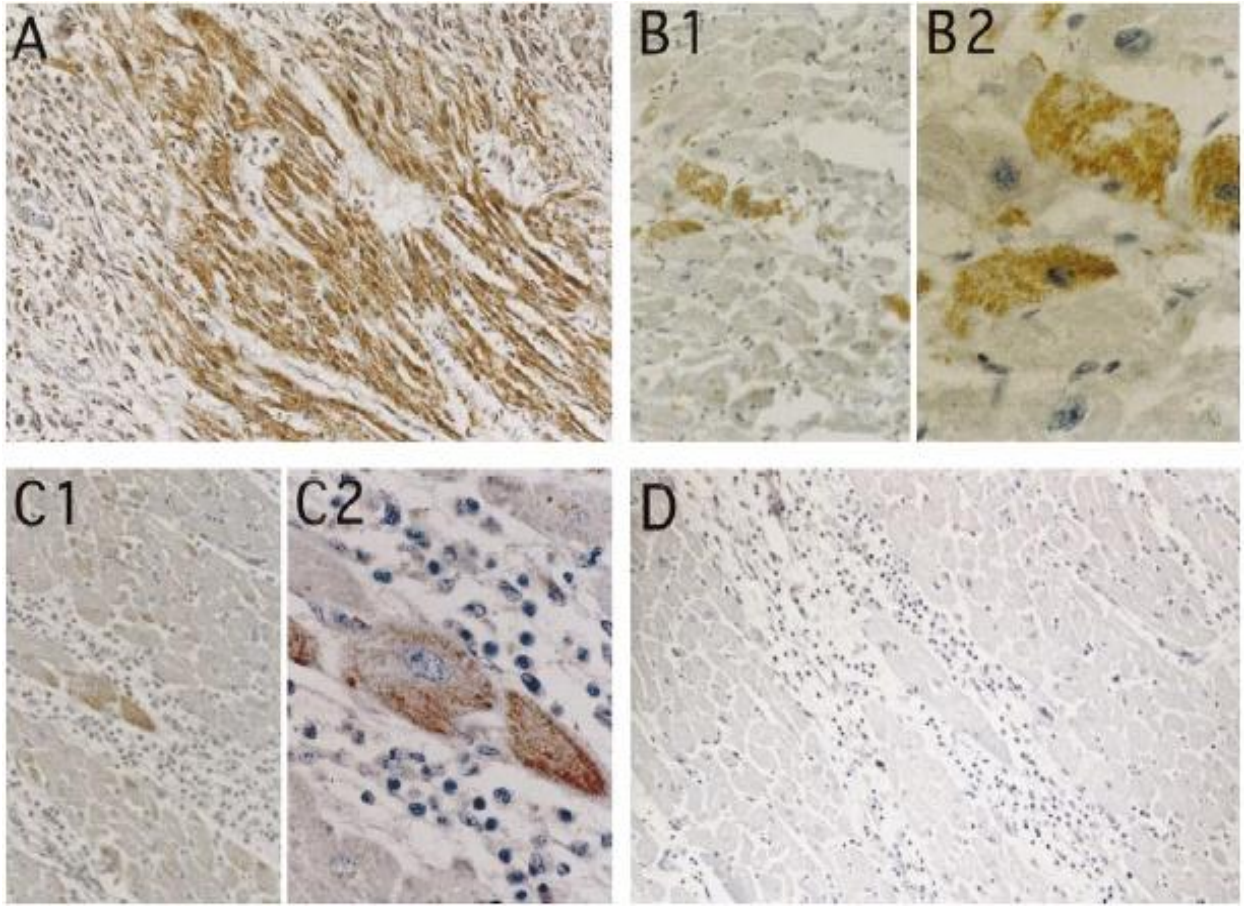
Badorff C. Med Microbiol Immunol (Berl). 2003 Aug 12

Diagnostic evaluation

3. Immunologic Approaches

- Immunohistochemical staining for Lc
- MHC expression – chronic for of myocarditis

Detection of enteroviral capsid protein VP1 by immunohistochemistry



A : fatal myocarditis

B : D-CMP

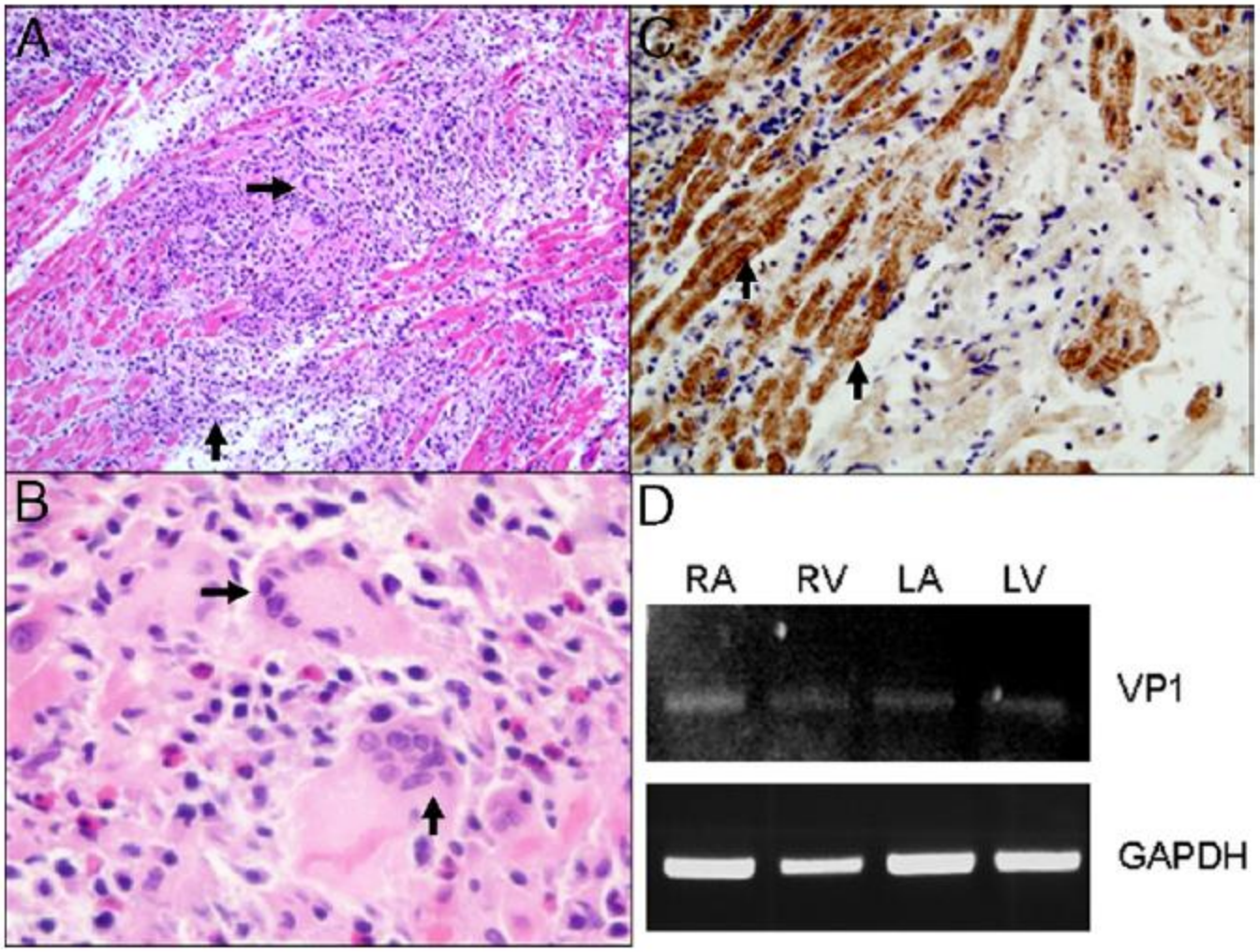
C: Chronic myocarditis

D : Negative control with IgG2a

x200 : A, B1, C1, D

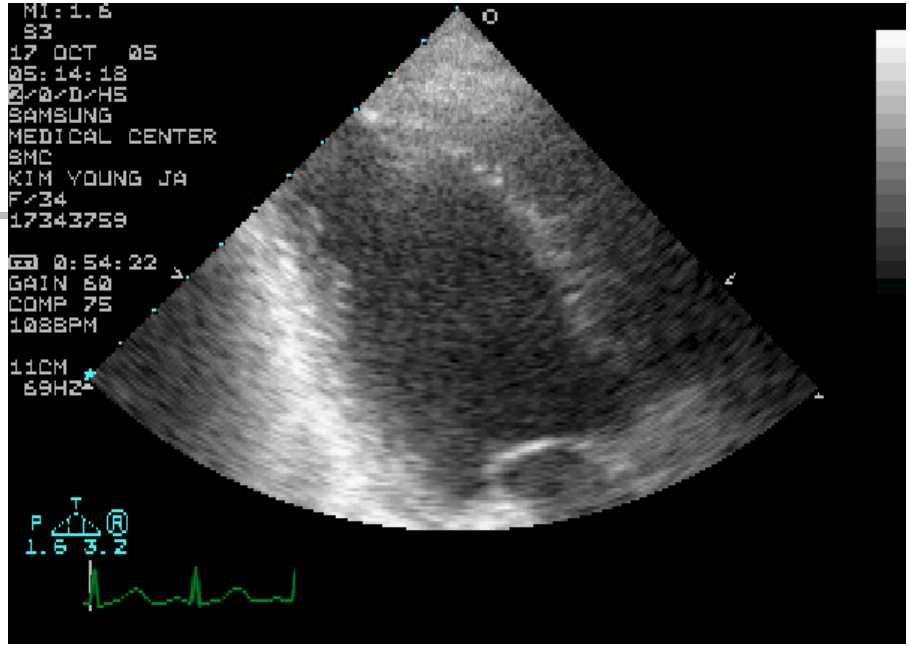
x600 : B2, C2

Circulation 2000;101:231

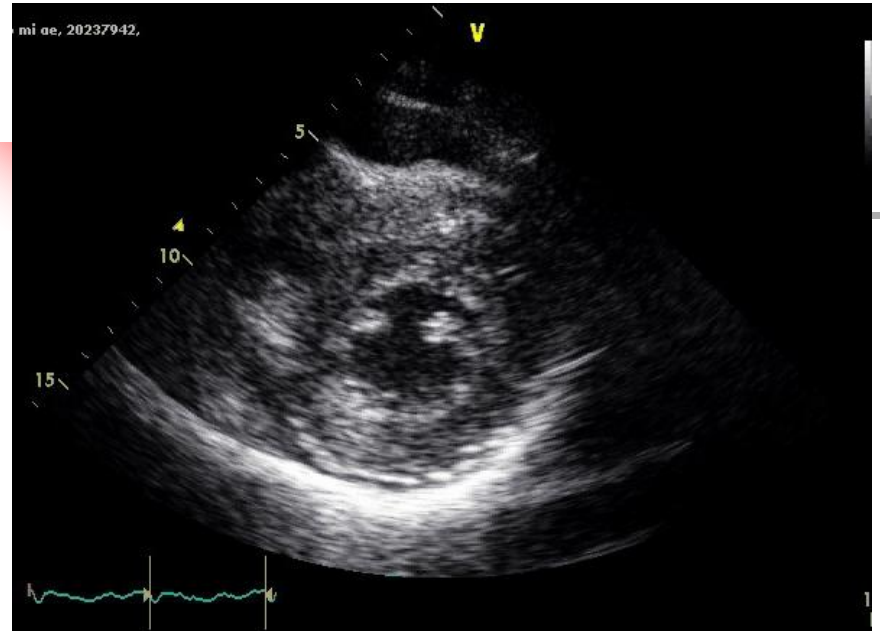


Biopsy of the left atrium at day 5 showed severe inflammation and myocardial necrosis **(A, arrows) with multinucleated giant cells (B, arrows), and enteroviral VP1 protein** was detected in immunohistochemistry **(C, arrows). By reverse transcriptase–polymerase chain** reaction, CVB VP1 ribonucleic acid could be detected in all chambers of the explanted heart at day 10 **(D, upper panel). The titers of neutralization antibody for CVB3 in the patient's** serum at 7 days were increased 8 times that at 1 day. (J Am Coll Cardiol 2010:56;e19)

Diagnosis



4. Myocardial imaging
   - Echocardiography
   - RI imaging
     - Gallium scan for inflammation
     - In111 labeled anti-myosin RI scan for necrosis
   - Contrast enhanced MRI



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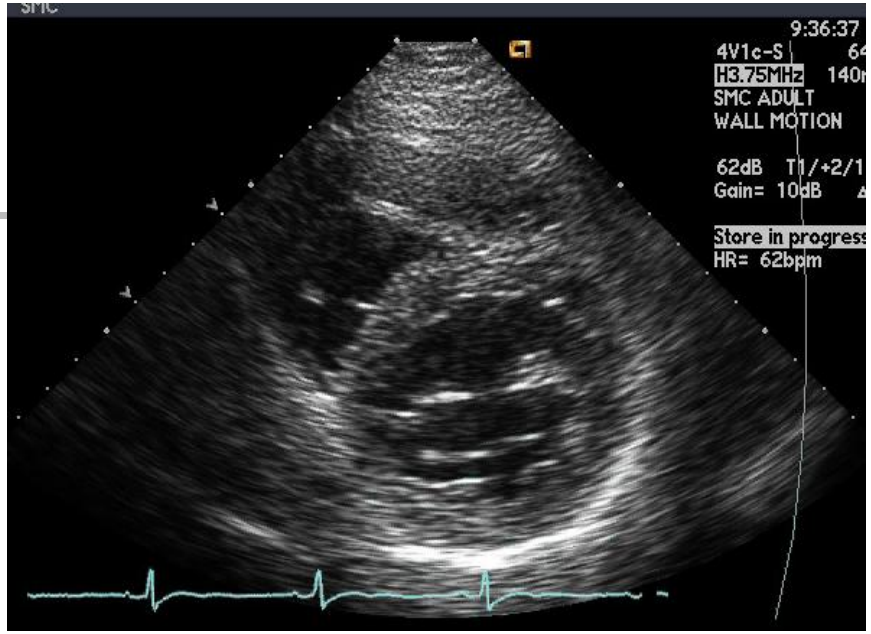

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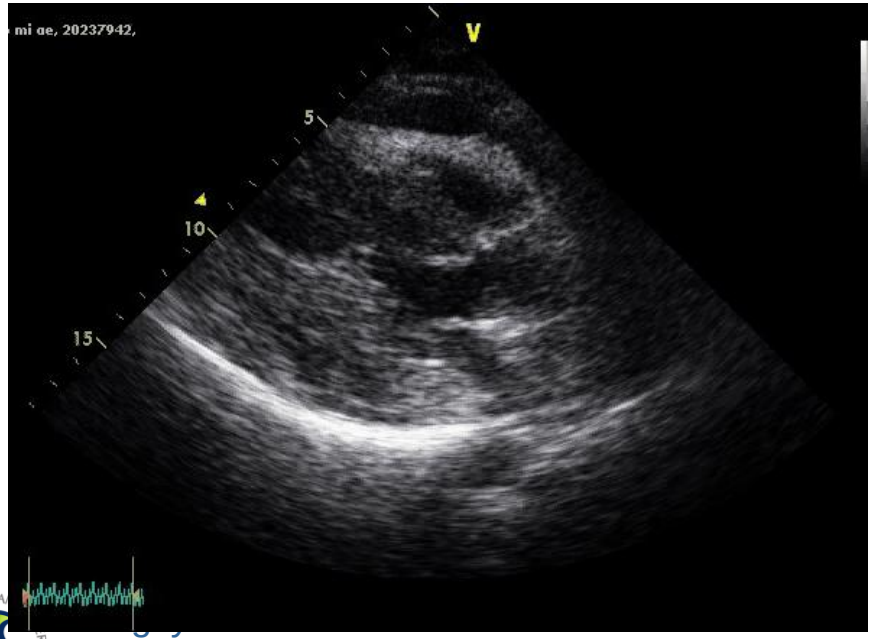
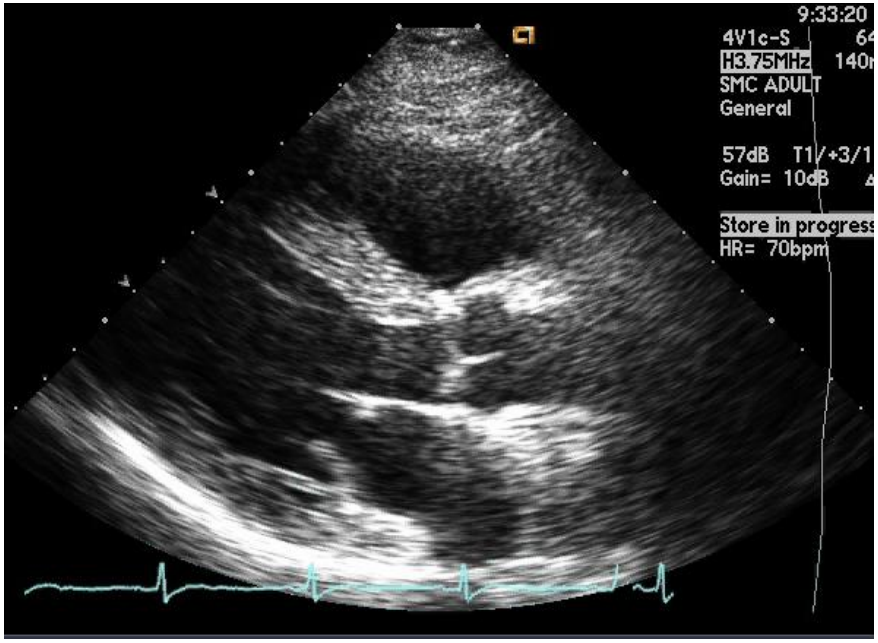
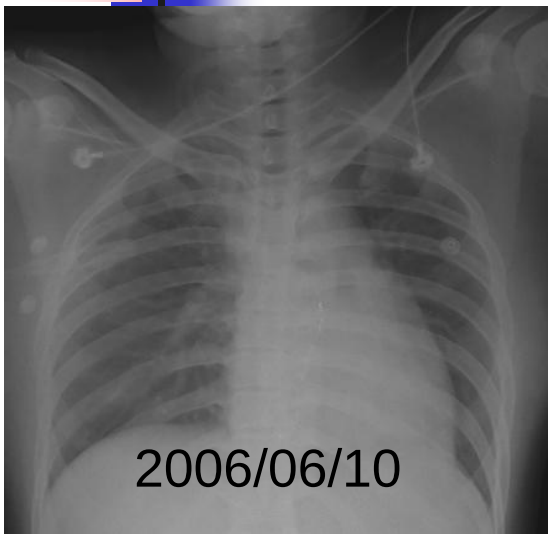
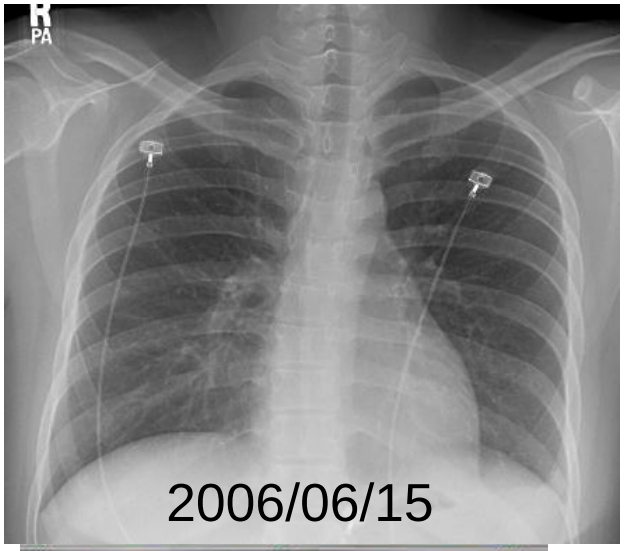
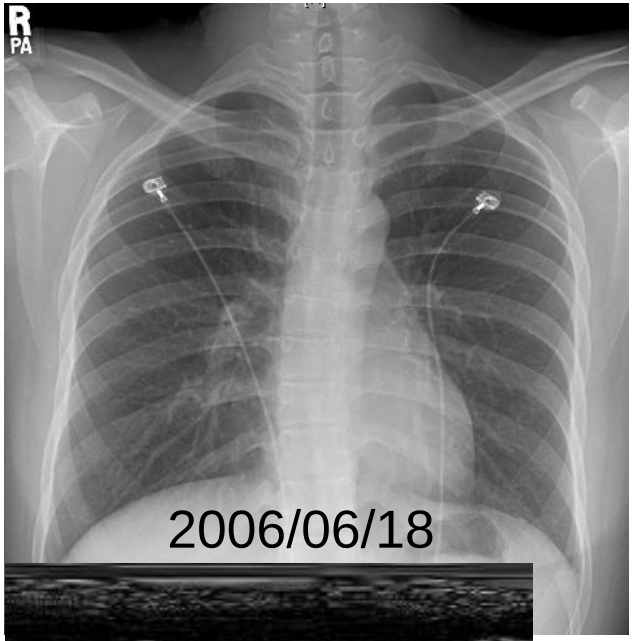


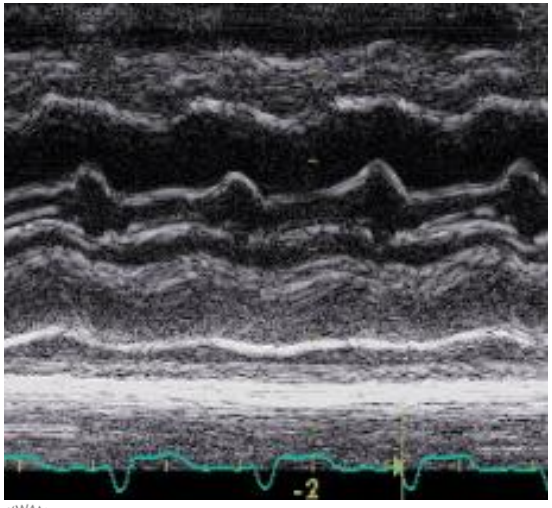


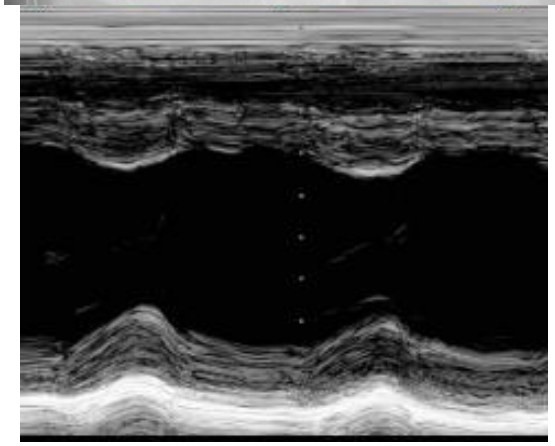
Chest PA

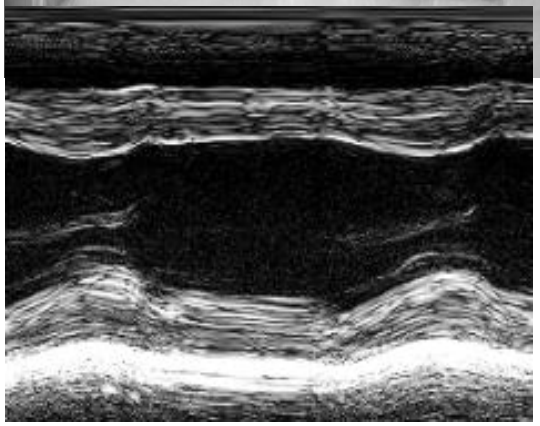


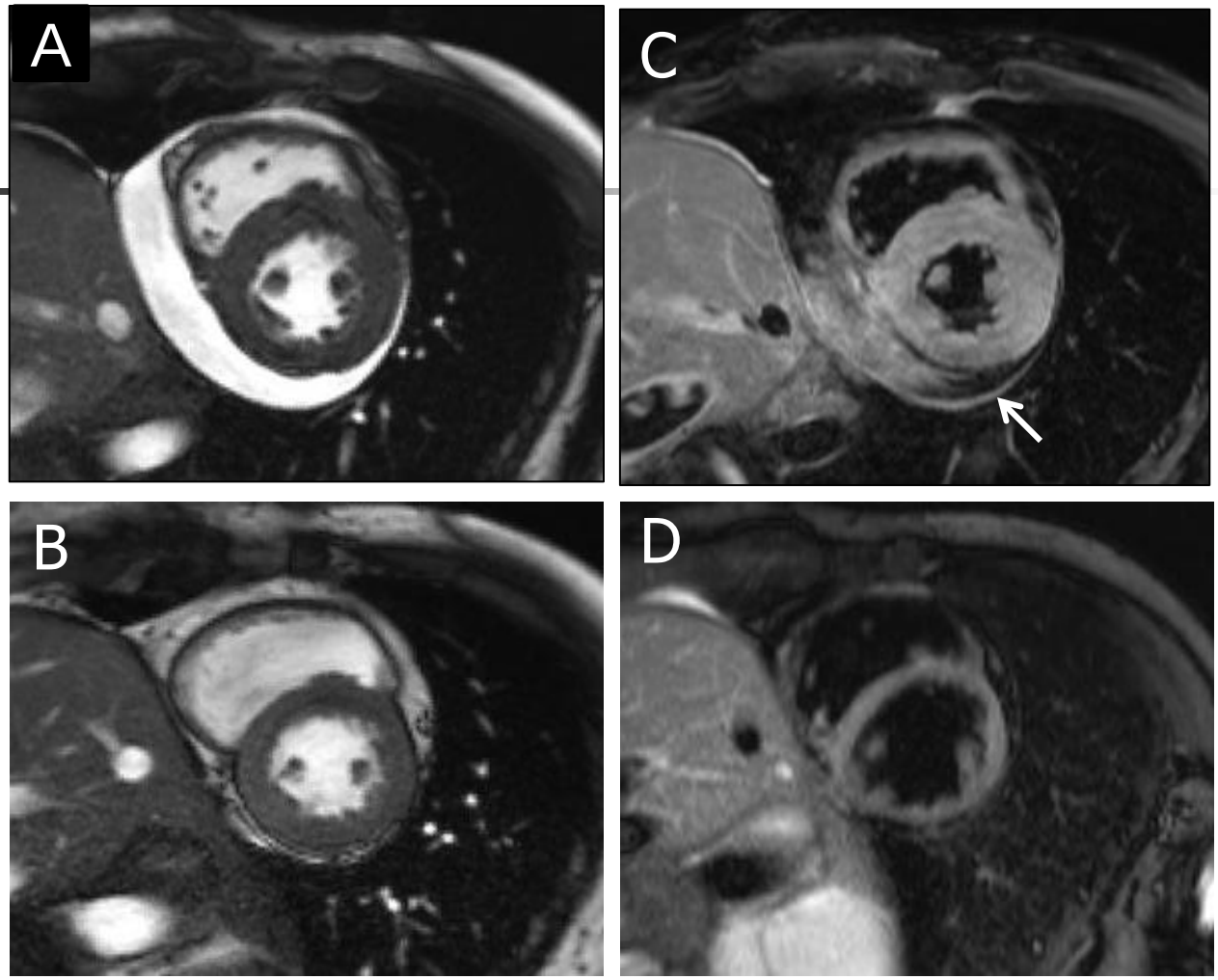












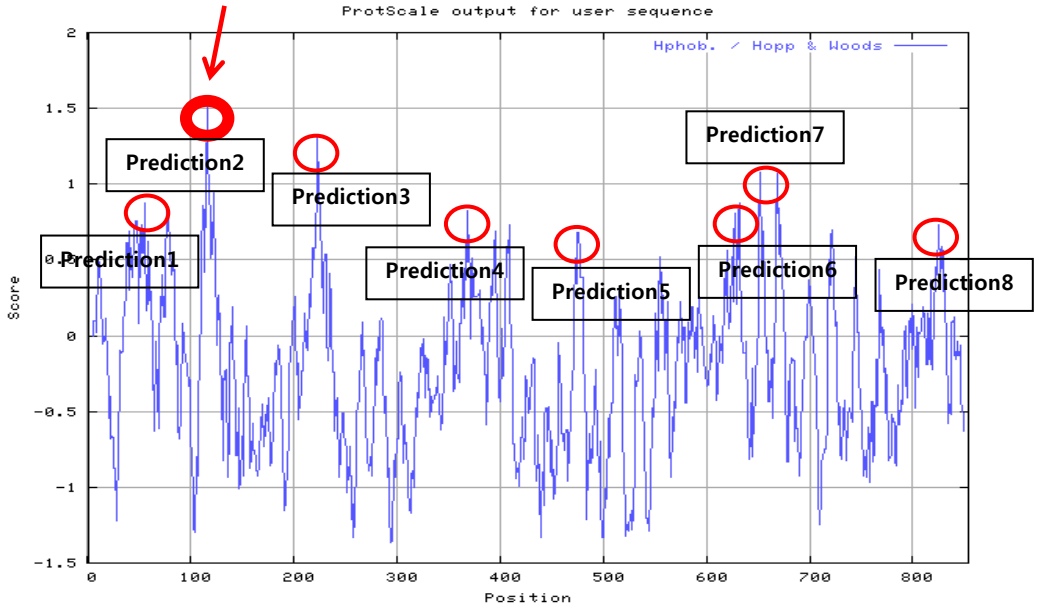
Cine CMR (A) mildly increased LV wall thickness and moderate amount of pericardial effusion.
→ (B) LV wall thickness was normalized and pericardial effusion was disappeared.
T2 signals were increased in LV myocardium and pericardium(C) → T2 signal was normalized in myocardium and pericardium at 2 months later.

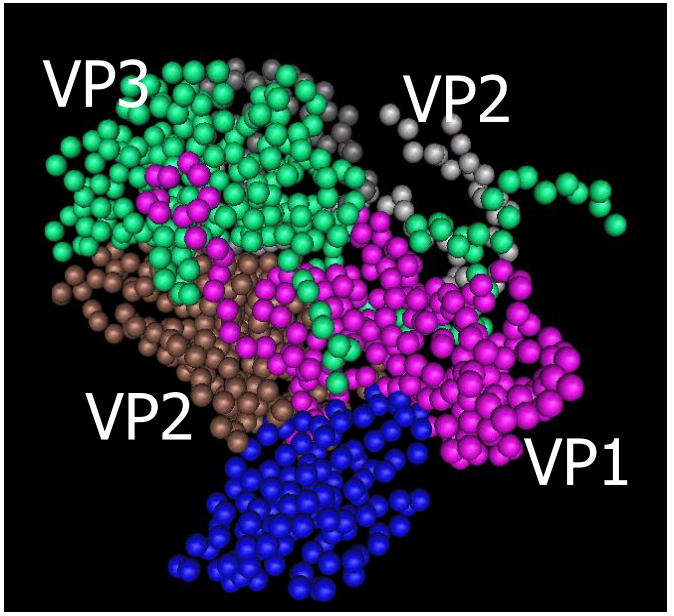
Enteroviral capisid protein ELISA kit to detect anti-enteroviral antibody

Enteroviral capisid protein ELISA kit to detect anti-enteroviral antibody

- 바이러스 캡시드 단백질 서열에서 **antigenicity**가 높은 **peptide** 선정

ELISA 기법을 이용한 바이러스 진단은 기본적으로 항체-항원 반응을 이용한 것으로 전체 단백질을 이용하여 detection하는 것보다 항체-항원 반응에 감수성이 높은 부위를 찾아서 펩타이드 형태로 합성하여 연구를 하는 것이 효율적이다.
따라서, 항원성(antigenicity) 이 높은 서열을 찾는 프로그램을 이용하여 8개의 펩타이드 서열을 찾았고 이중에서 가장 높고 여러 엔테로바이러스에 호환이 되는 1개의 펩타이드 (VP2)를 합성하였다.



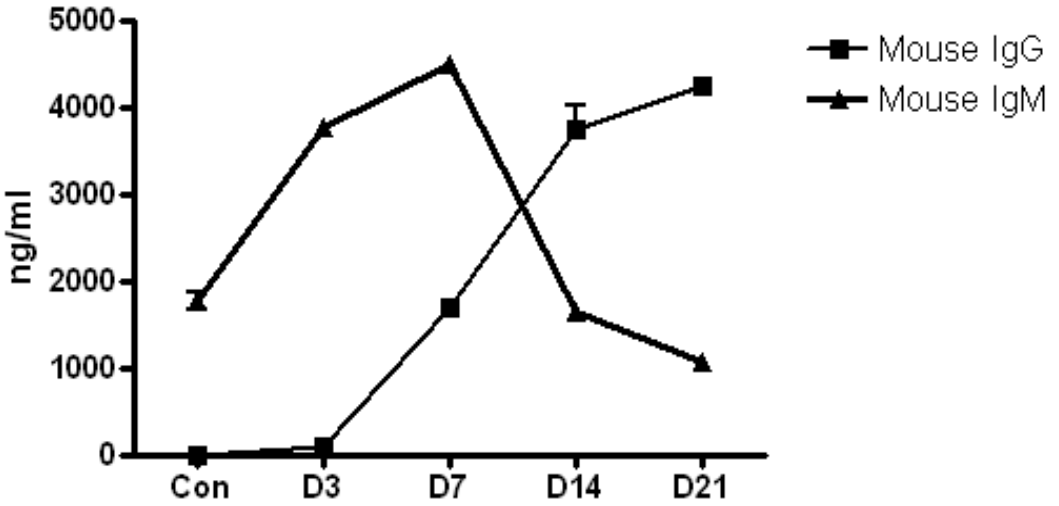


->이상의 결과로 예측 되어진 8개의 prediction region중에서 **Prediction 2, 3번**이 antigenicity와 hydrophobicity면에서 다른 candidate에 비해 항체 제작 가능성이 높을 것으로 판단되어짐.

Enteroviral capisid protein ELISA kit to detect anti-enteroviral antibody

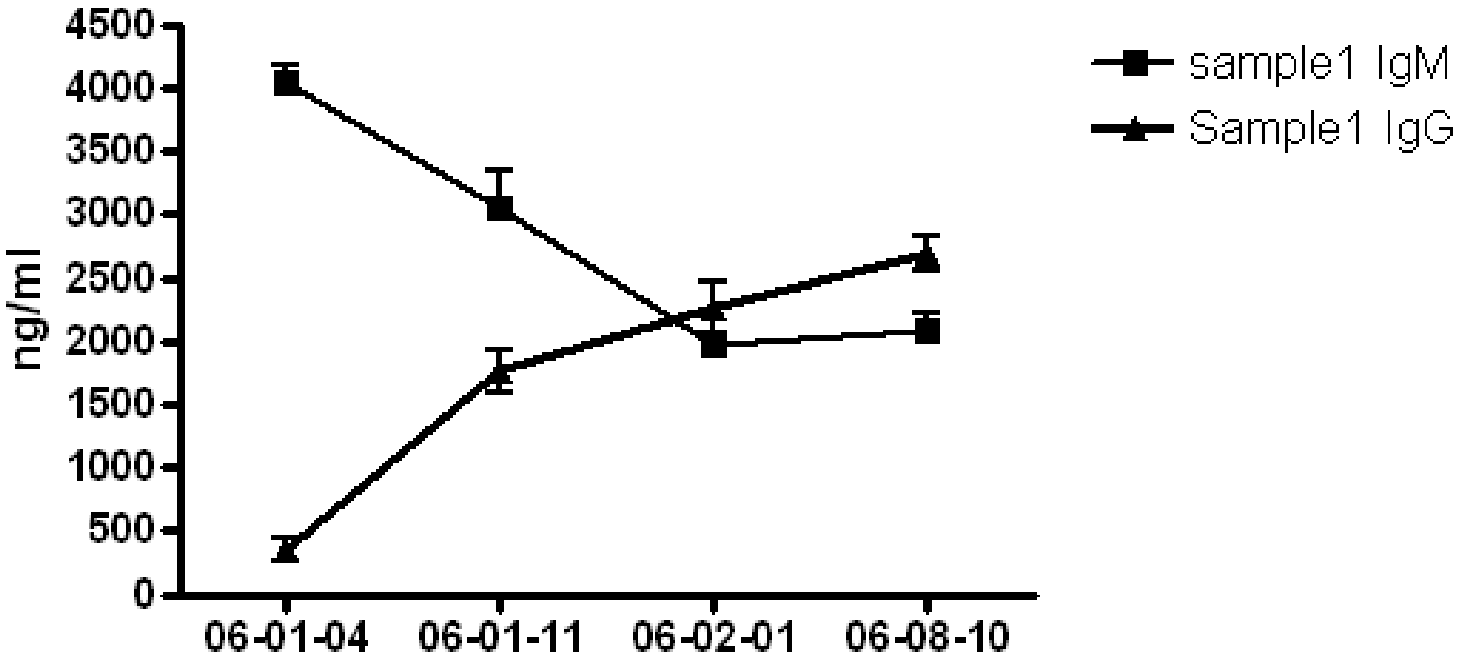
- **ELISA** 기법을 이용해 **Mouse** 혈청에서 콕사키 바이러스 항원**(VP2)**에 대한 **IgG, IgM**의 양상 변화 확인

콕사키바이러스 항원에 대한 IgG, IgM의 양상 변화를 확인하기 위해 먼저 Mouse 혈청에서 ELISA를 시행하였다. uncoated 96well plate에 제작된 peptide를 coating 하고 CVB3 바이러스 감염 생쥐의 혈청을 100배 희석하여 반응시킨 후, mouse IgG, IgM-HRP로 반응시키고 흡광도를 450nm에서 측정하였다. 그 결과, IgG는 H3를 감염시켰을 때 초기 3일째에는 항체 반응이 없었으나 감염 7일 후부터 콕사키바이러스 항원에 대한 IgG가 증가하는 것을 확인하였다. 또한, IgM은 감염 즉시 증가하기 시작한 후, 7일부터는 급격히 감소하는 것을 확인하였다.

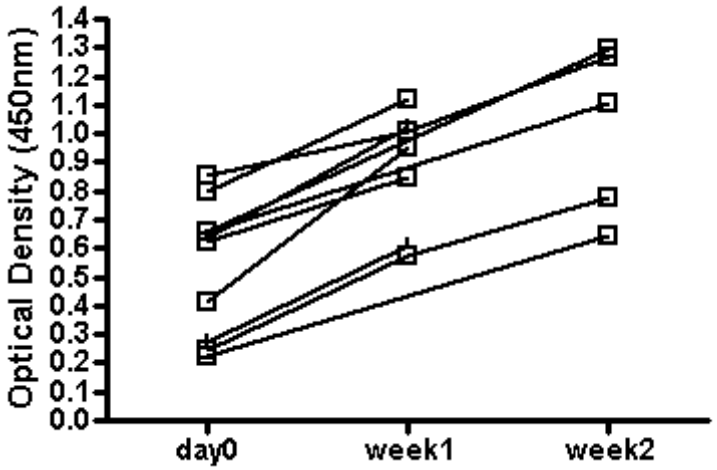


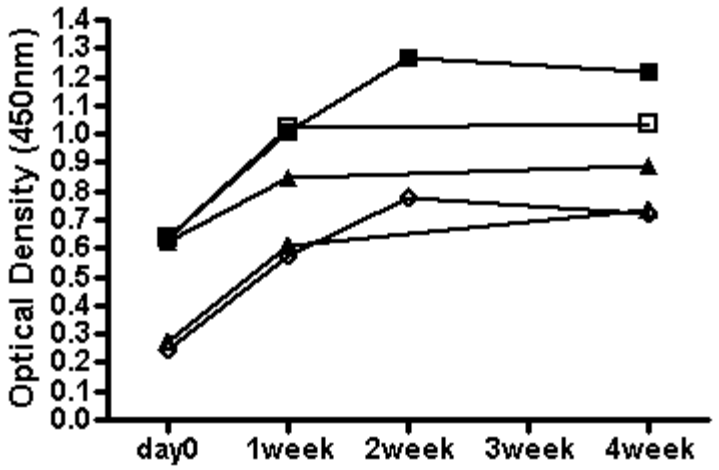
Enteroviral capisid protein ELISA kit to detect anti-enteroviral antibody

- 바이러스성 심근염 확진 환자 혈청에서 **anti-CVB3 IgG/IgM** 항체 분석

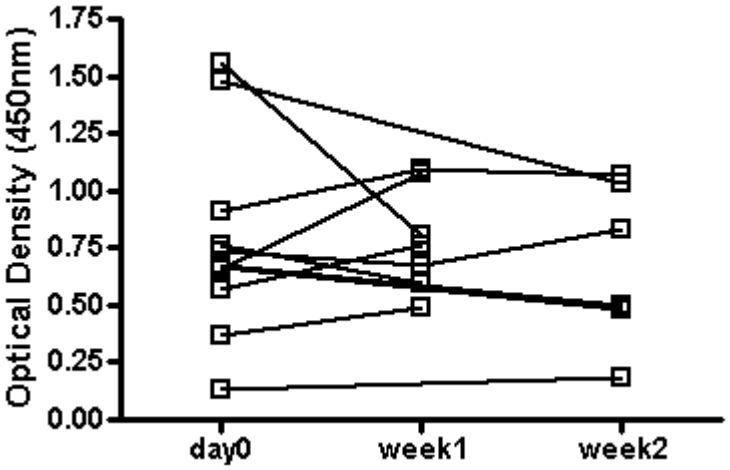


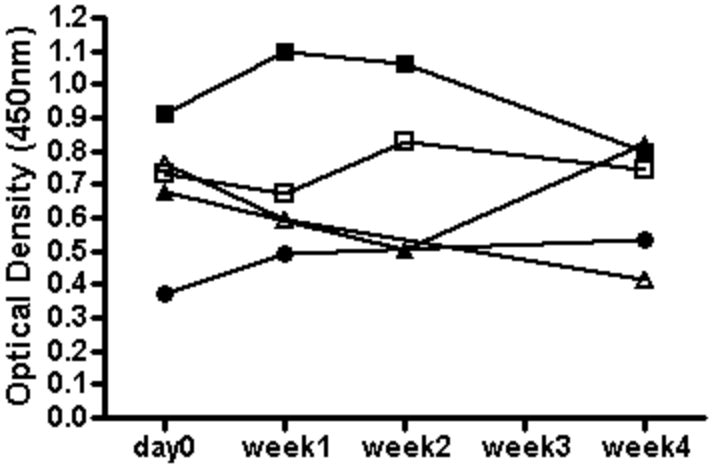
바이러스성 심근염 확진 환자 혈청에서 anti-CVB3 IgG 항체 분석 (VP2)



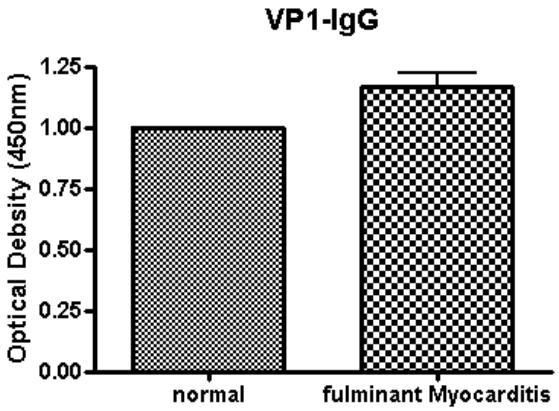


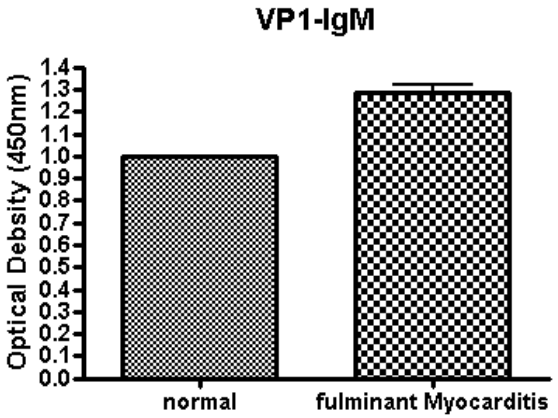
바이러스성 심근염 확진 환자 혈청에서 anti-CVB3 IgM 항체 분석 (VP2)

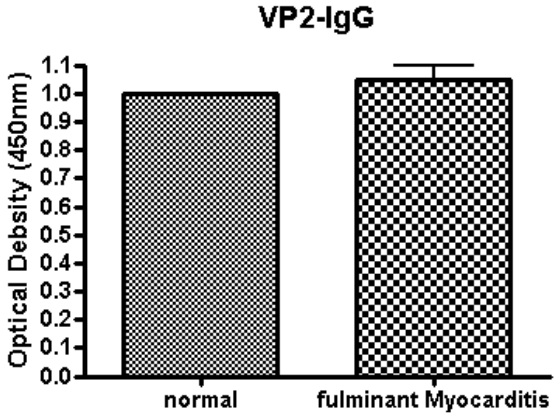


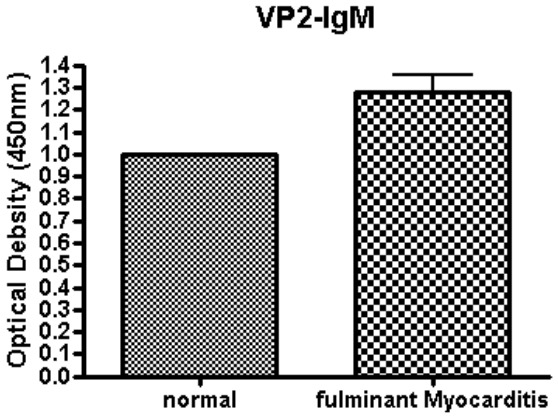


바이러스성 심근염 확진 환자 혈청과 정상인 비교 anti-CVB3 IgG/IgM 항체 분석









Clinical Presentation & Evolution

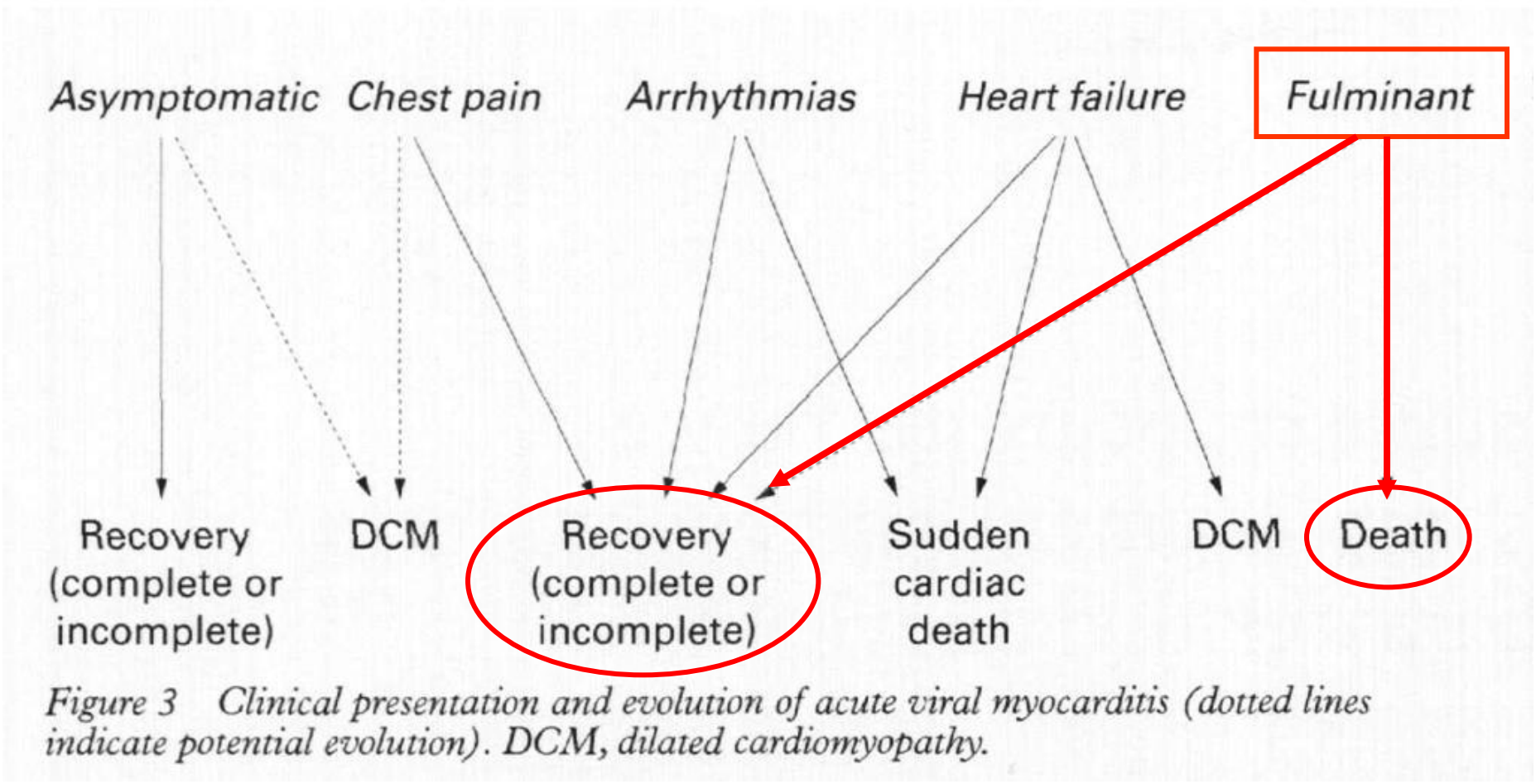
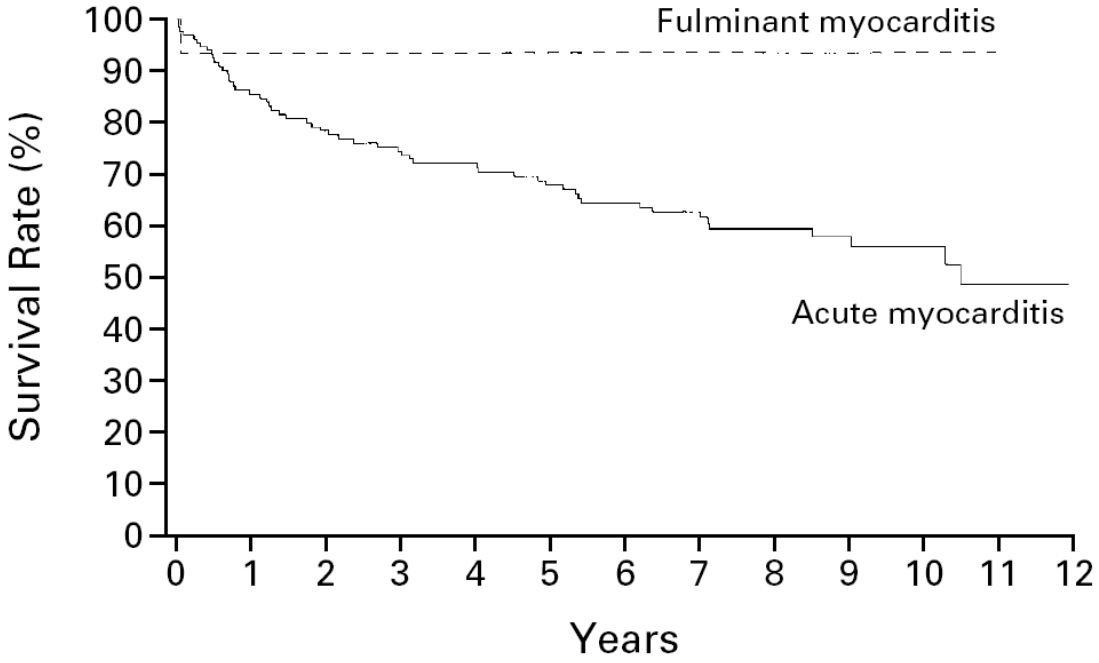


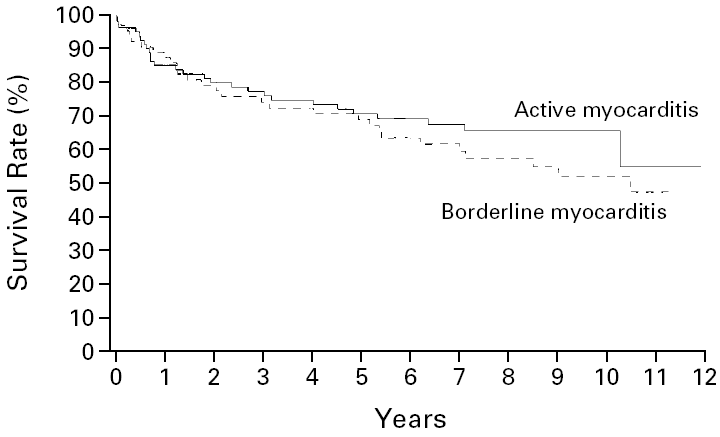
Figure 3 Clinical presentation and evolution of acute viral myocarditis (dotted lines indicate potential evolution). DCM, dilated cardiomyopathy.

Definition of FM

- McCarthy (NEJM 2000;342:690) : FM should have
  1. severe hemodynamic compromise requiring
     >5 μg of dopamine or dobutamine /kg/min) or LVAD
  2. histopathologically borderline or active myocarditis
  3. at least two of the following clinical features
     1) fever
     2) distinct onset of symptoms of heart failure (fatigue, dyspnea on exertion or at rest, or edema that could be dated specifically to a one-to-two-day period)
     3) history consistent with the presence of a viral illness within the two weeks before hospitalization

Prognosis of FM





Acute fulminant myocarditis (FM) is a distant clinical entity when compared with acute non-fulminant myocarditis (NFM), because has an excellent long-term prognosis when recovered.

Case Presentation

환자 : 16세 여자

C.C) 좌측 흉부 통증 for 1day

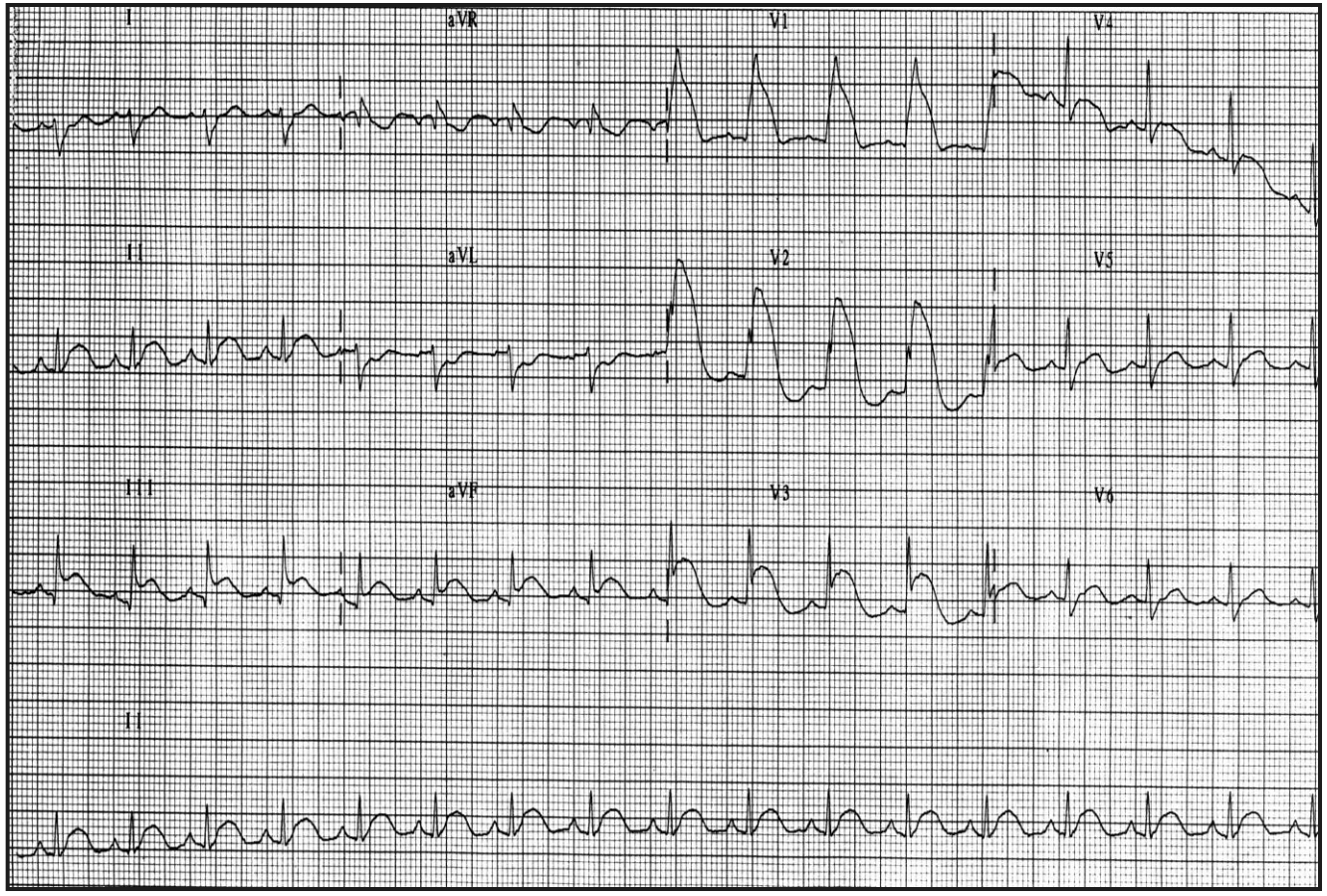
P.I) 평소에 건강하였고, 2주 전 2일 간 감기 증상

P.M.Hx) not significant

P/E) acute ill-looking, JVP elevation (-)

RHB w/o murmur, no rales

Laboratory Findings at ER



검사소견 :

Troponin T (+)

AST 223 IU/L

CPK 1323 IU/L

Laboratory Test

1. Lab test
   - Troponin T (+) CPK 1323 IU/L
2. Coronary Angiography :
   - normal
   - Ergonovine test : normal
3. Echocardiography
   - LV size : 35/50
   - EF : 77 %

Hospital Course

At day 1.

During conventional CHF with diuretics

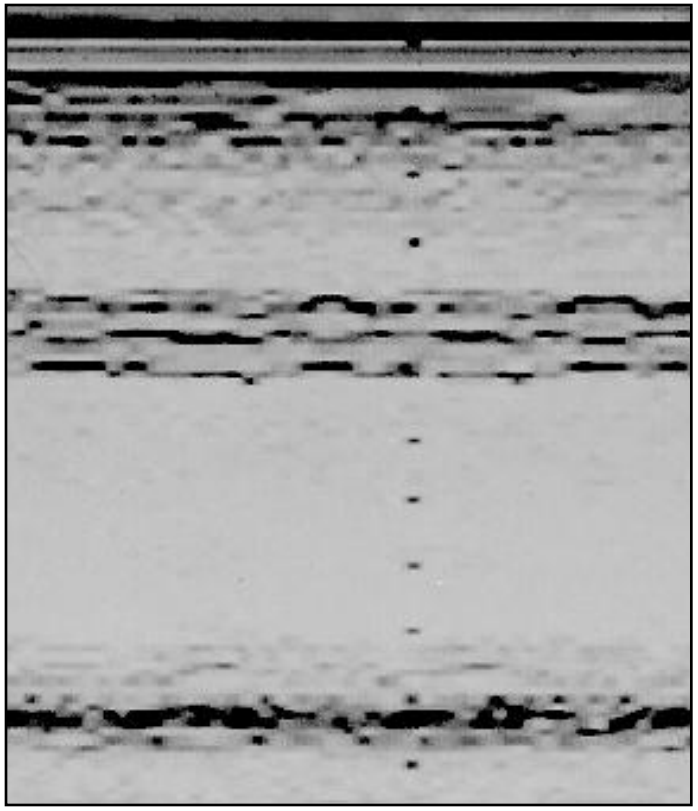
S) Pulmonary edema, oliguria,

hypotension (SBP 60 mmHg)

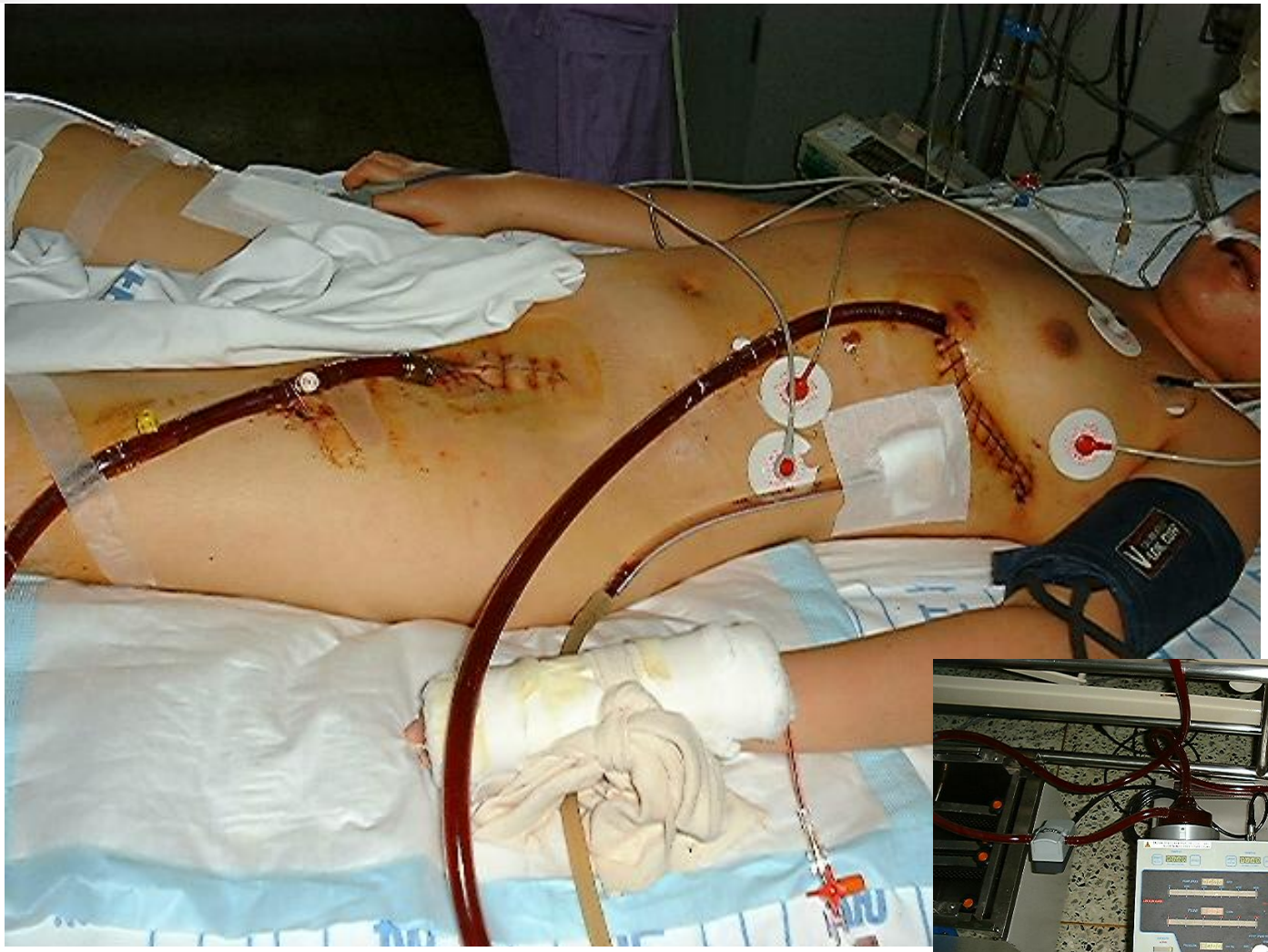
O) Echocardiography

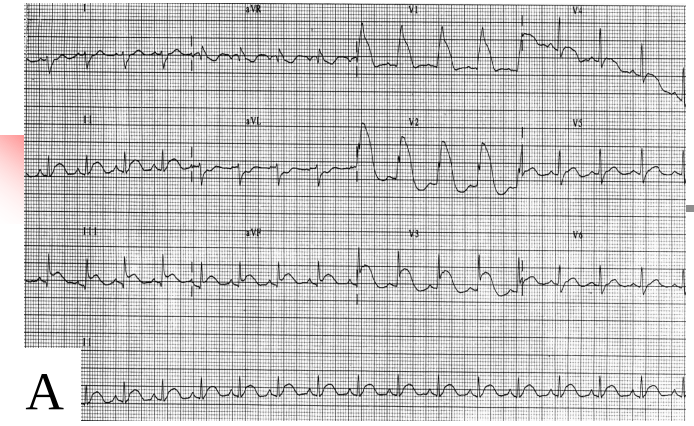
A) Cardiogenic shock

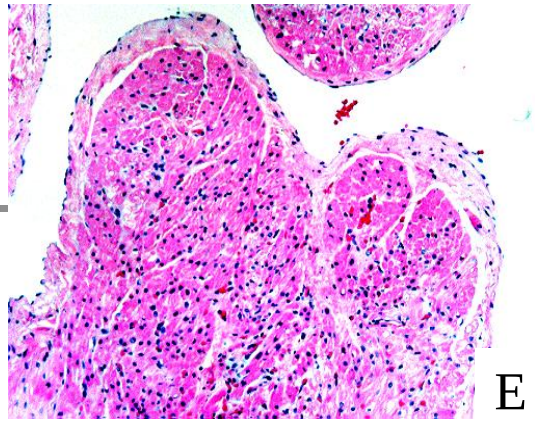
P) IABP

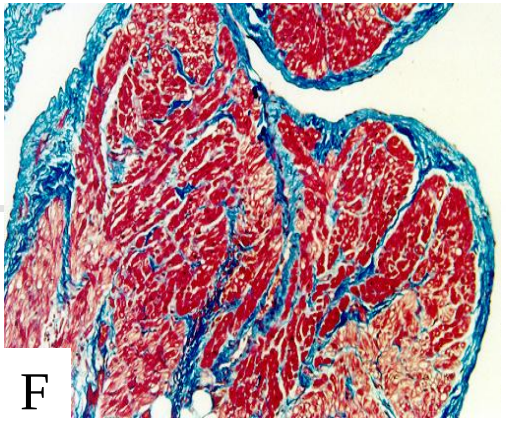


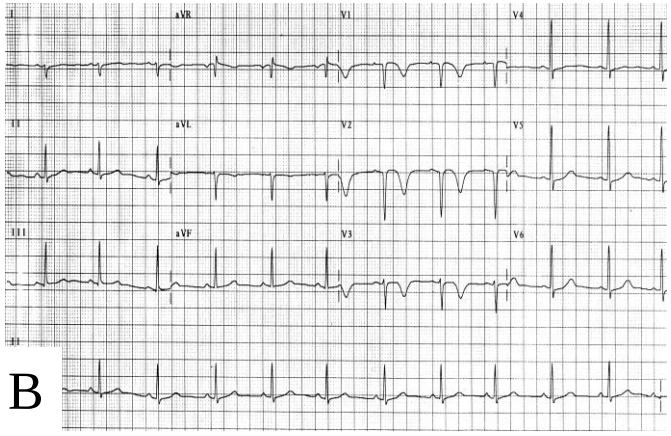
Ventricular Assistant Device

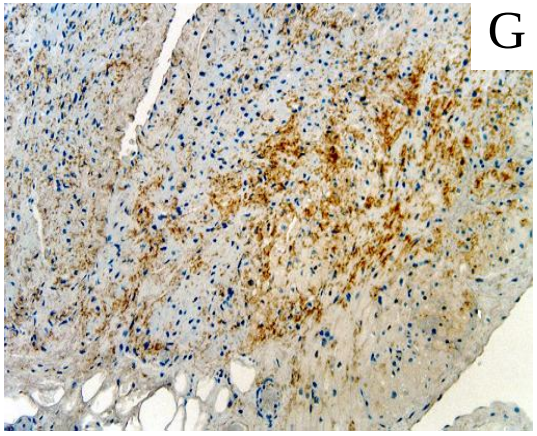


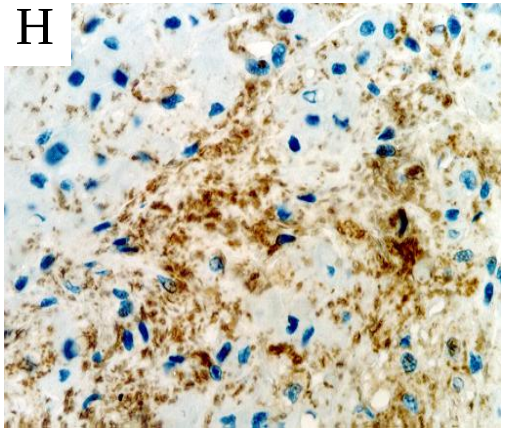


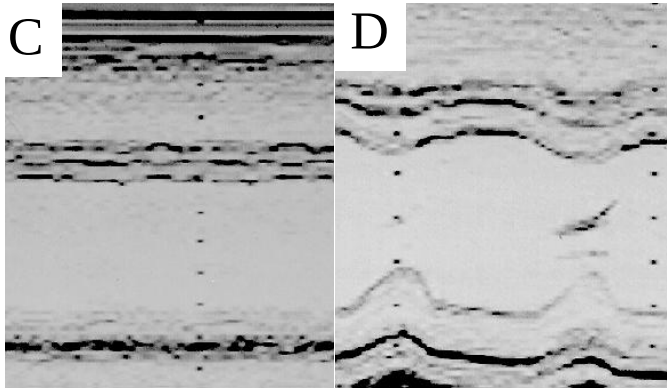












Case of fulminant Coxsackieviral myocarditis

A, C) EKG and Echo at ER

B, D) EKG and Echo at day 10

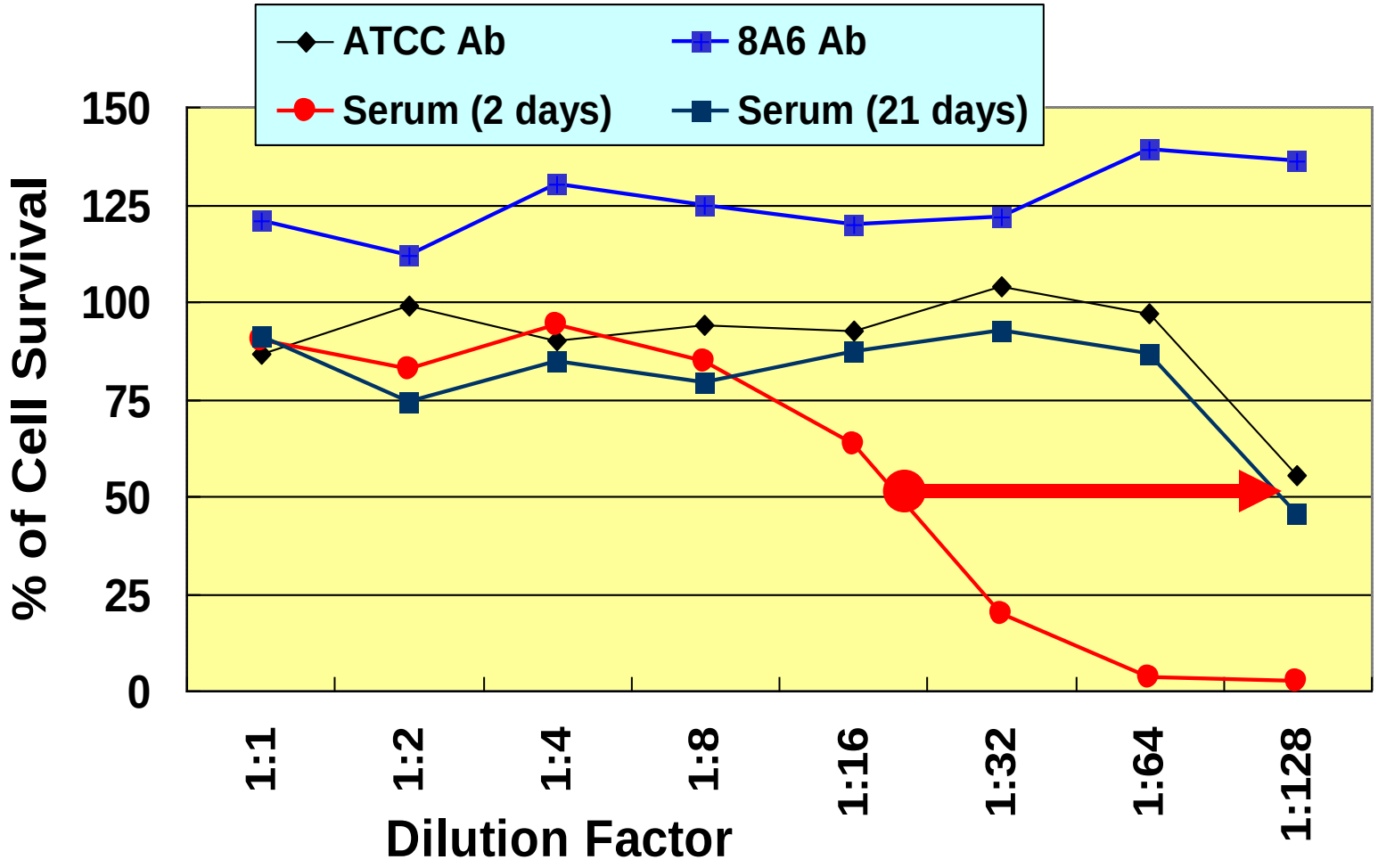
E) Left auricle (H& E stain, x200)

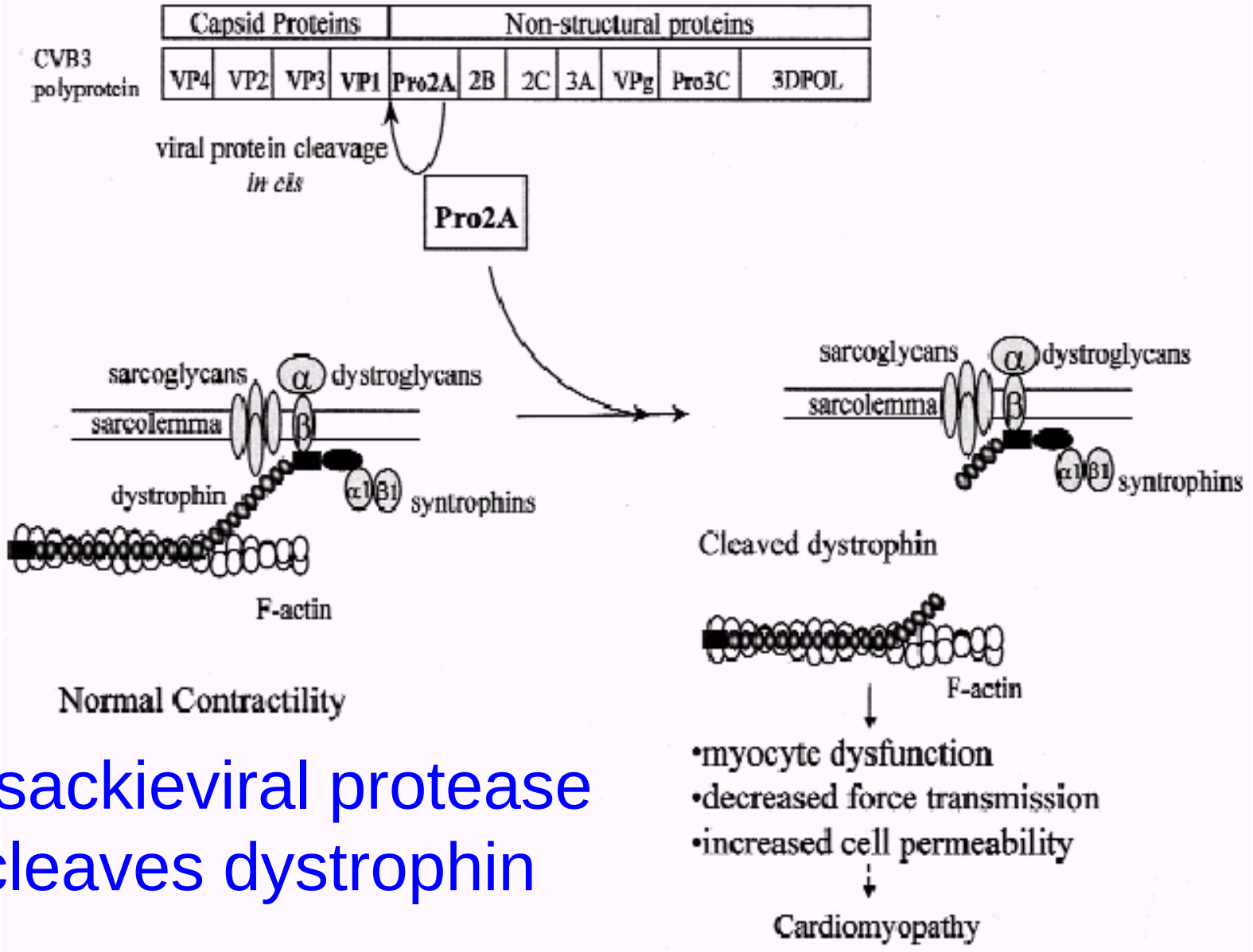
F) Left Auricle (Masson's Trichrome stain, x200)

G,H) Immunohistochemistry probed by anti-enteroviral VP1 Ab.(G; x100,H; x400)

Jeon ES. NEJM 345:179, 2001

Results of Neutralization Test

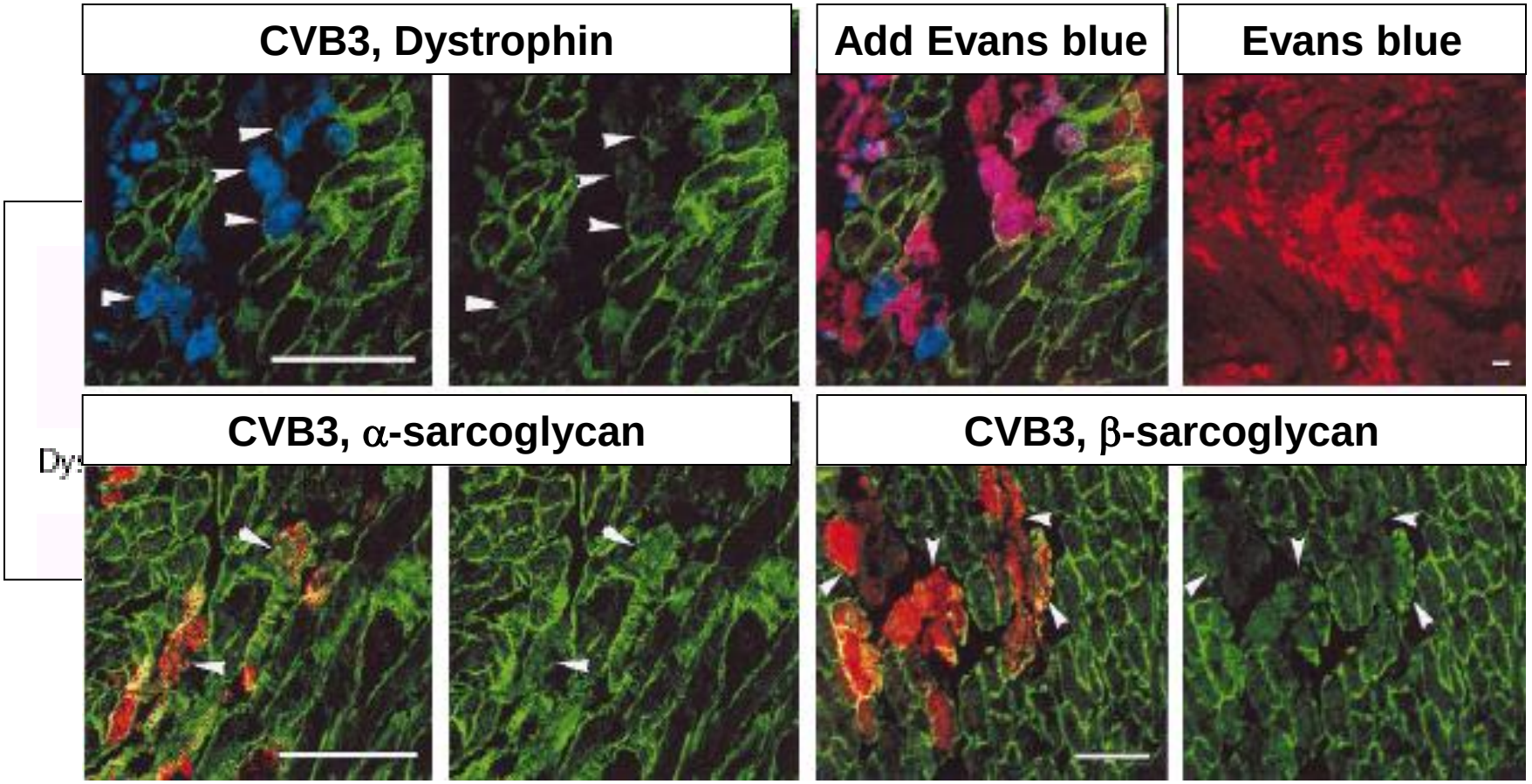




Coxsackieviral protease 2A cleaves dystrophin

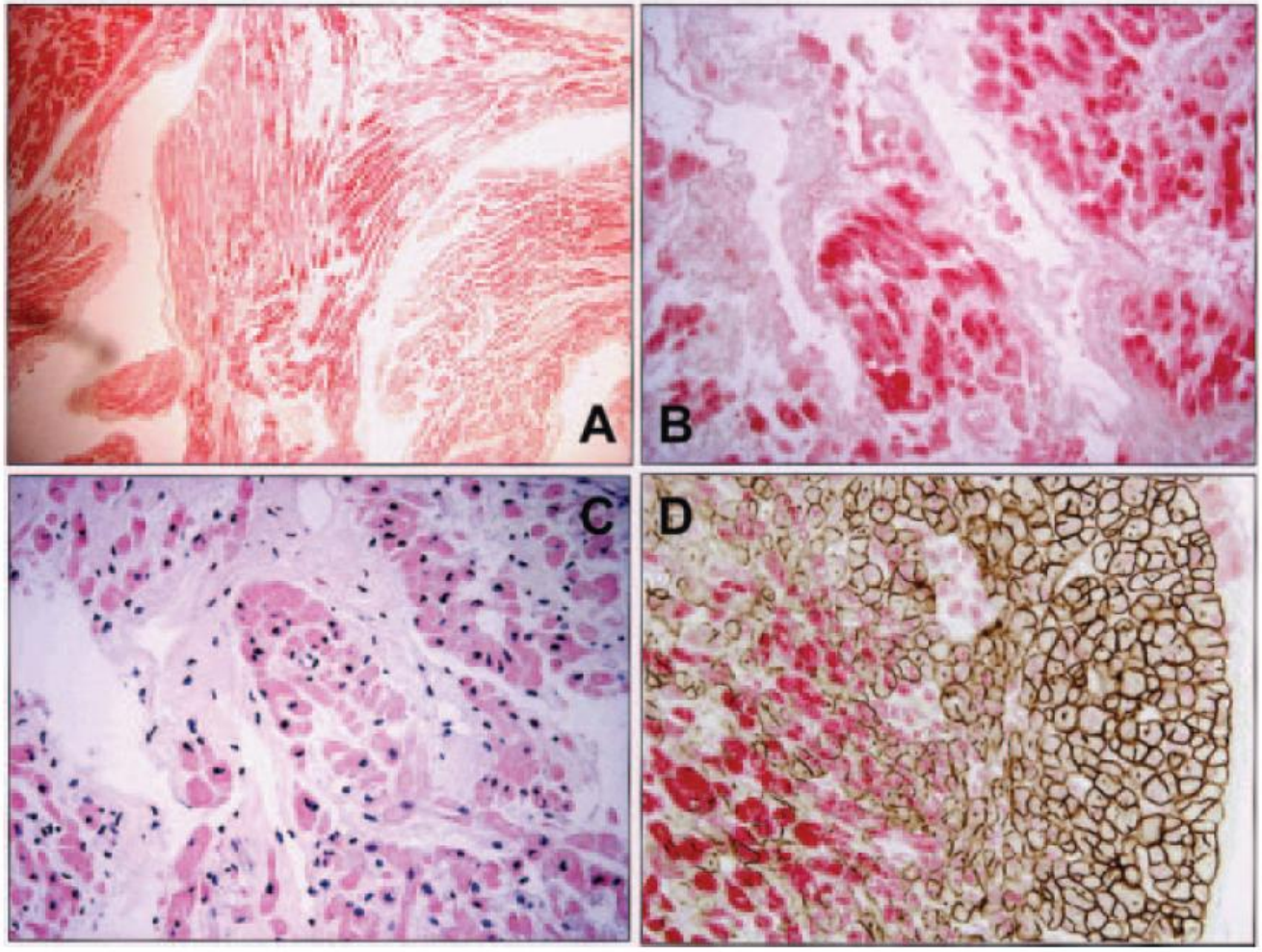
Badorff, Nat Med 5:320, 1999

CVB3 protease 2A cleaves dystrophin-Sarcoglycan Complexes



Badorff, Nat Med 5:320, 1999

Serial histological and immunohistochemical analyses of left appendage showed the enteroviral VP1 capsid proteins over the entire heart (A, day 1, 100; B, day 6, 400), with scant lymphocytes observed at day 5. C, Hematoxylin and eosin stain, 400. Focal areas of myocardium displayed a loss of the sarcolemmal staining pattern for dystrophin using antidystrophin Ab (NCLDYSA) that colocalized with enteroviral capsid protein (D, merged image for serial sections, 200).



Jeon es. Circulation. 2006 Feb 14;113(6):e76-7

Thioredoxin, adiponectin and clinical course of acute fulminant myocarditis

Jin-Oh Choi,[1] Soo-Hyeon Yun,[1] Kiick
Eun-Seon Ju,[1] Sang-Chol Lee,[1] Seun
Eun-Seok Jeon[1]

ABSTRACT

Background In an animal model of viral myocarditis, plasma levels of thioredoxin and adiponectin have been reported to be associated with the severity of inflammation and recovery of ventricular dysfunction, respectively. However, there have been few reports about the clinical significance of these cytokine levels in human myocarditis.

Objectives To examine the hypothesis that cytokine levels correlate with clinical courses of patients with acute fulminant myocarditis (FM).

Methods A total of 33 consecutive patients with biopsy-proven acute myocarditis were evaluated. Twenty patients were ascribed to an FM group and the other 13 patients were grouped as a non-fulminant group (NFM). Plasma cytokine levels at the time of admission and after 2 weeks were evaluated and correlated with the duration of mechanical circulatory support application.

Choi JH & Jeon ES.
Heart 2011 Jul;97(13):1067-73

Etiology of FM and NFM

- 15 (75%) patients with FM (n=20) had CVB myocarditis
  - 5 on immunohistochemical analysis
  - 5 on RT-PCR
  - 13 on a neutralization test
- 5 (38%) patients with NFM (13) had evidence of CVB myocarditis (1on IHC, 3 on RT-PCR, 1 one on NT test)

Method	FM (n=20)	NFM n=13
Immunohistochemistry	5	1
RT-PCR for Coxsackievirus	5	3
Neutralization test	13	1
Total (+) for CVB	15(75%)	5(38%)

Choi JH & Jeon ES. Heart 2011 Jul;97(13):1067-73

Etiology of FM and NFM

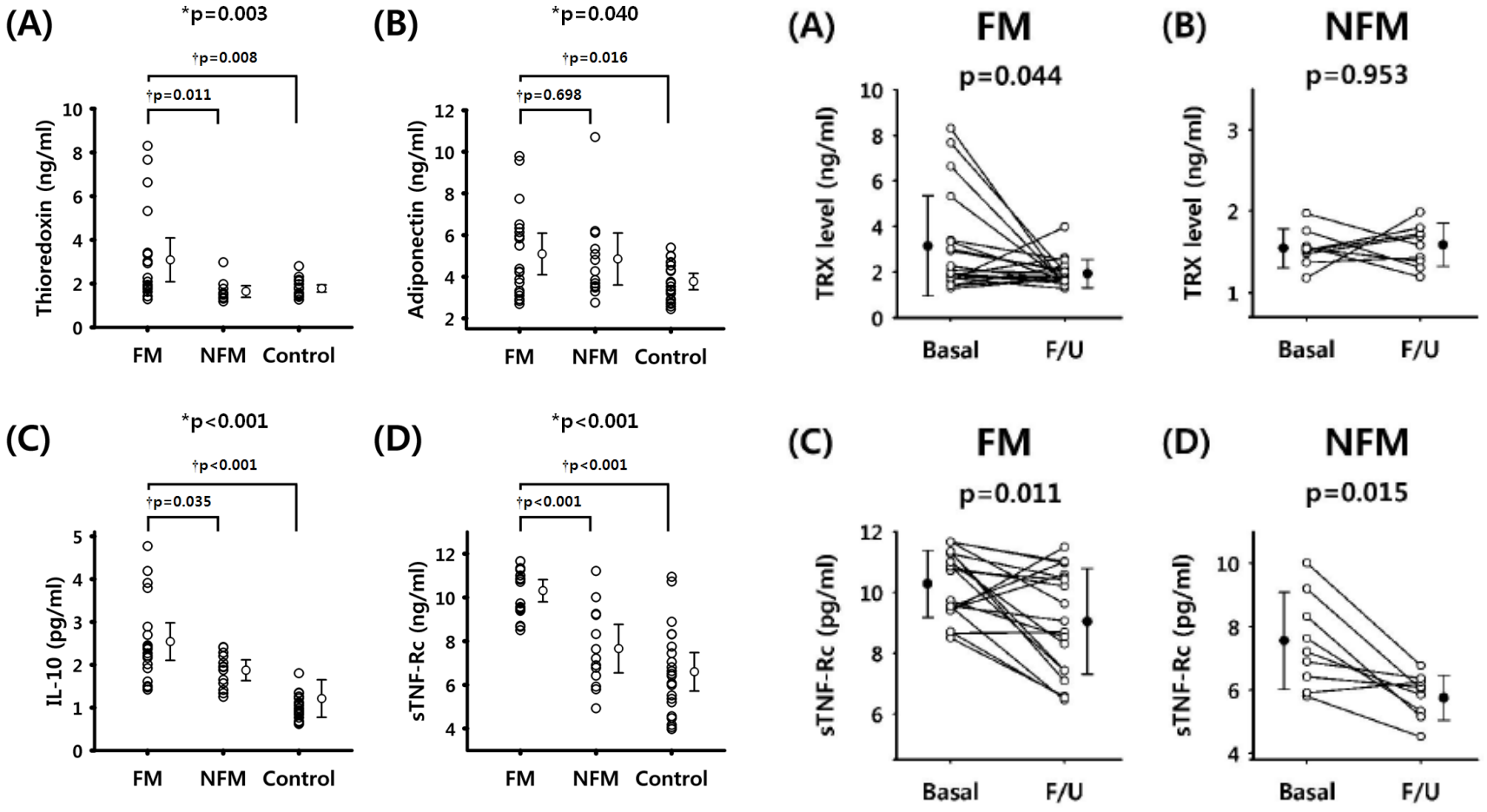
Table 1 Clinical features and laboratory findings of the individual study subjects

Patient No	Group	Sex	Age (years)	Hx	SBP (mm Hg)	DBP (mm Hg)	VT/VF	AVB	PE	LVEF (%)	Cr (mg/dl)	NT-ProBNP (pg/ml)	TRX (ng/ml)	ADPN (ng/ml)	IL-10 (pg/ml)	TNFα (pg/ml)	sTNFR-II (pg/ml)	IABP	PCPS	D of MCS (days)	Outcome
1	FM	F	18.1	LC	53	42	Yes	No	No	25	1.15	7972	5.32	3.22	4.19	4.69	9.46	Yes	Yes	30	Expire
2	FM	M	37.2	LC	60	40	Yes	Yes	No	15	1.85	31 029	1.86	3.32	2.45	4.14	11.6	Yes	Yes	25	Expire
3	FM	M	72.8	LC	84	64	Yes	No	Yes	10	2.16	8028	1.62	2.82	2.89	4.76	11.7	Yes	Yes	21	Expire
4	FM	F	56.5	LC	85	50	No	Yes	No	25	1.03	33 446	2.92	5.49	2.01	4.68	10.8	Yes	Yes	16	Survive
5	FM	F	54.1	LC	46	37	No	Yes	No	15	1.37	9737	3.01	5.94	3.79	3.04	11.3	No	Yes	14	Survive
6	FM	M	71.5	LC	145	82	Yes	No	Yes	35	1.06	13 276	1.82	3.71	2.26	2.96	10.9	Yes	Yes	12	HT/expire
7	FM	M	52.2	LC	110	90	Yes	No	Yes	20	1.22	30 650	1.89	2.91	1.92	2.36	9.56	Yes	Yes	11	Survive
8	FM	F	41.5	LC	73	44	Yes	Yes	No	45	1.14	>35 000	1.74	4.24	2.16	6.48	11.7	No	Yes	10	Survive
9	FM	F	39.6	GC	89	52	Yes	No	No	25	0.77	>35 000	2.08	7.74	2.42	3.06	9.51	Yes	Yes	10	HT
10	FM	F	29.9	LC	77	48	Yes	Yes	No	30	1.18	4939	1.75	4.20	1.50	2.27	8.65	Yes	Yes	8	Survive
11	FM	F	27.7	LC	90	40	Yes	No	Yes	25	0.80	4245	1.41	4.38	1.47	2.39	9.39	Yes	Yes	8	Survive
12	FM	M	27.5	LC	143	68	No	No	Yes	28	0.97	17 100	7.66	3.09	3.91	4.01	8.50	Yes	Yes	7	Survive
13	FM	F	56.5	LC	91	63	Yes	No	No	18	1.52	11 492	1.28	6.33	2.38	2.99	11.3	Yes	Yes	6	Survive
14	FM	M	32.9	LC	70	40	No	Yes	No	15	1.44	9110	1.41	3.88	2.21	3.49	11.3	Yes	Yes	6	Survive
15	FM	F	41.5	LC	110	66	No	No	Yes	25	1.51	34 684	3.40	5.84	2.69	2.72	10.7	No	Yes	6	Survive
16	FM	F	44.8	LC	150	76	Yes	No	Yes	10	1.03	20 456	1.94	2.68	1.61	2.44	9.72	No	Yes	6	Survive
17	FM	F	29.0	LC	92	45	No	No	No	19	0.93	13 455	2.26	9.57	2.36	2.02	9.54	No	Yes	5	Survive
18	FM	M	14.3	LC	85	40	Yes	Yes	Yes	23	1.30	2093	6.63	6.51	2.39	2.97	10.8	No	Yes	3	Survive
19	FM	M	15.3	LC	90	60	Yes	Yes	Yes	25	8.12	>35 000	8.30	9.79	4.77	6.05	11.0	No	No	0	Survive
20	FM	M	37.2	LC	82	52	No	No	No	42	0.84	10 592	3.34	6.13	1.42	2.14	8.70	No	No	0	Survive

- FM vs NFM 20: 13
- 3 patients expired / 2 patients - Heart transplantation
- 19 Lymphocytic myocardtis / 1 Giant cell myocarditis
- MCS duration: 0-30 days

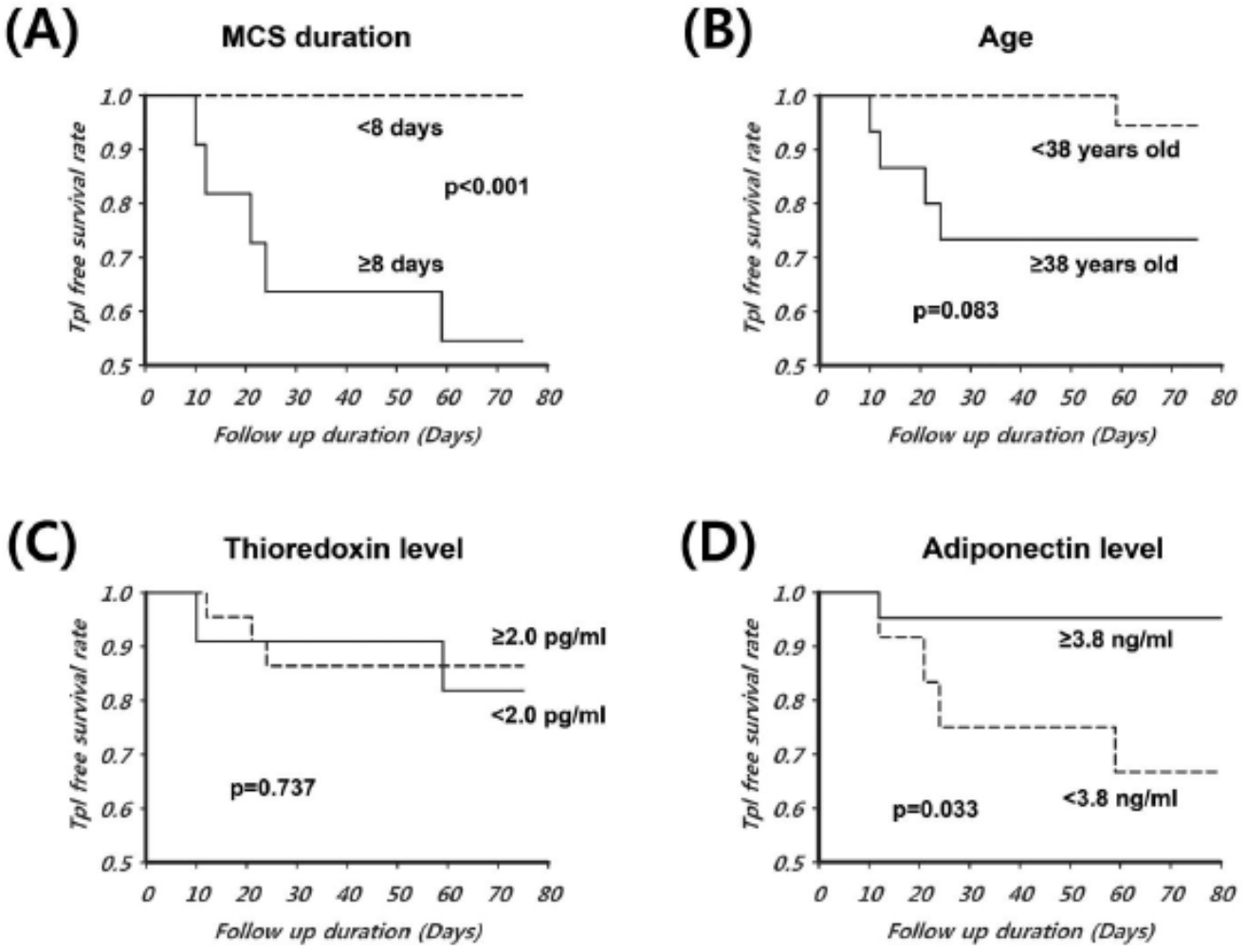
Choi JH & Jeon ES. Heart 2011 Jul;97(13):1067-73

Cytokines as prognostic factor



Choi JH & Jeon ES. Heart 2011 Jul;97(13):1067-73

Prognostic factors in FM survival



Choi JH & Jeon ES. Heart 2011 Jul;97(13):1067-73

Treatment of Viral Myocardtis

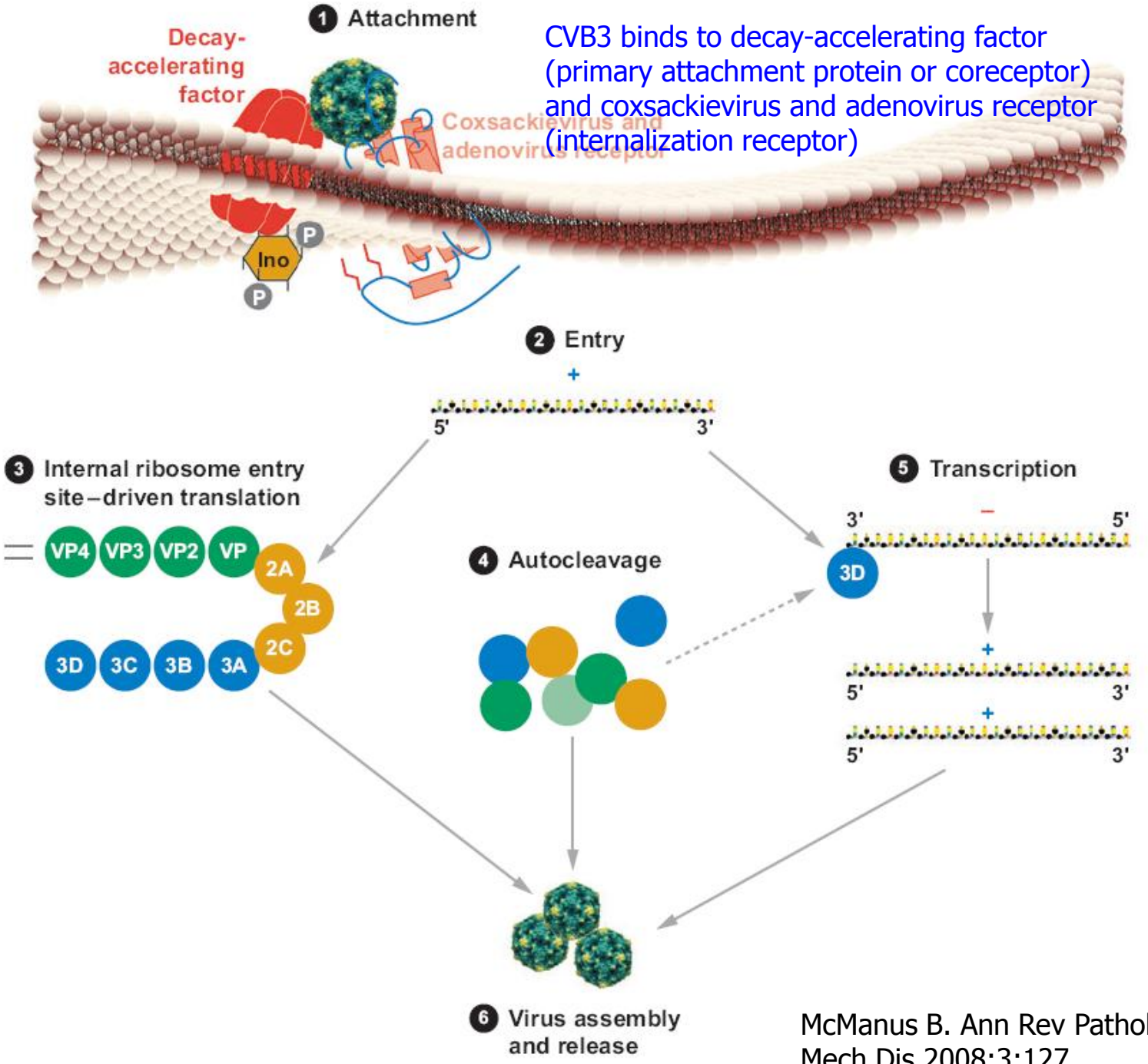
1. Supportive care
   - Hemodynamic stabilization
2. ACC/AHA guideline for LV dysfunction
   - β-adrenergic blocker / ACEI for all
   - Aldosterone antagonist in NYHA FC III-IV
3. Immunosuppressive agents

AHA/ACC Guidelines for HF. Circulation 2006;113:876

Antiviral Agent Targeting Enterovirus

전은석 / 임병관 / 윤수현 /주은선 / 신재옥
광주과기원 김용철
한국화학연구원 정영식

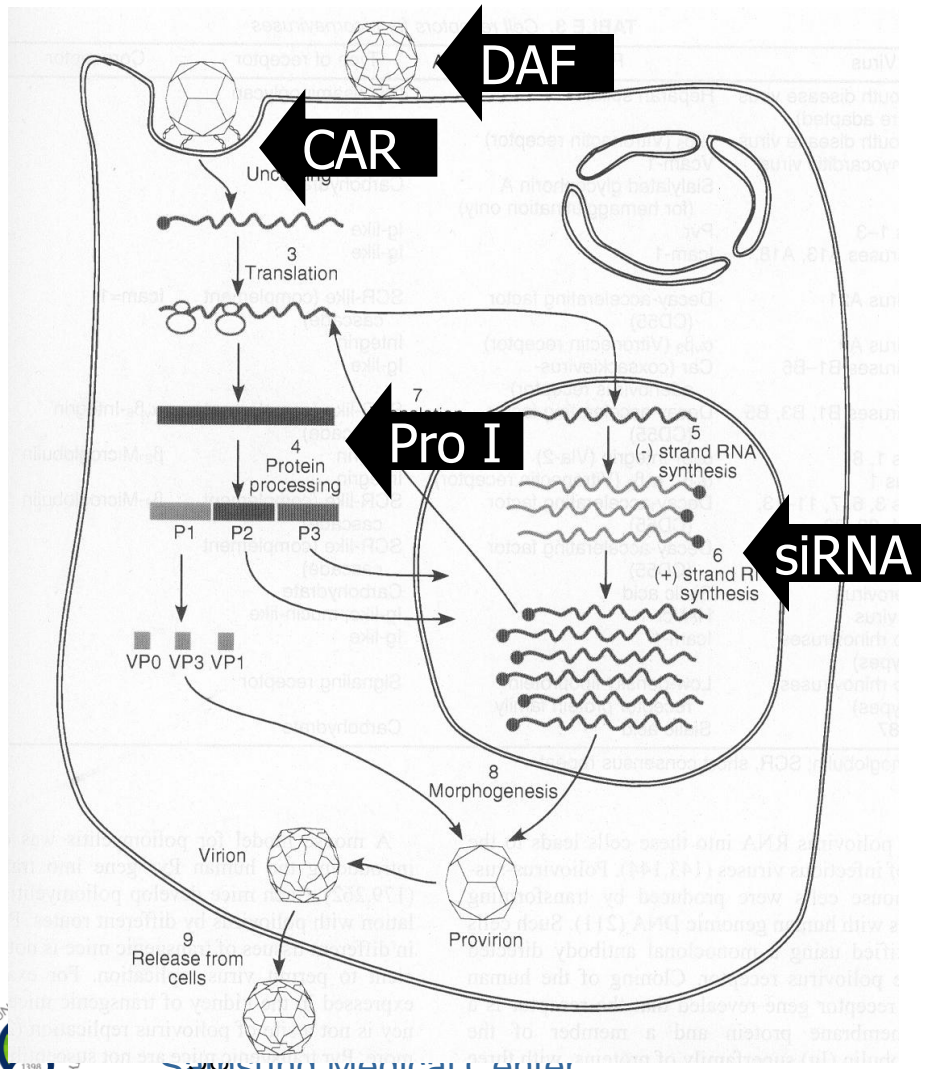
CVB life cycle



CVB3 binds to decay-accelerating factor (primary attachment protein or coreceptor) and coxsackievirus and adenovirus receptor (internalization receptor)

McManus B. Ann Rev Pathol Mech Dis 2008;3:127

Anti-picornaviral agents



1. Capsid-binding :
   - pleconaril, pirodavir (R 77975), SCH 48973, SDZ 35-682, Chalcone amides …
2. Receptor binding:
   - soluble ICAM-1
   - CAR-DAF Ab
   - CAR-DAF receptor trap
3. RNA synthesis
   - (+) plus-strand viral RNA synthesis inhibition by 3A coding region control: enviroxime …
4. Viral protease inhibitors
   - 3C protease inhibitor
   - 2A protease inhibitor
   - Polymerase inhibitor

Time Course of Viral Myocarditis

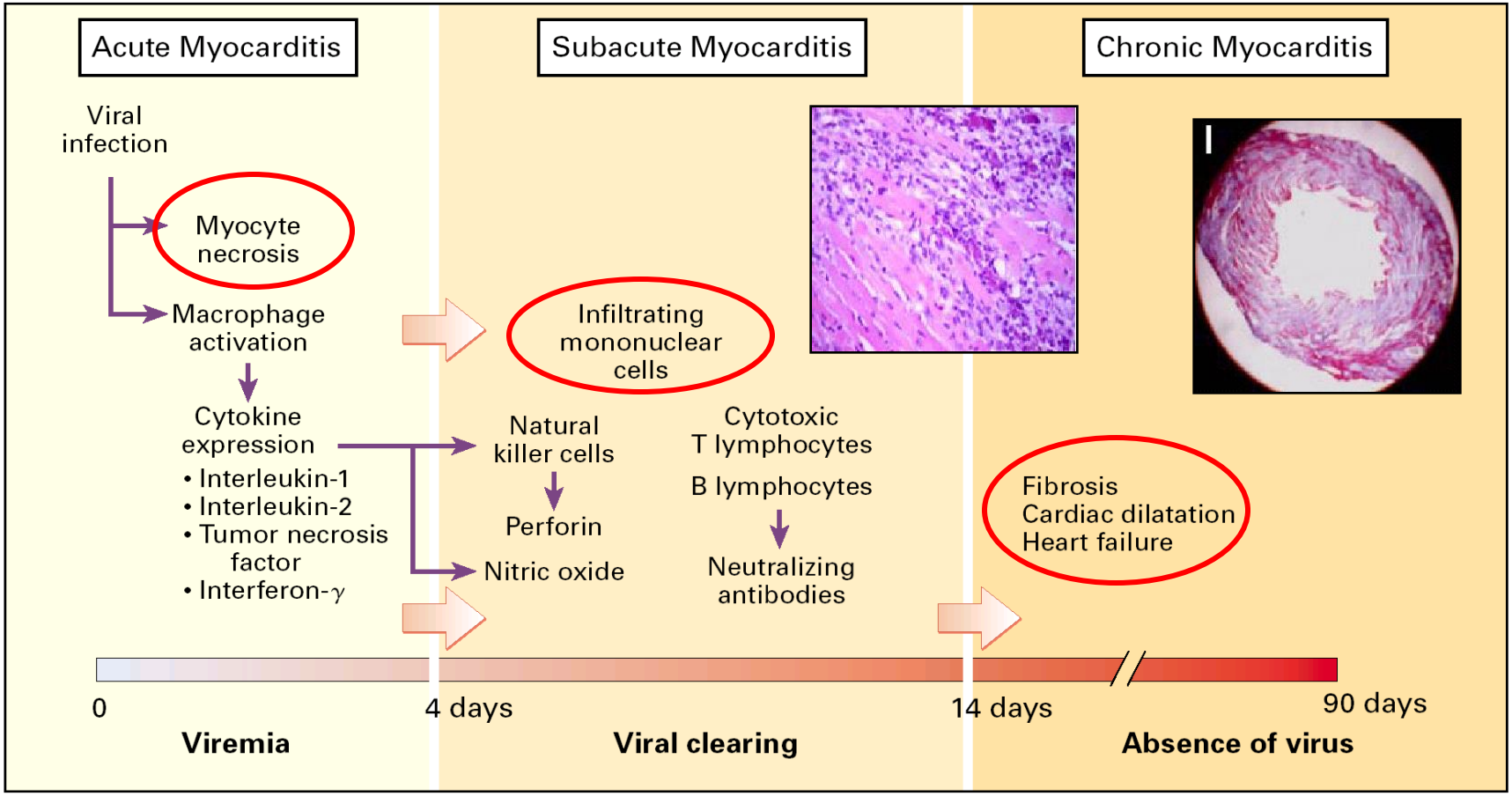
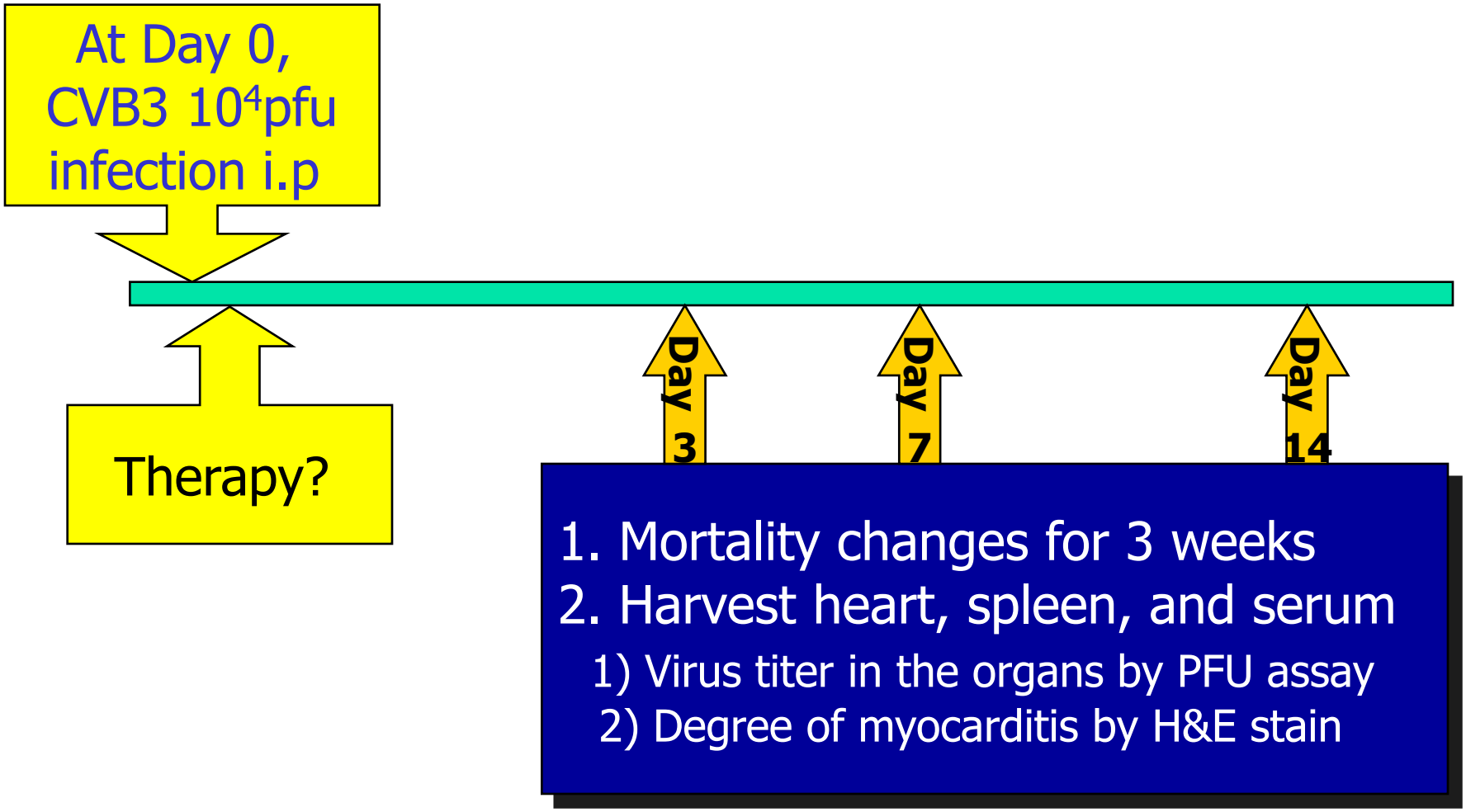
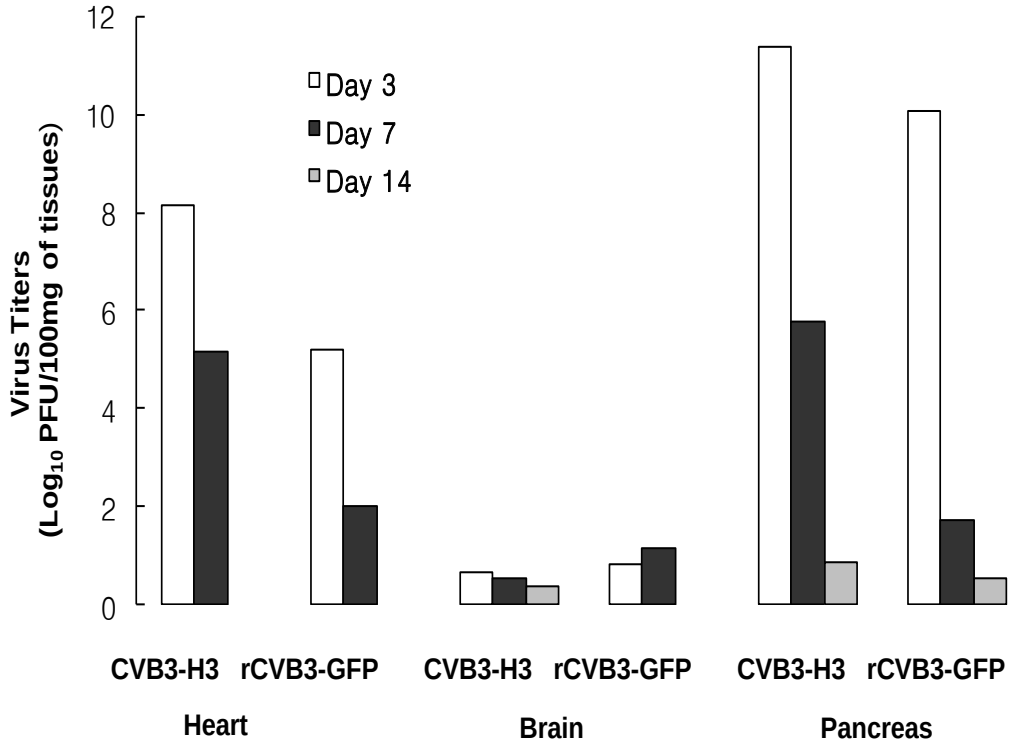


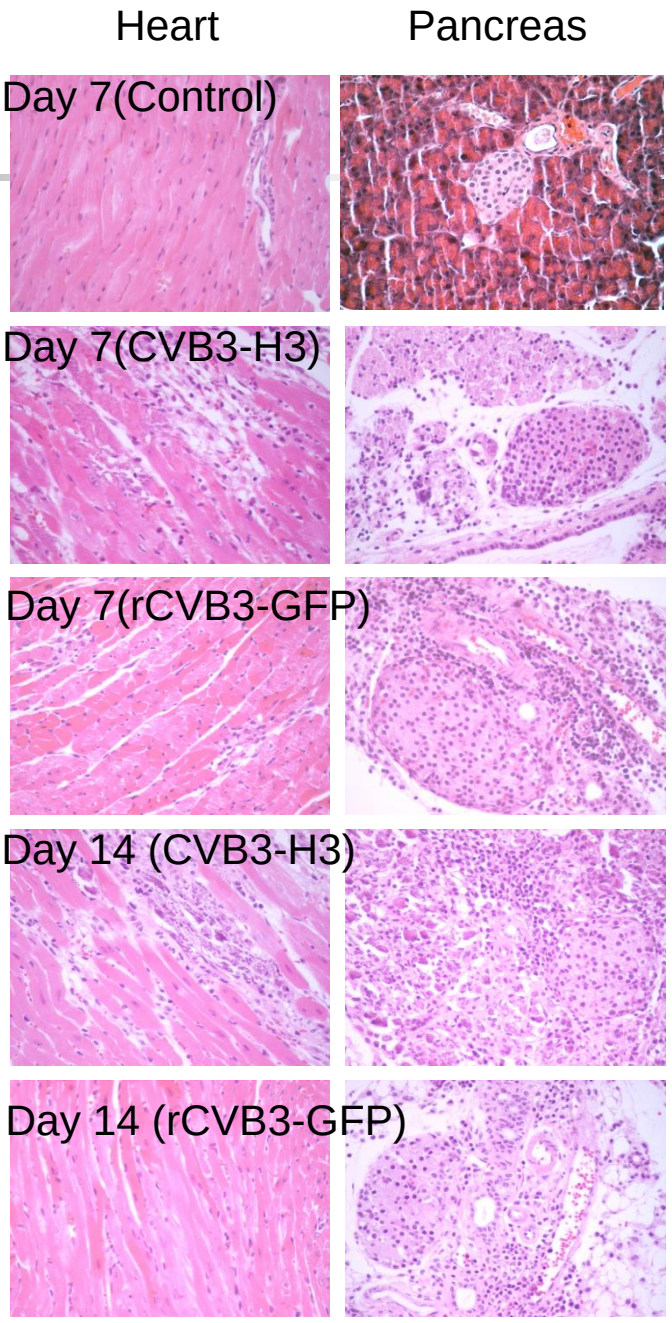
Figure 2. Time Course of Experimental Viral Myocarditis in Mice.
Adapted from Kawai[11] with the permission of the publisher. The timeline is not drawn to scale.

CVB3 mycardtis model

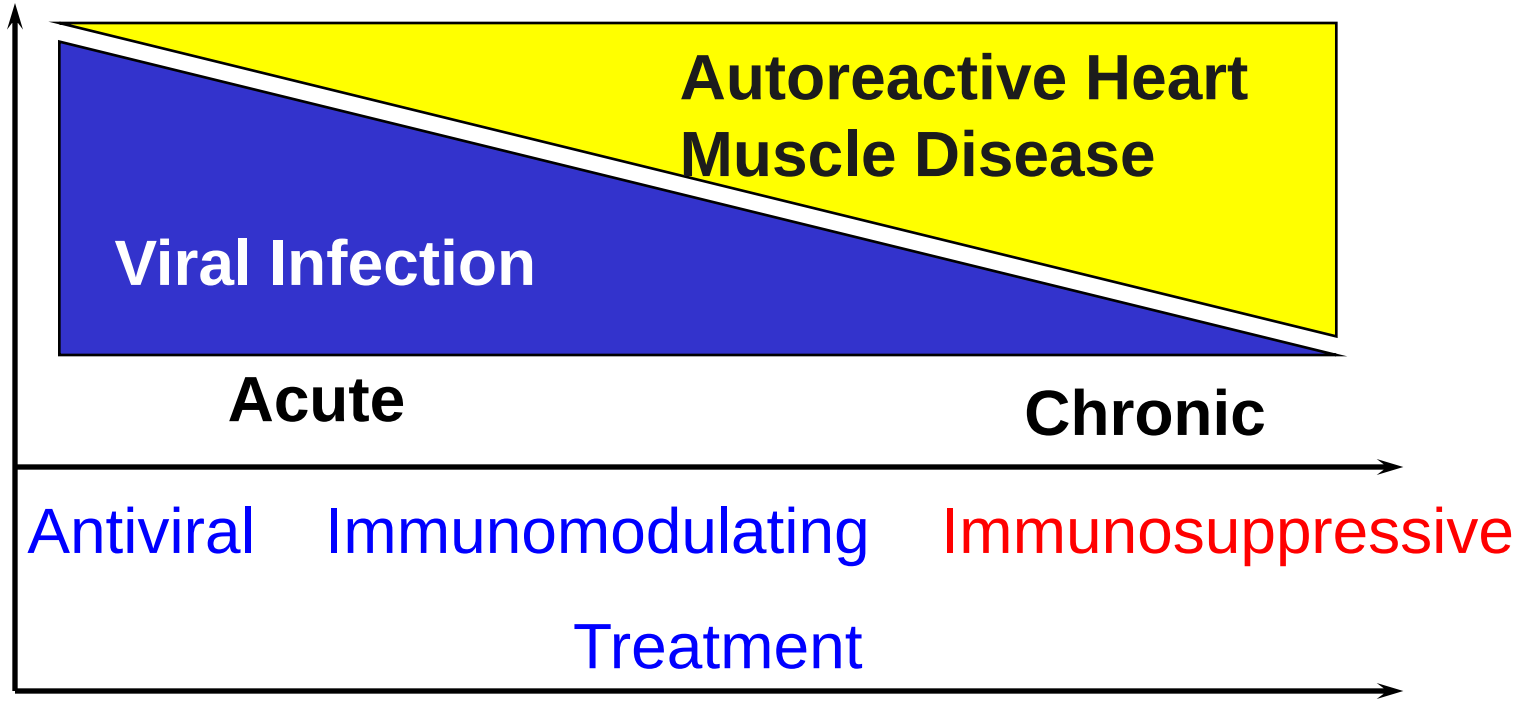


Pathology and Virus titers In organs of H3 infected mice

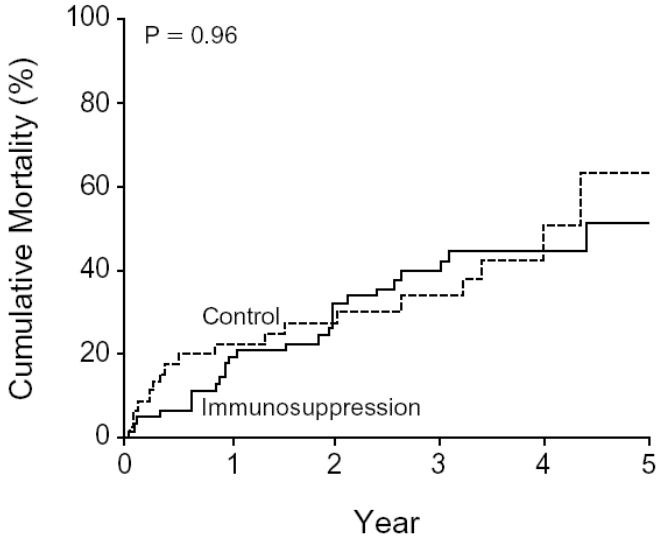




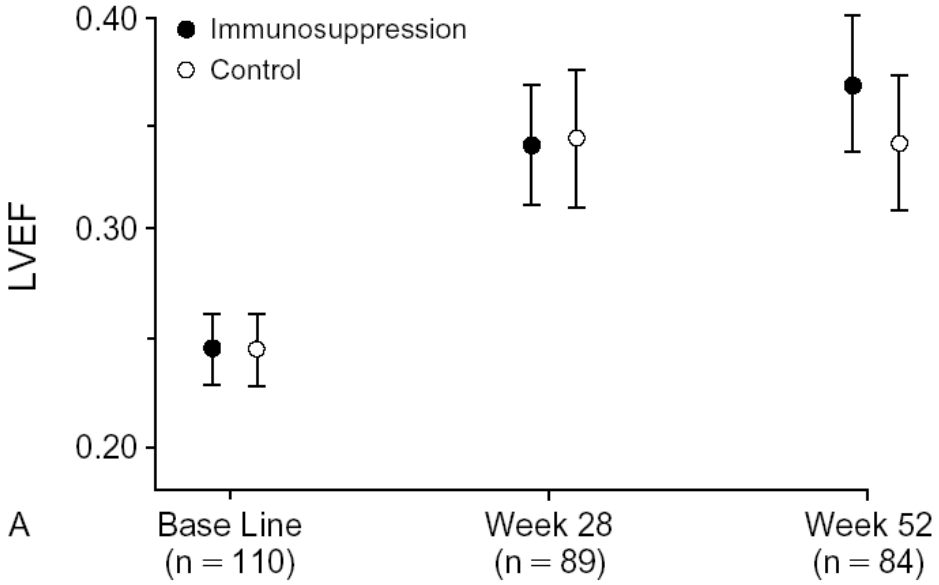
Pathogenic Mechanism of Myocarditis



Myocarditis Treatment Trial



Immuno-suppression	64	49	37	23	12	0
Control	47	32	23	16	6	0

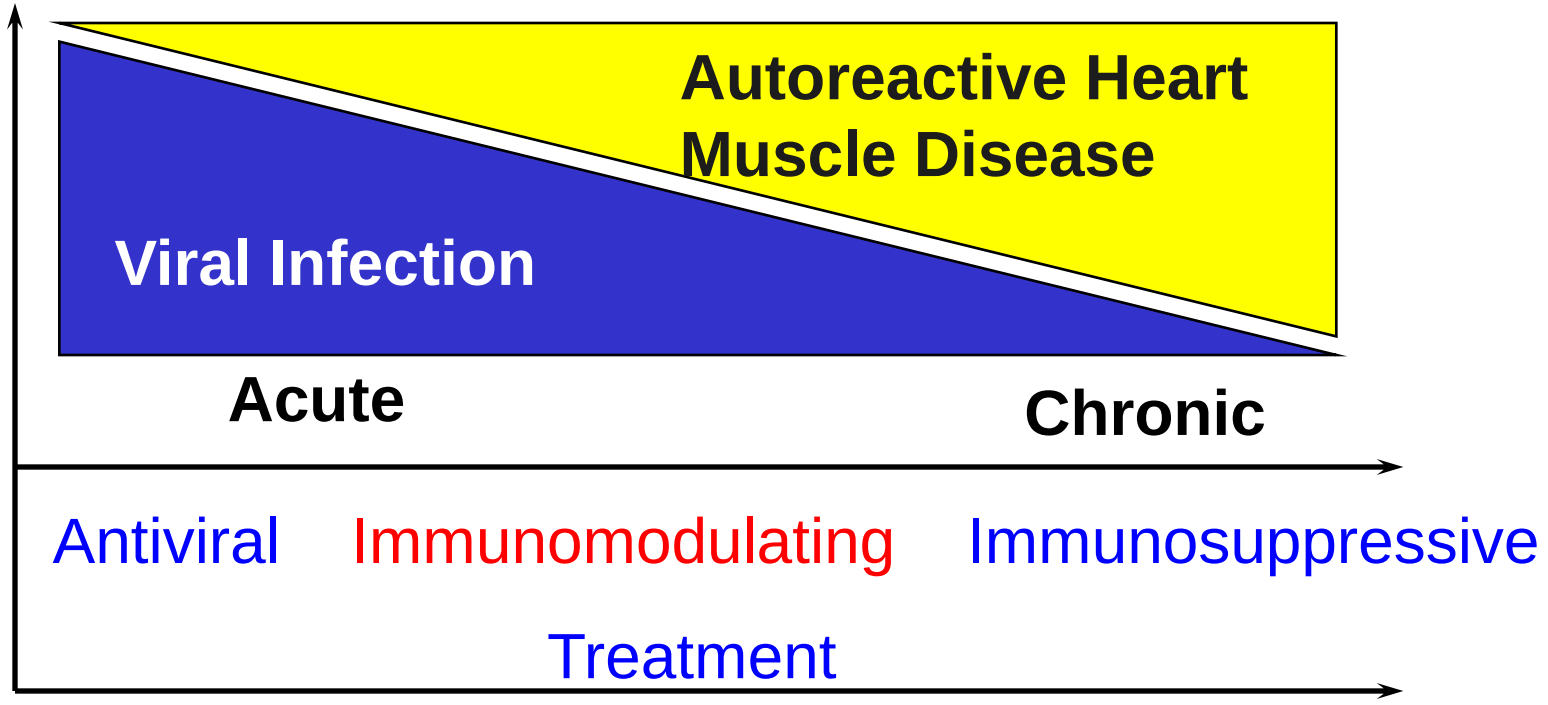


A

- Results : No significant effects of immunosuppression on NYHA FC, LVEF, Survival, PWP, LV dimension

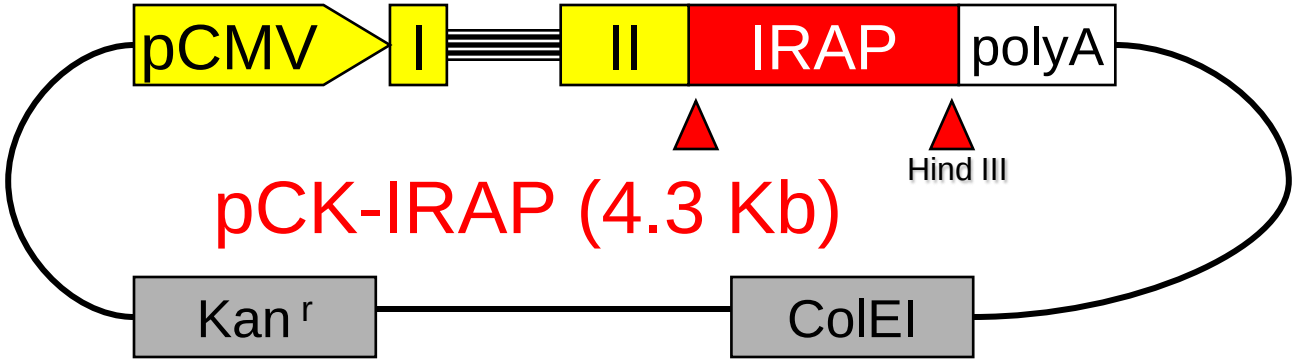
Mason, NEJM 333:269, 1995

Pathogenic Mechanism of Myocarditis



IRAP Clone : pCK-IRAP

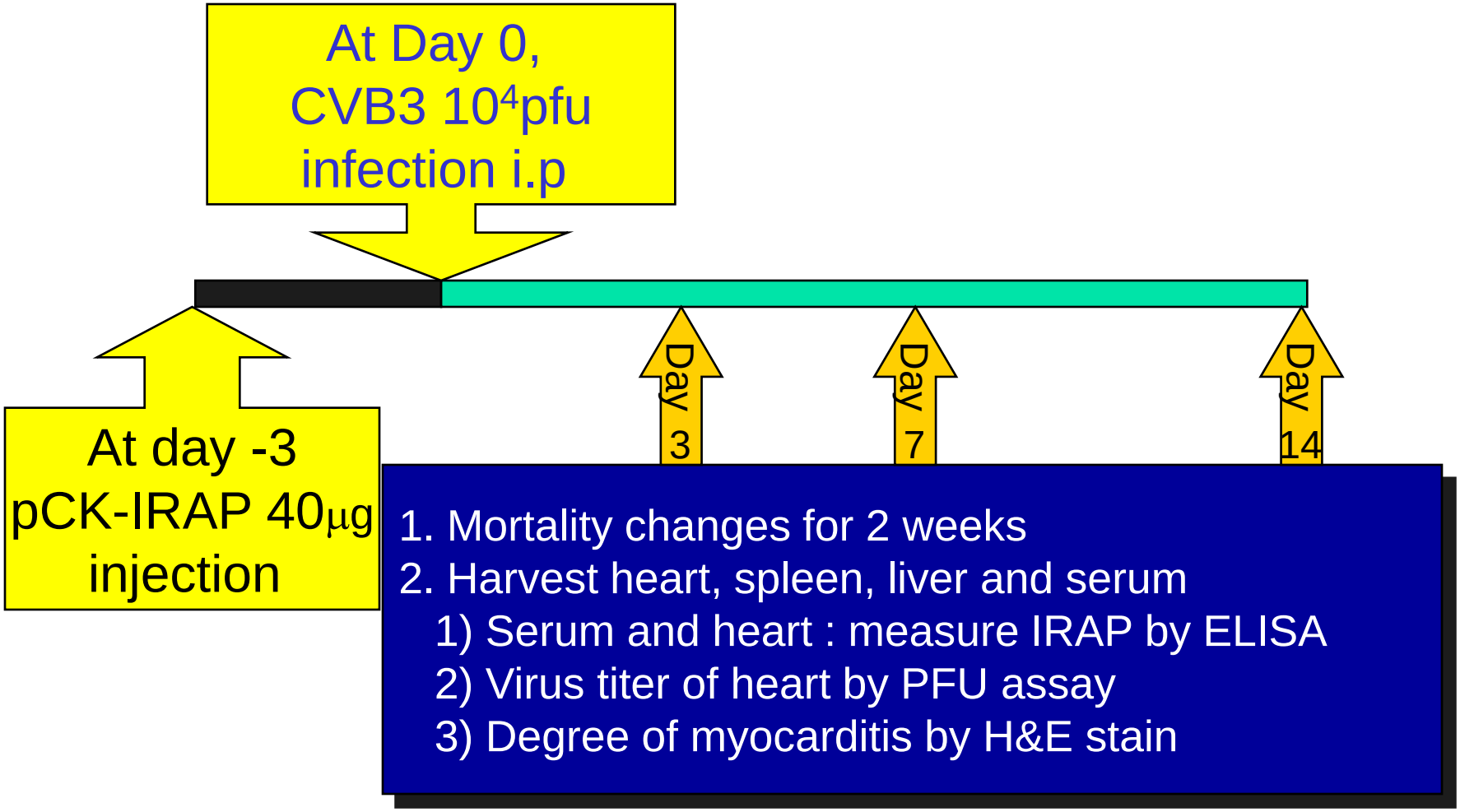
- gDNA from peripheral blood lymphocytes
- Reverse transcription of human IRAP
  - N-term : 5'-*AAGCTT*ATGGAAATCTGCAGAGGCCTCCGCAGTACAC-3'
  - C-term : 5'-*GTCGAC*CTACTCGTCCTCCTGGAAGTAGAATTTGGT-3'
- Clone human IRAP into pCK plasmid



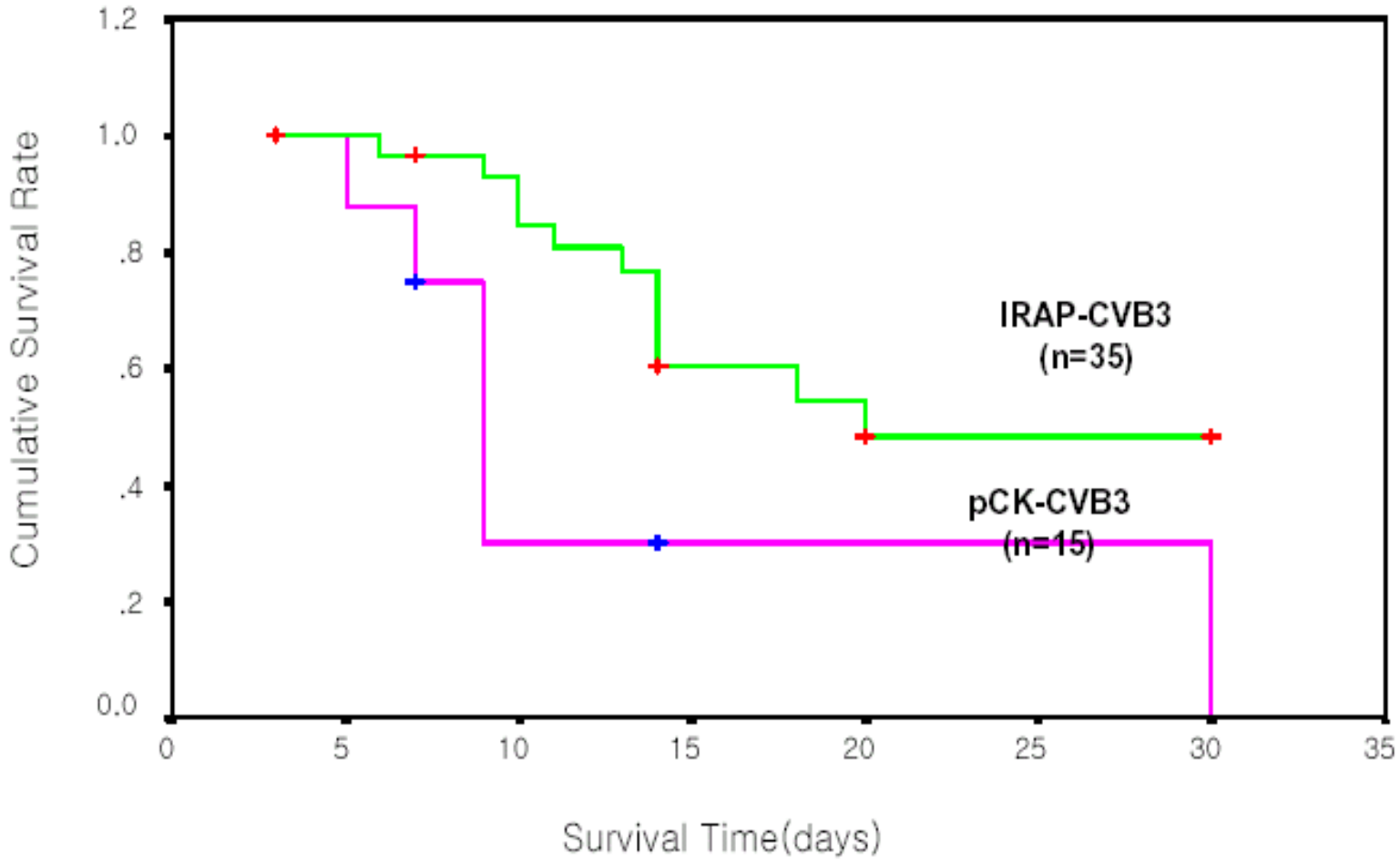
바이로메드㈜ 김종묵, 김선영

Lee et al., BBRC 272:230, 2000

Experimental Scheme of IRAP effect on Myocarditis

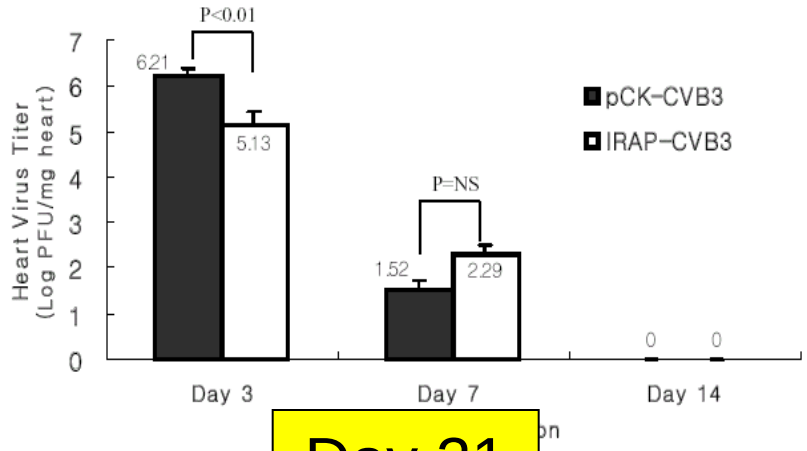


Expressed IRAP decreased mortality in CVB3 myocarditis

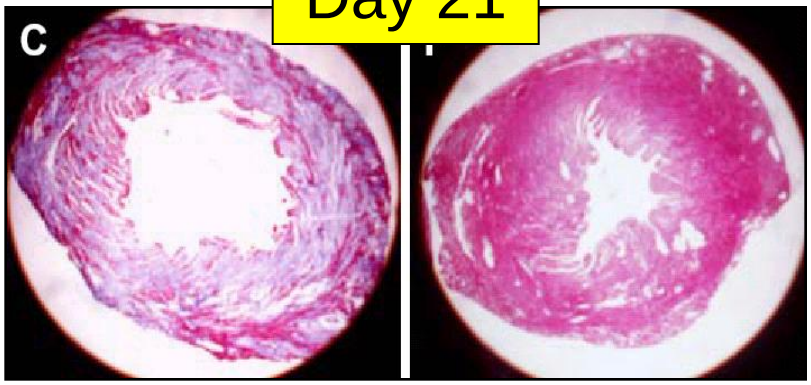


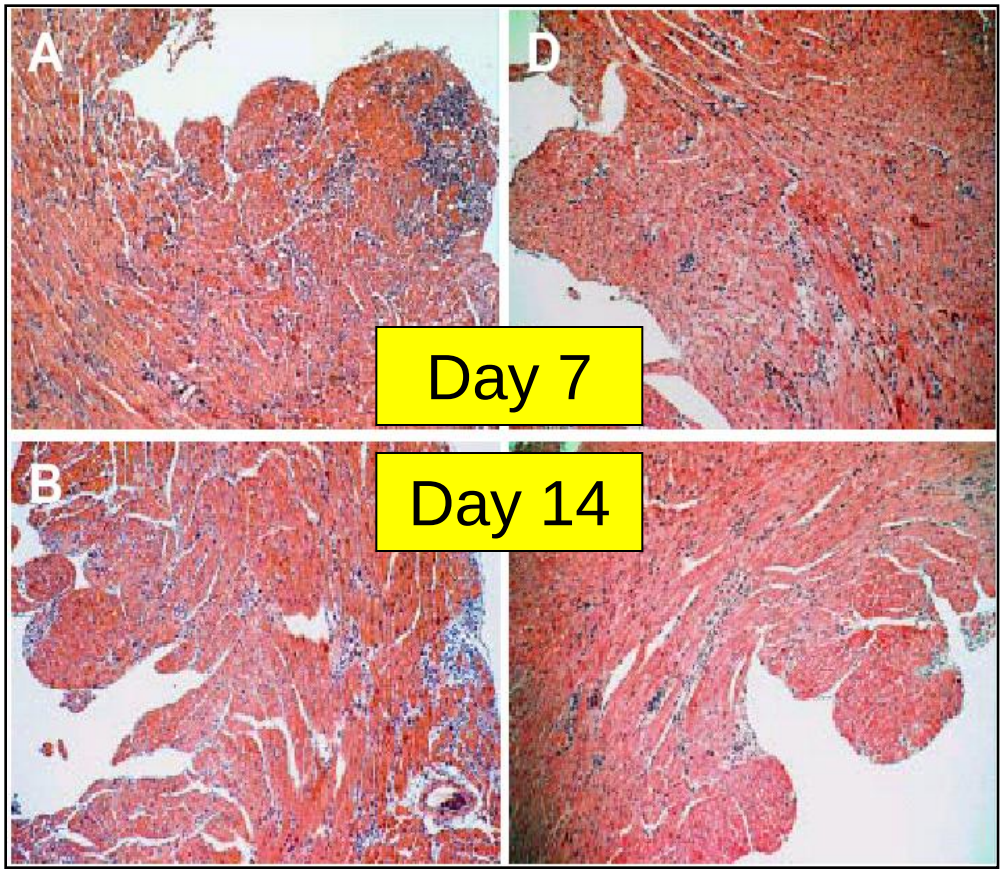
Jeon ES et al. Circulation 2002:105:1278

Expressed IRAP decreased virus titer, inflammation and fibrosis



Day 21





Day 7

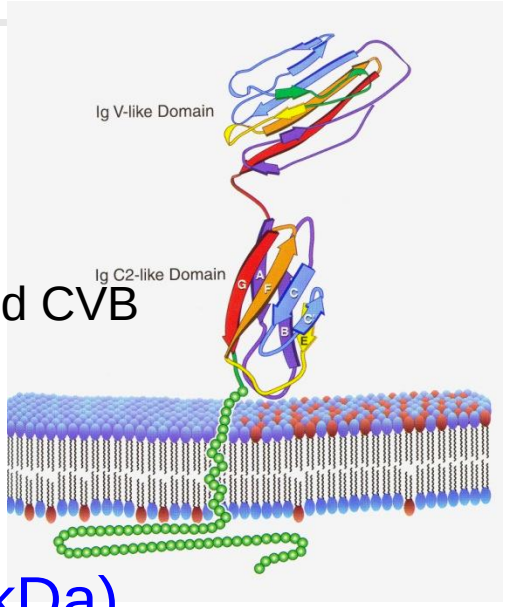
Day 14

pCK-CVB3 pCK-IRAP pCK-CVB3 pCK-IRAP

Jeon ES et al. Circulation 2002:105:1278

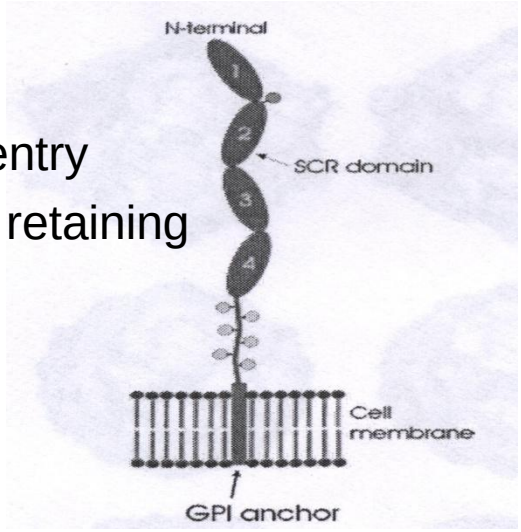
Receptors of Coxsackievirus

1. CAR: Coxsackievirus and Adenovirus Receptor(48kDa)



- High affinity receptor for both subgroup C adenovirus and CVB
- CAR mediates attachment and infection by CVB
  (*Science* 1999;286:1579)

2. DAF: Decay-accelerating factor: CD55 (70kDa)



- A member of complement control protein family
- Regulators of complement activation
- Act as an attachment receptor preliminary to viral cell entry
- Some CVB group also have DAF binding activity while retaining binding activity of CAR
  (*J Virol* 1995;69:1903)

CAR and DAF

Collaboration of receptors

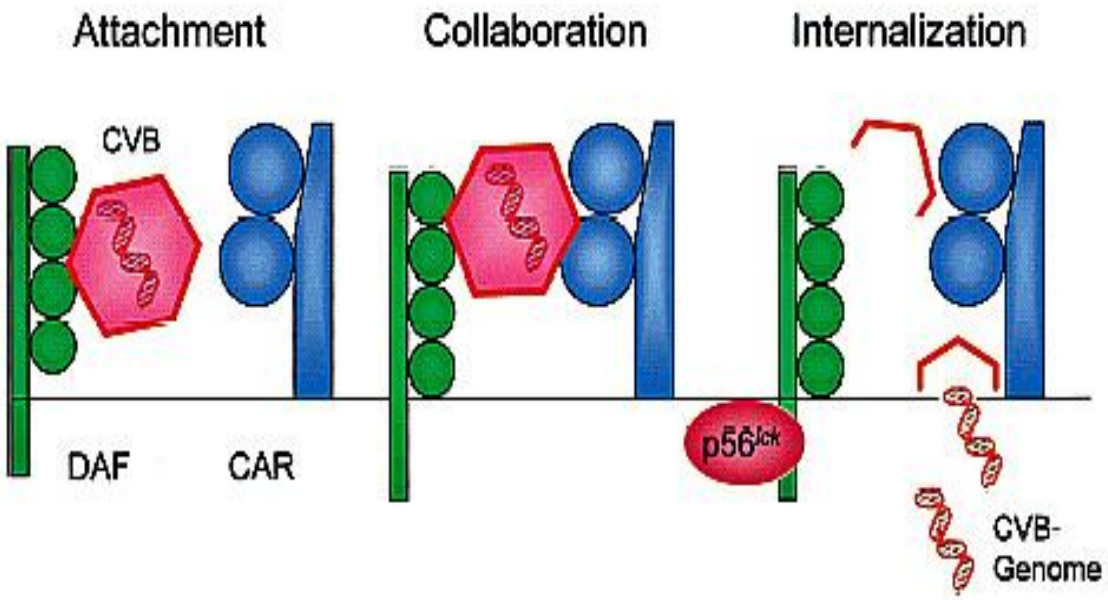


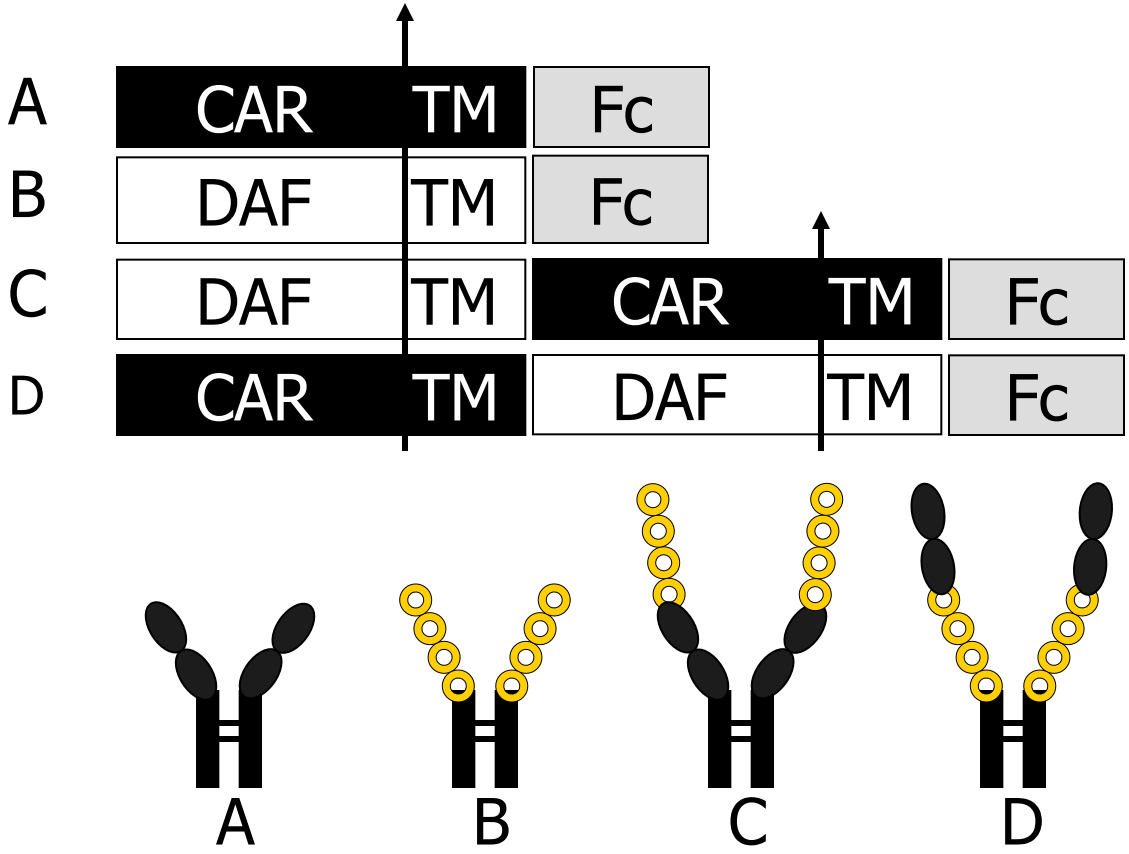
Figure 3. Collaboration of receptors. CAR and DAF receptors cooperate to permit uncoating of viral genome and internalization.

Virus Receptor Traps, express both CAR and DAF attenuate coxsackievirus infection *in vitro and in vivo*

Lim BK, Choi JH, Nam JH, Gil CO, Shin JO, Yun SH, Kim DK, Jeon ES.

Cardiovascular Research 2006;17(3):517-26.

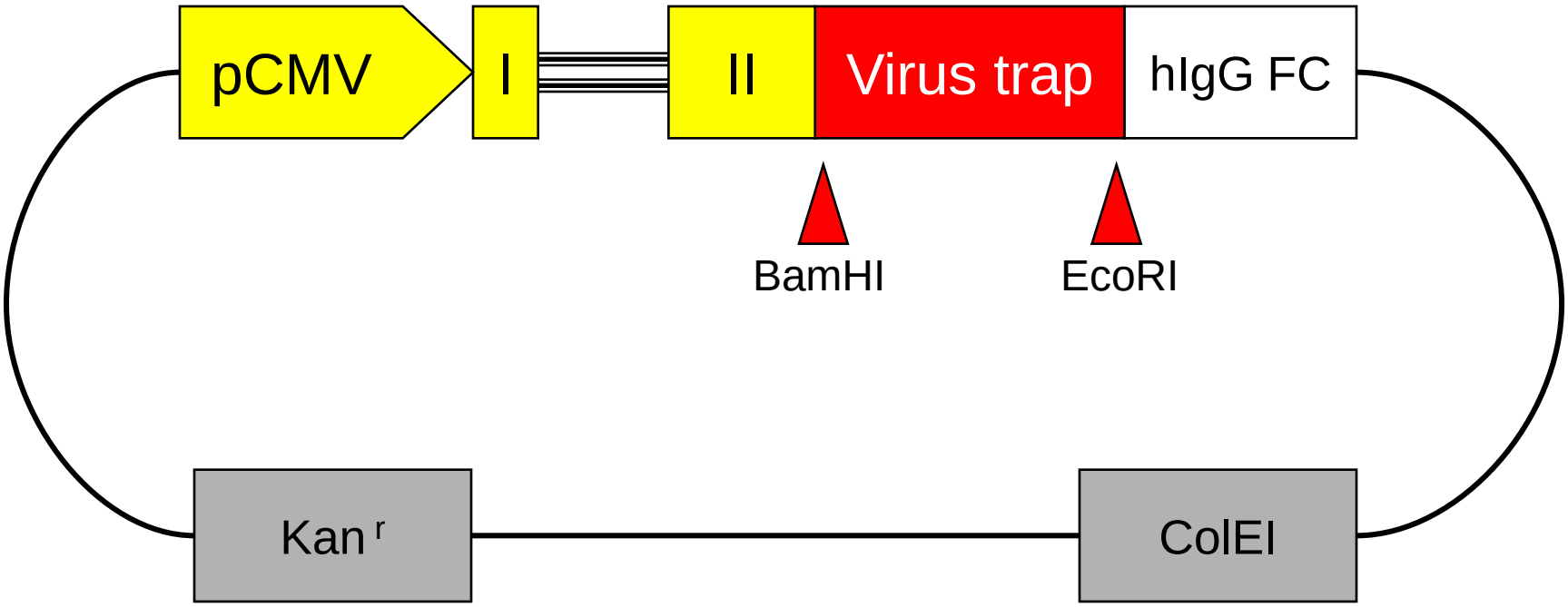
Clone virus traps into pCK:Fc vector



A) CAR-Fc, B) DAF-Fc, C) DAF-CAR-Fc, D) CAR-DAF-Fc

, with or without TM (transmembrane domain)

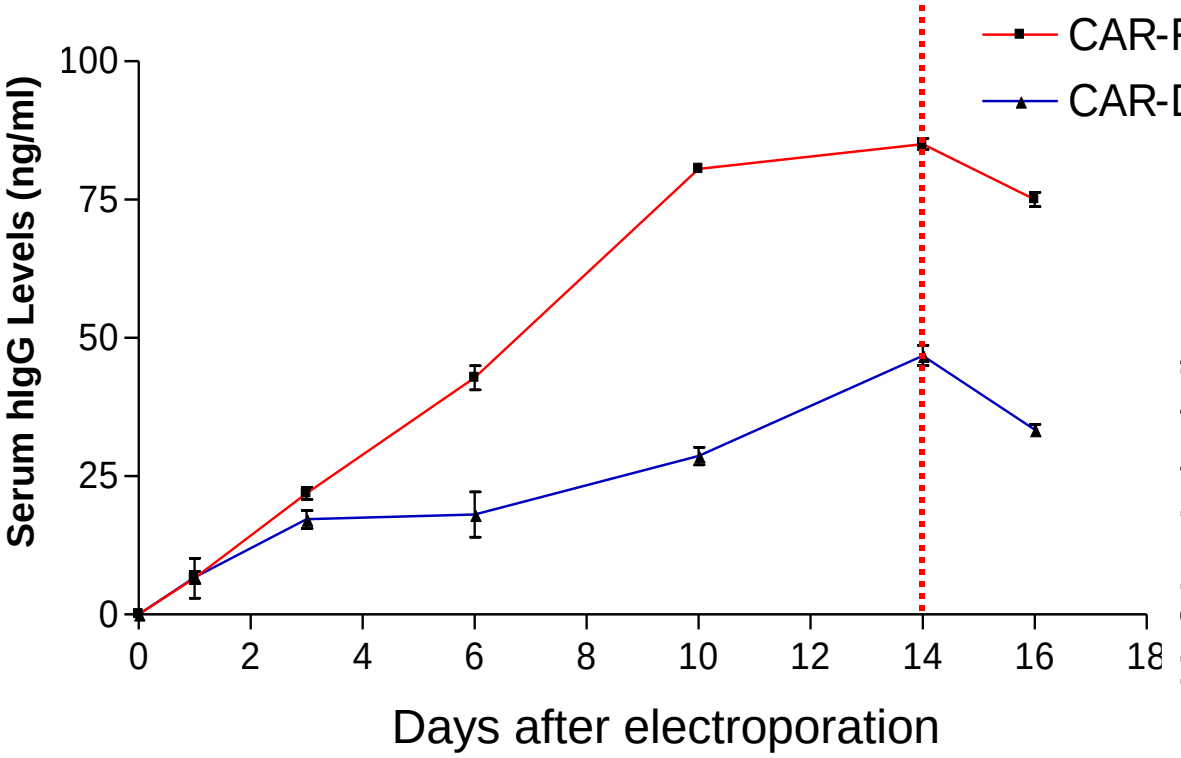
pCK - Virus Trap Vector

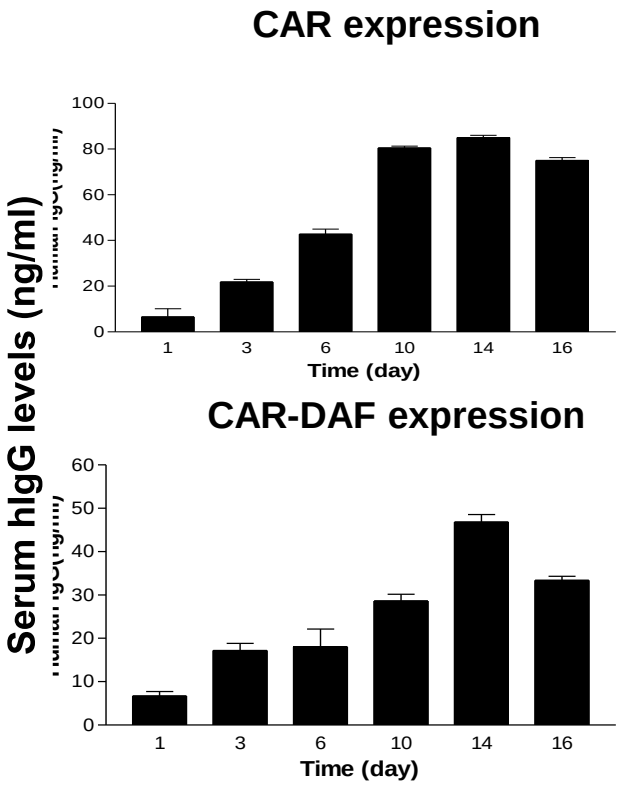


pCK-Virus Trap

Lee et al., BBRS 272:230, 2000

Culmulative Expression of Virus Traps after Electroporation *in vivo* measured by hIgG ELISA

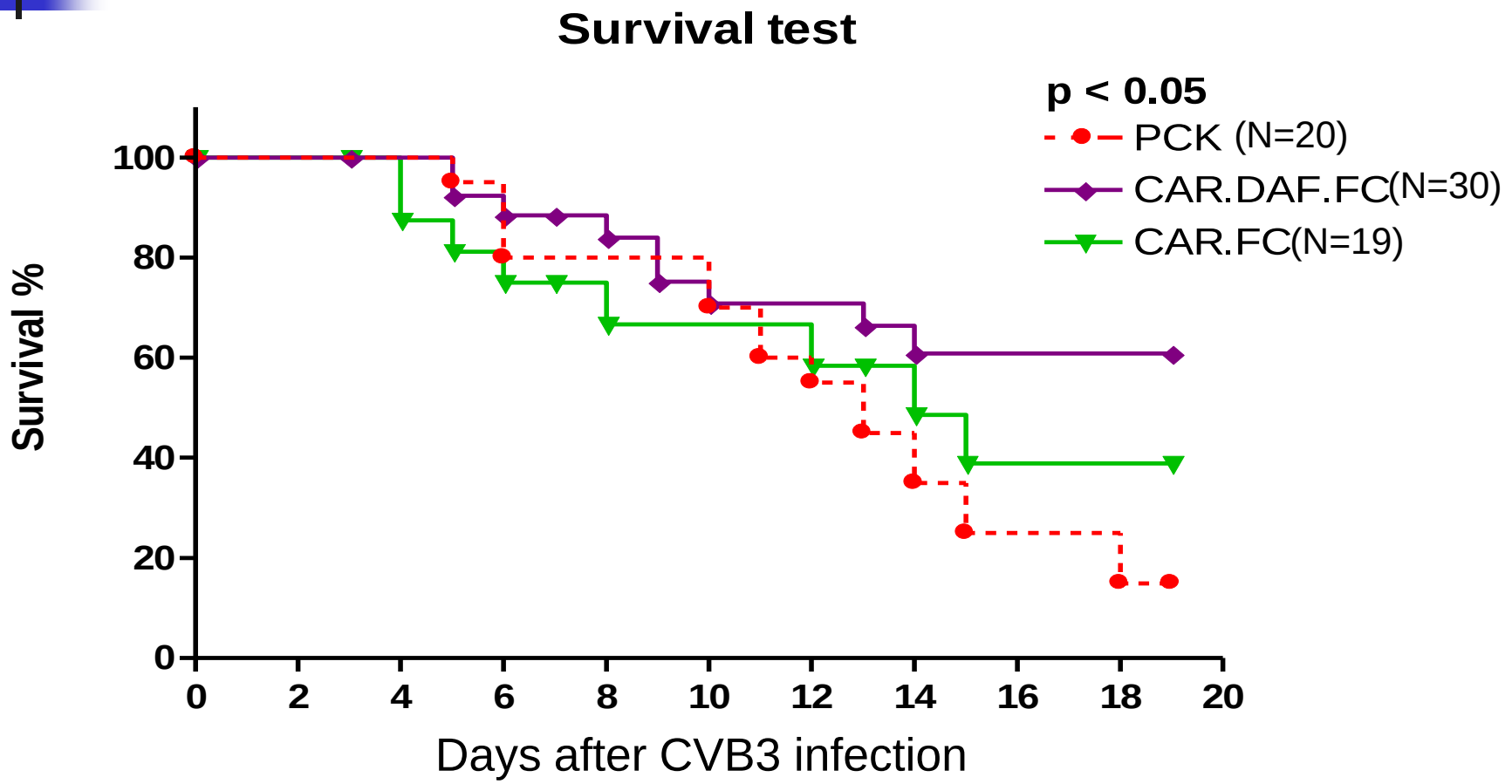




On day 14, serum levels of CAR:Fc increased 12.9-fold (85.1±1.0 ng/ml) and CAR-DAF:Fc increased 7.1-fold (46.8±1.8 ng/ml) compared with the baseline (6.6±1.2 ng/ml).

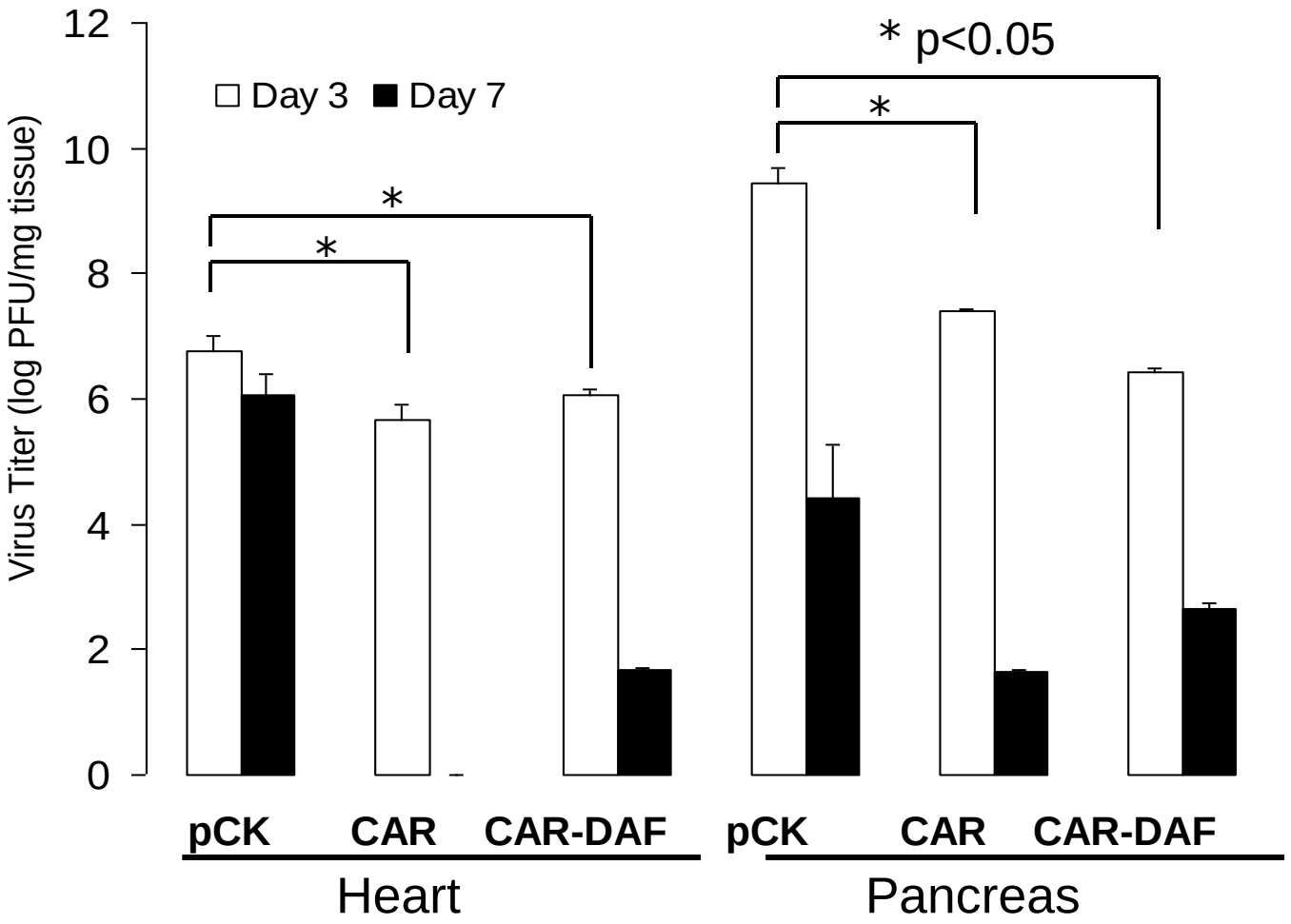
Jeon ES et al. J Mole Cell Cardiol 2005;38:745

Expressed Virus Trap by Electroporation decreased Mortality in CVB3 myocarditis

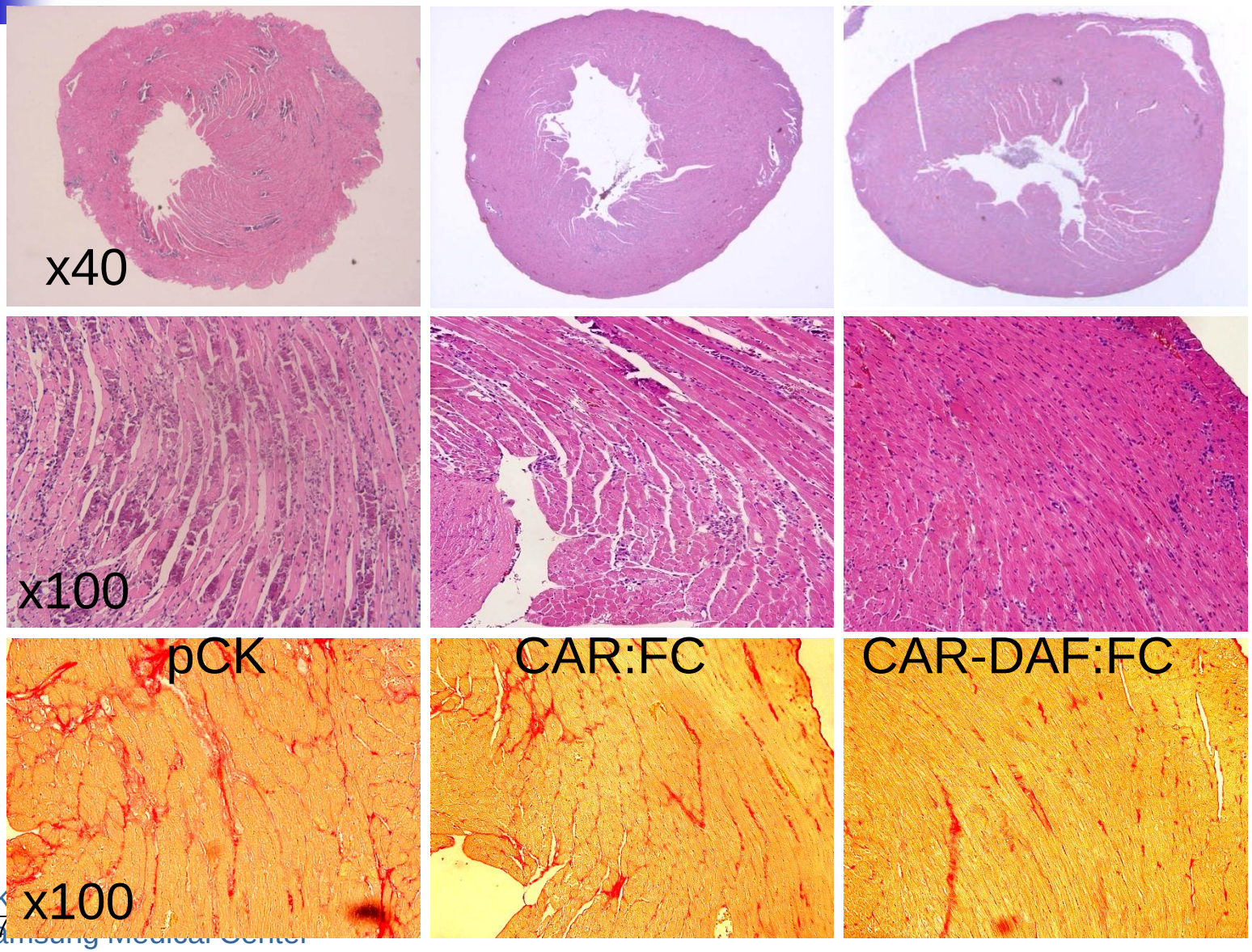


The 20-day survival rates were higher in virus-trap-treated mice (CAR-DAF:Fc, 61%; CAR:Fc, 29%; controls, 15%; $p<0.05$)

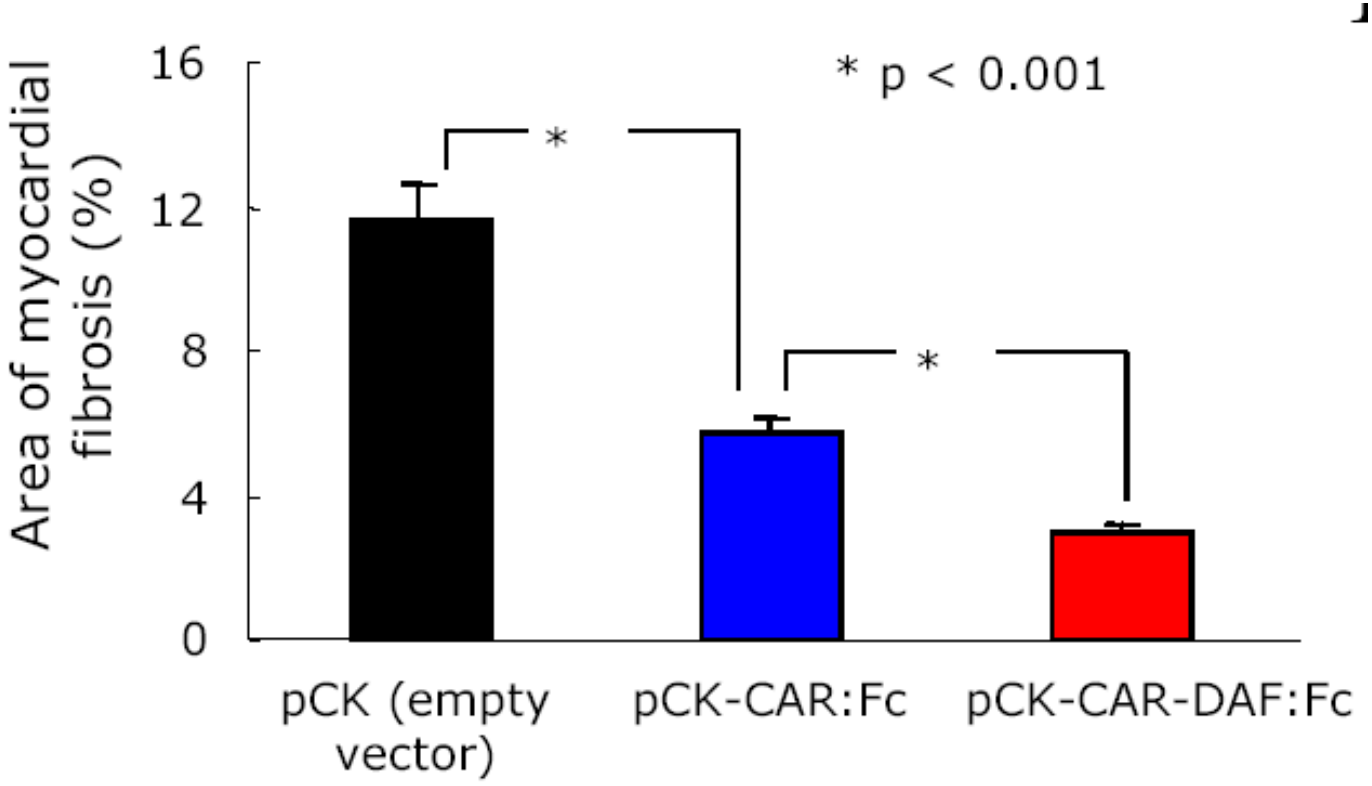
Organ Virus Titers at Day 3 and Day 7 after CVB3-H3 Infection



Pathologic findings of Hearts at day 7 and day 14 after CVB3-H3 Infection



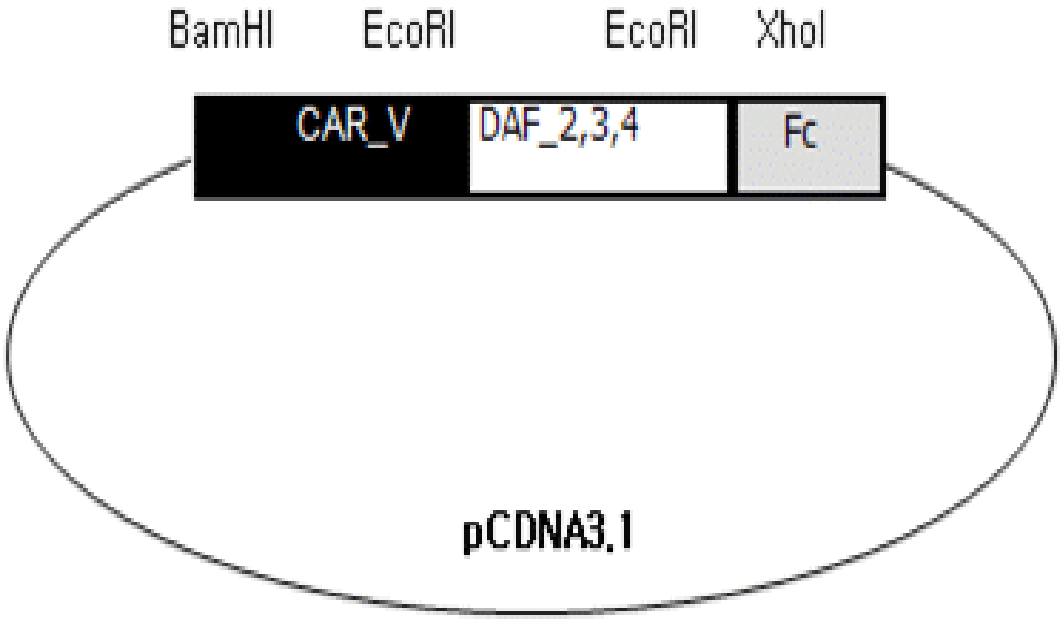
Areas of fibrosis at day 14



Computer-assisted quantitation of the areas of fibrosis showed significantly reduced myocardial fibrosis in the treatment groups, and less fibrosis in the CAR-DAF:Fc group than in the CAR:Fc group ($p<0.001$).

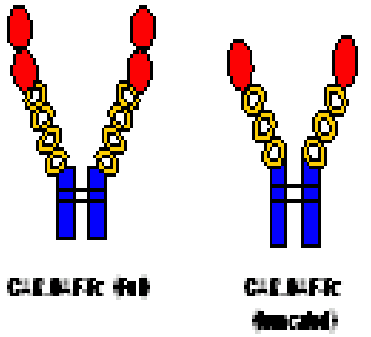
수용성 바이러스 수용체 유전자 재조합 plasmid

A

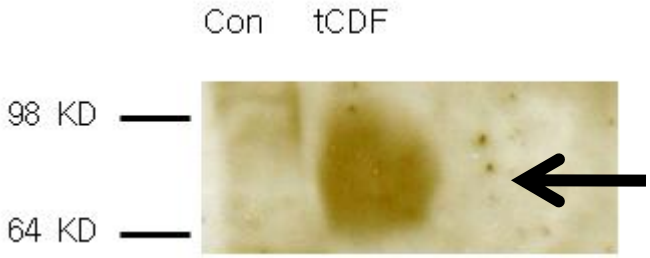


Truncated CAR.DAF:Fc

- CAR-Ig V like domain
- DAF- 2,3,4 domain

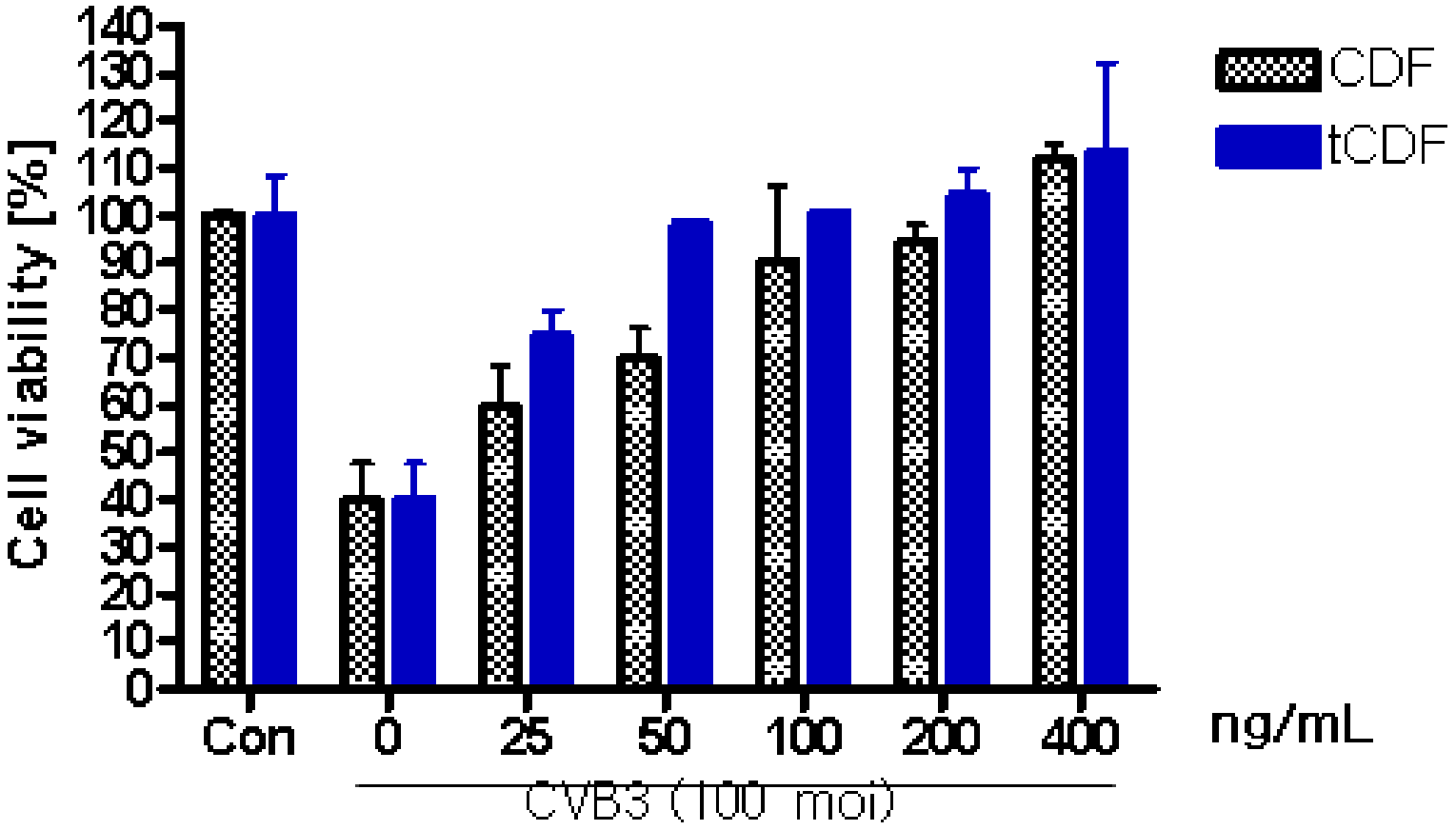


B



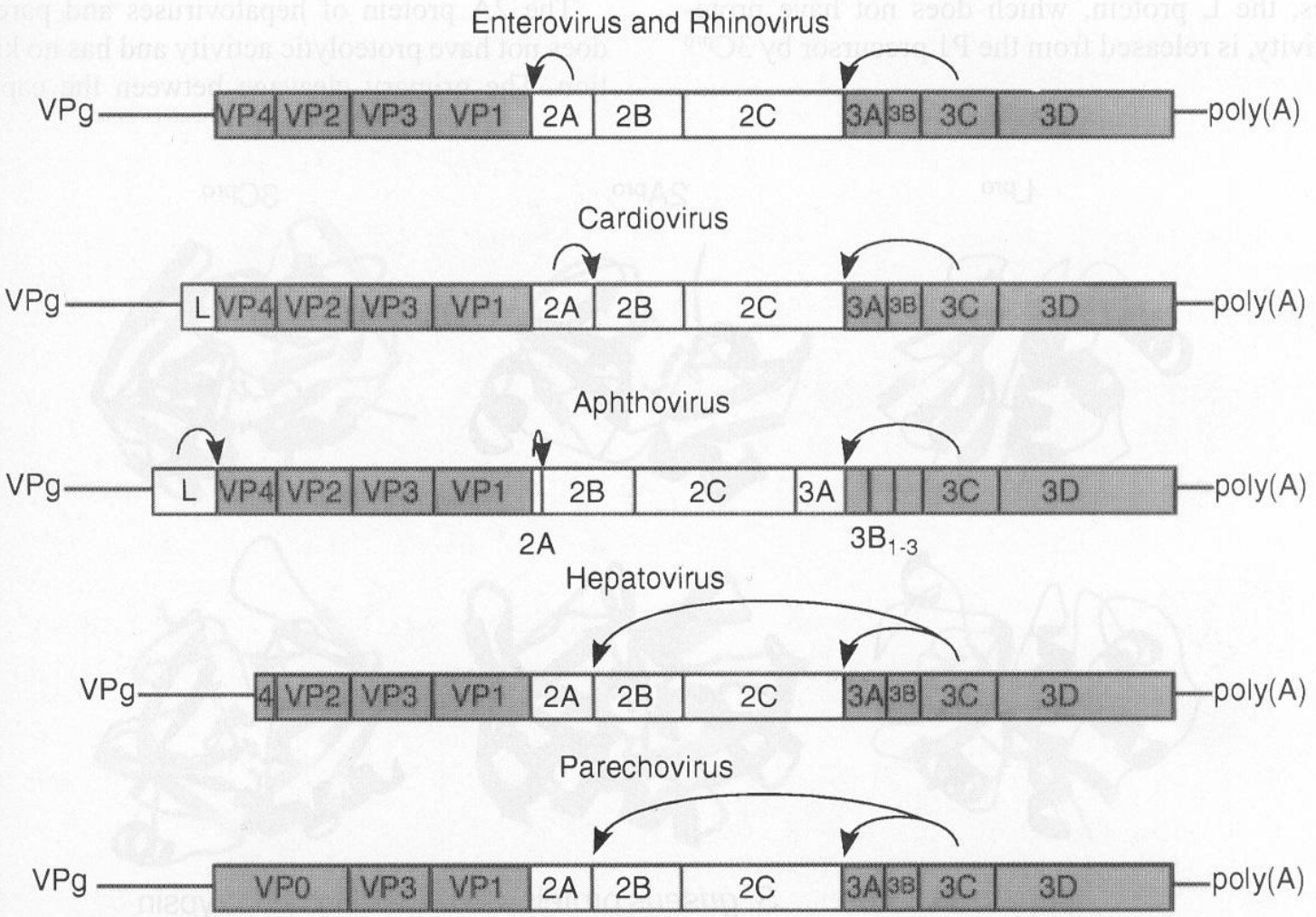
cell lines 발현 단백질

수용성 바이러스 수용체의 항 바이러스 효꽈

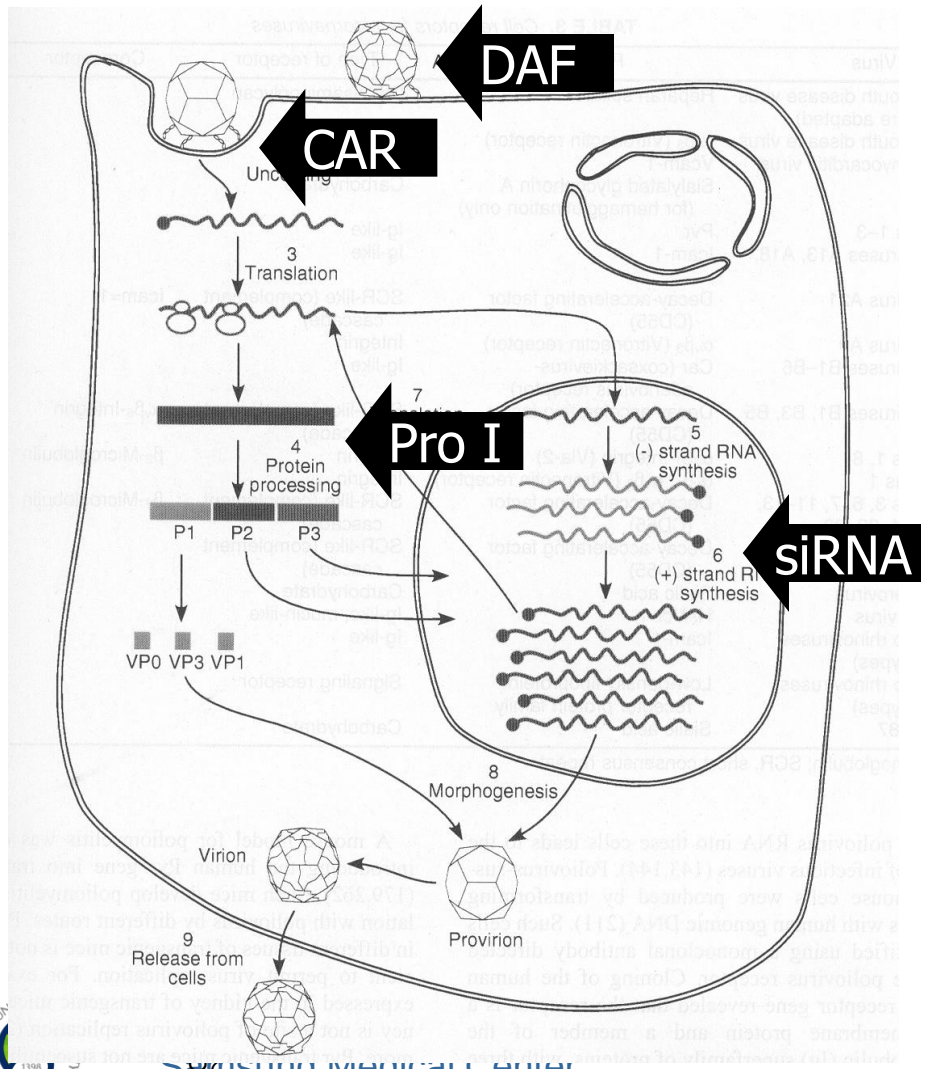


기존의 수용체 보다 우수한 효과를 나타냄

Primary cleavages of picornavirus polyprotein



Anti-picornaviral agents



1. Capsid-binding :
   - pleconaril, pirodavir (R 77975), SCH 48973, SDZ 35-682, Chalcone amides …
2. Receptor binding:
   - soluble ICAM-1
   - CAR-DAF Ab
   - CAR-DAF receptor trap
3. RNA synthesis
   - (+) plus-strand viral RNA synthesis inhibition by 3A coding region control: enviroxime …
4. Viral protease inhibitors
   - **3C protease inhibitor**
   - 2A protease inhibitor
   - Polymerase inhibitor

콕사키바이러스 3C protease 저해제를 이용한 항바이러스 치료제 개발

Development of antiviral agent using 3C protease inhibitor of Coxsackievirus

제 1 세부 : 삼성서울병원 순환기내과 전은석

Antiviral effect of 3C protease inhibitor in experimental myocarditis

제 2 세부 : 광주과학기술원 약품화학 김용철

Development of new soluble coxsackievirus 3C protease inhibitor

Chemical structure of HRV 3CP inhibitor

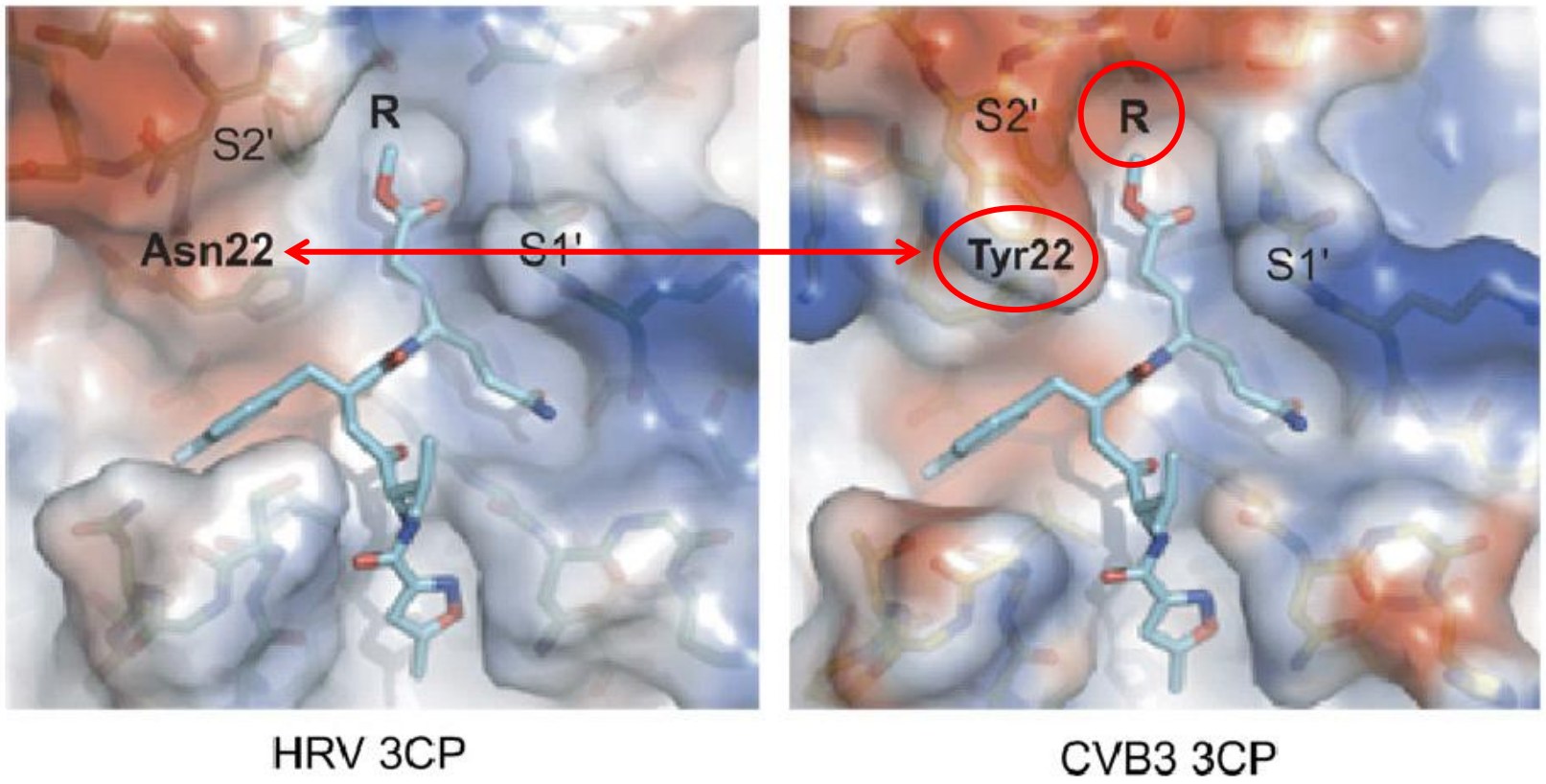
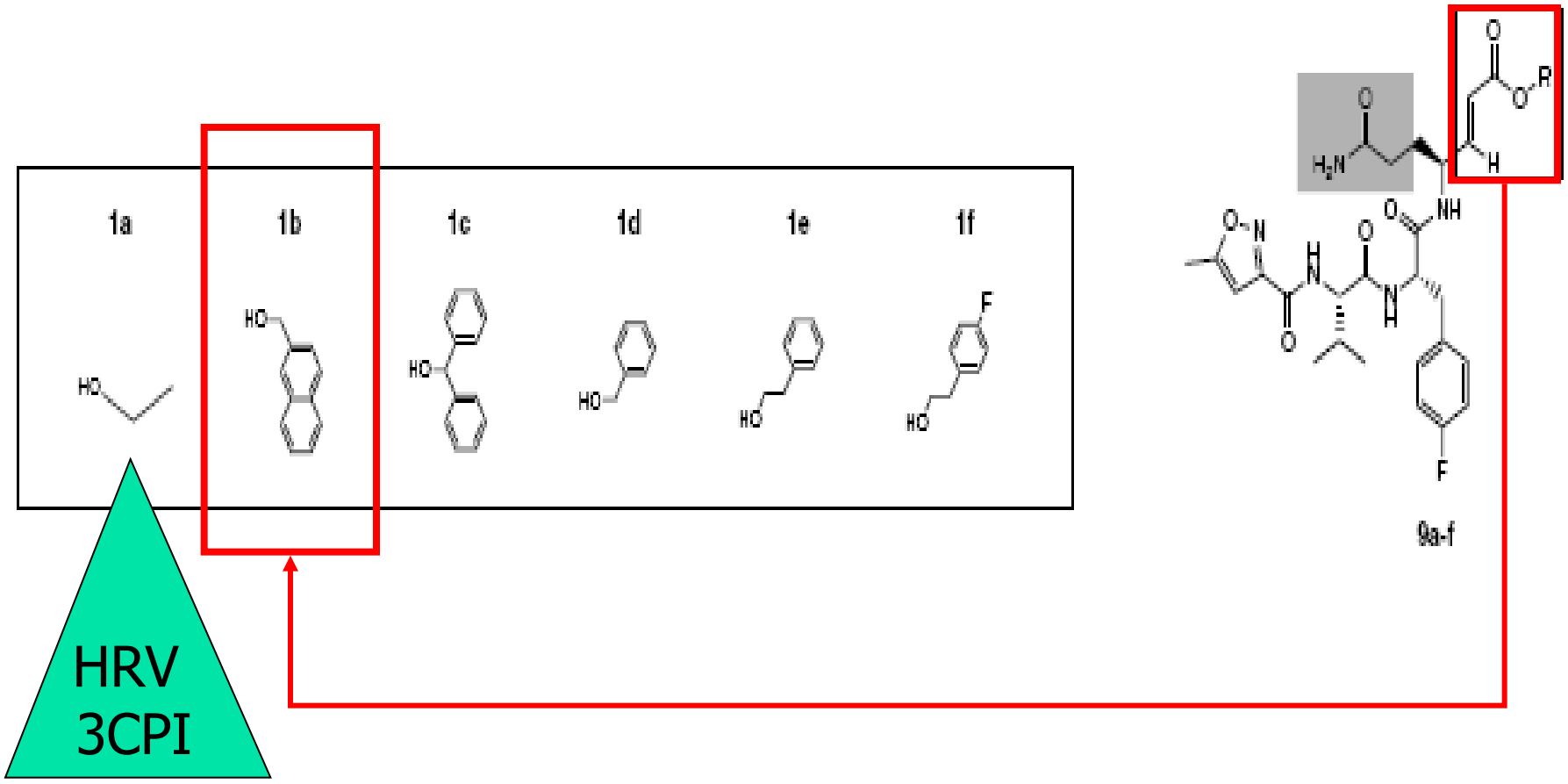


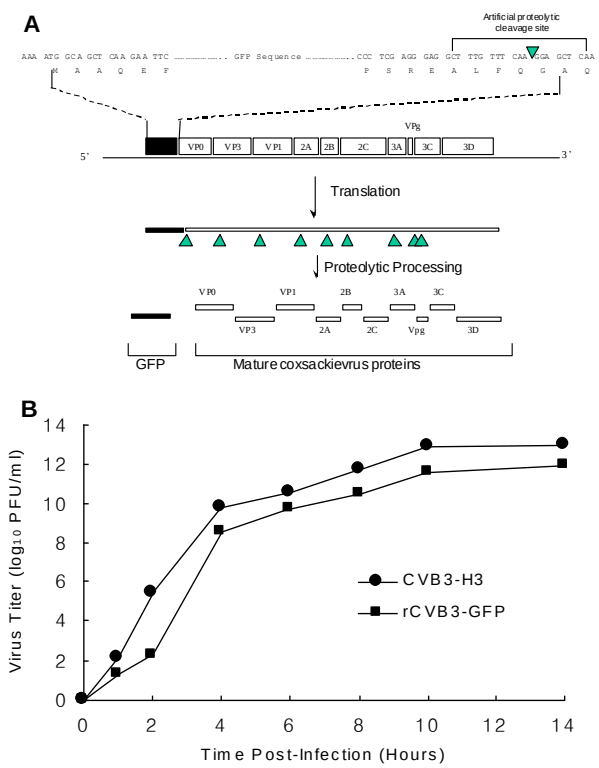
Fig. 1. Comparative modeling of CVB3 3CP. Structures of both HRV 3CP and CVB3 3CP docked with a HRV 3CP inhibitor, AG7088, are shown. **Note the presence of Tyr22 at S20 of CVB3 3CP (right panel).** We hypothesized that Tyr22 would interact preferentially with aromatic R groups in potential inhibitors. The electrostatic calculation of models and the preparation were performed using the PyMol software .

Biochem Biophys Res Commun. 2007;358:7

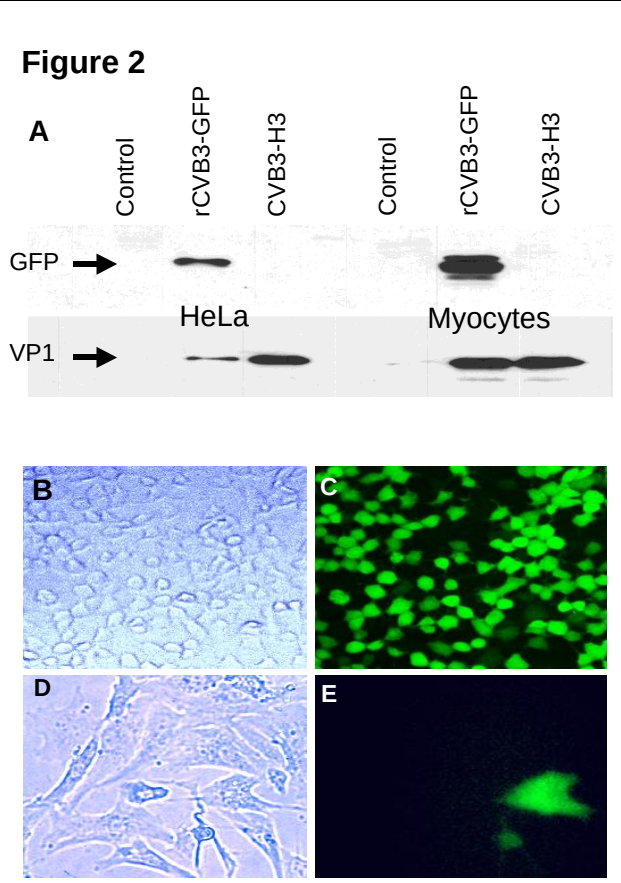
Chemical structure of CVB3 3CP inhibitor



Schematics of recombinant coxsackievirus and strategy for the expression of GFP.



One-step growth curve of rCVB3-GFP virus. Total virus production was determined at each time point by a plaque-forming assa



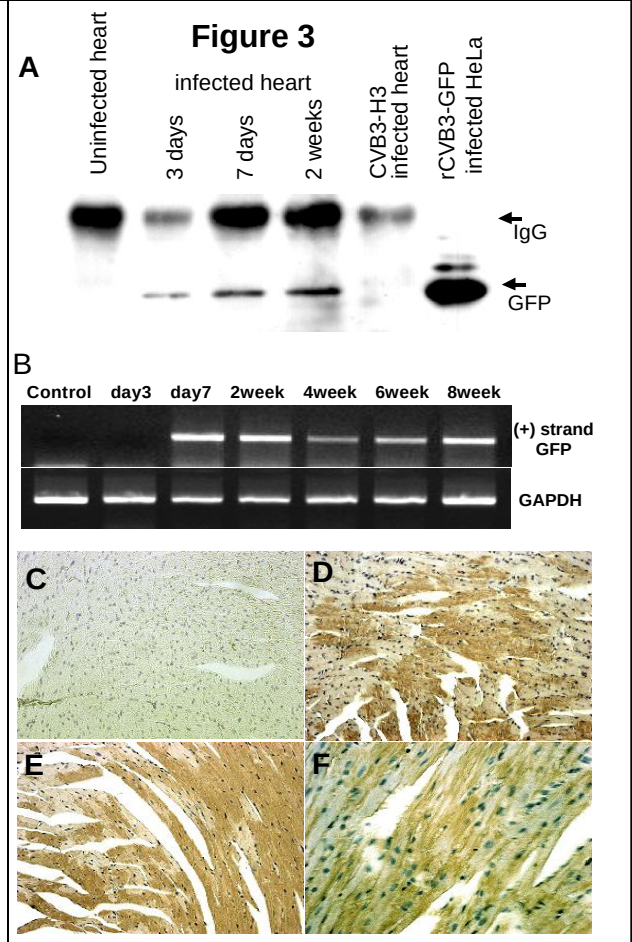
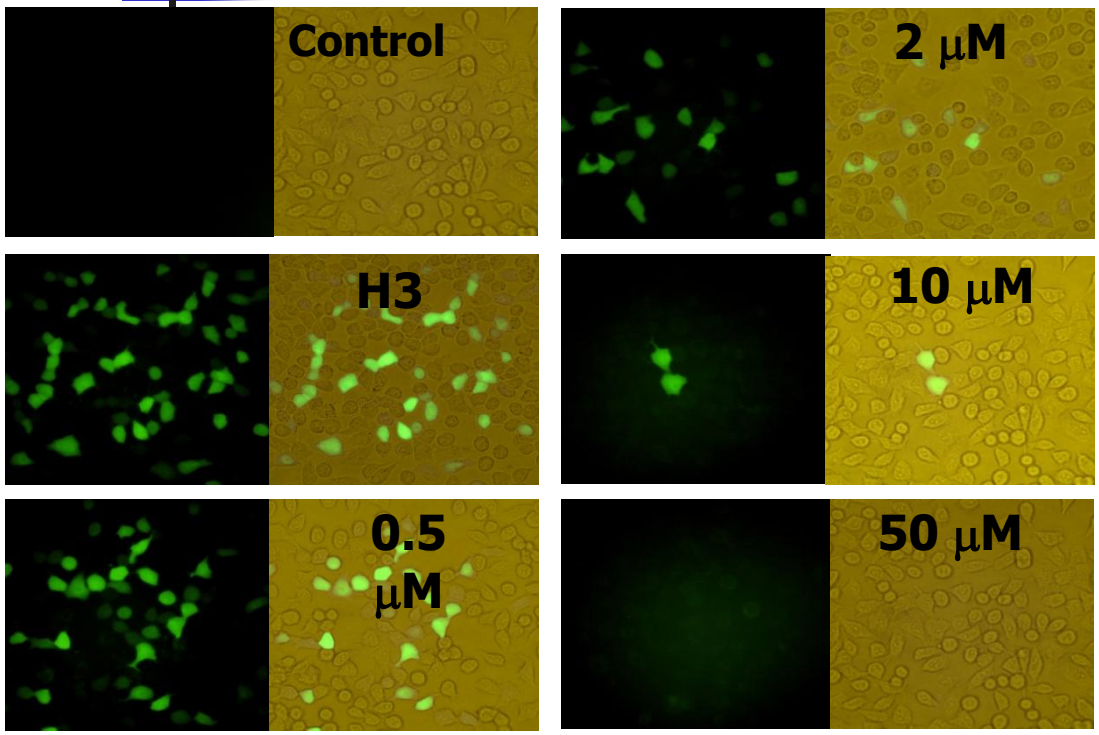


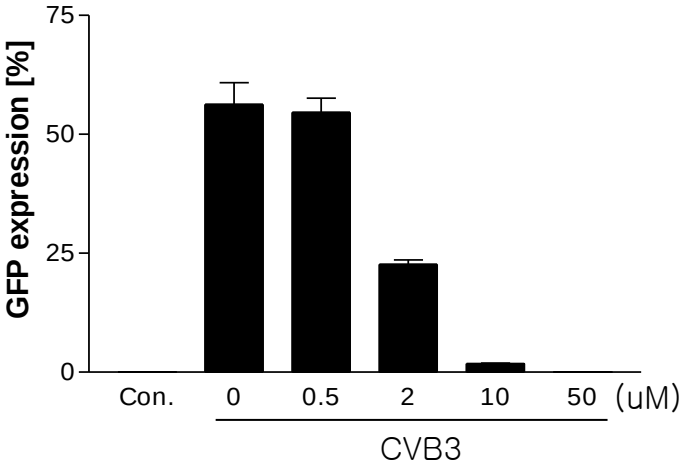
Figure 2. Exogenous GFP expression in cultured cells and in Neonatal rat myocytes. (A) GFP expression was detected by immunoblot in infected HeLa cells and in cultured rat neonatal myocytes. The upper panel was probed with anti-GFP antibody and the lower panel probed with anti-enteroviral VP1 antibody. (B–E) The fluorescence of functional GFP was detectable in infected HeLa cells (B and C: x 100) from six hours after infection (moi = 10), and in cultured rat neonatal myocytes (D and E: × 400) from 24 hours after infection (moi = 200). Both cell types were observed using the FITC fluorescence filter without fixation.

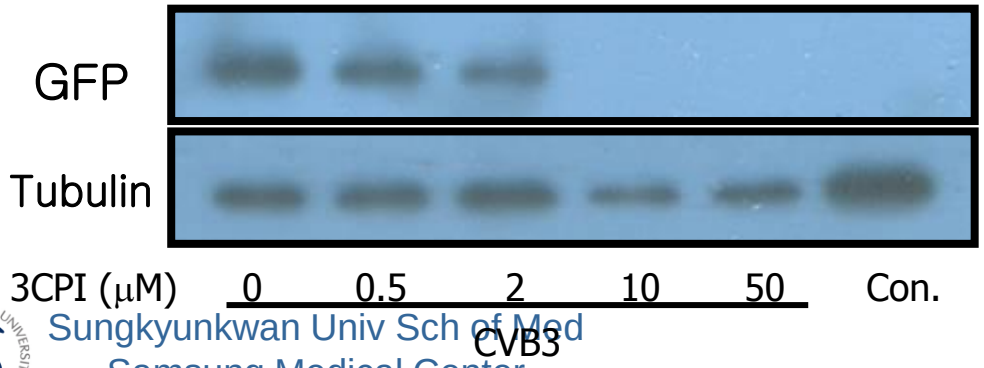
Figure 3. Expression of GFP in the heart was detected by immunoprecipitation, rT-PCR and immunohistochemistry. **(A)** Expression of GFP in the heart detected by immunoprecipitation. Cell lysates, obtained from uninfected and CVB3-H3 infected hearts, were used as negative controls. The extracts from rCVB3-GFP-infected HeLa cells were used as a positive control. GFP expression was detectable at three and seven days, and at two weeks after intraperitoneal injection. **(B)** Result of RT-RCR. The positive strands of GFP could be found from day 7 to 8 weeks pi. **(C–E)** In vivo expression of GFP was detected in the heart after intraperitoneal injection of rCVB3-GFP by immunohistochemistry. GFP expression was detectable from three days to eight weeks after intraperitoneal injection of rCVB3-GFP (10^6 pfu), and GFP was present in up to 30–40% of myocytes. (C: control heart, × 200, D: day 3 pi, × 200, E: 6 weeks, × 200, F: 8 weeks, × 400).

Jeon ES et al. J Mol Cell Cardiol 2005;38(5):745

GFP expression is inhibited by 3CI in the GFP-H3 infected HeLa cells





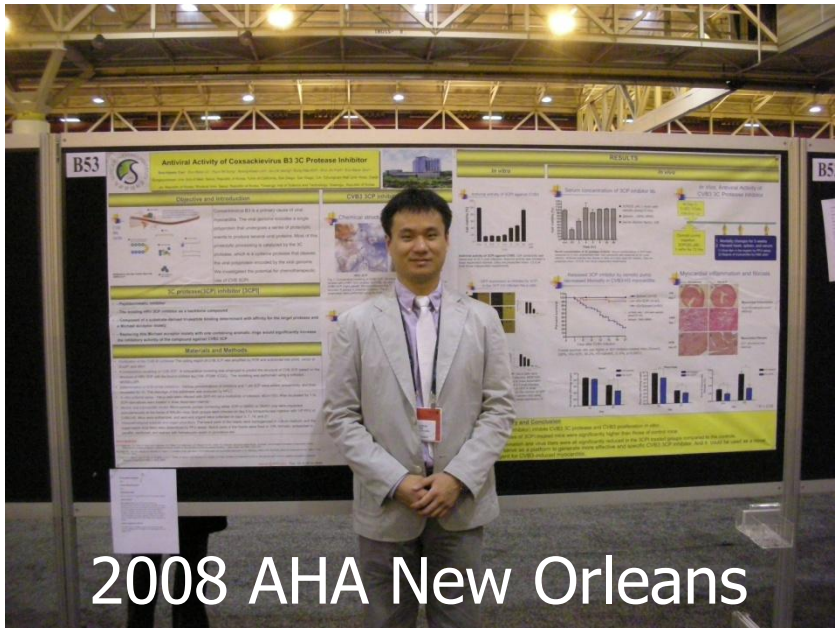


HeLa cells were infected with GFP-CVB3 (MOI, 100), and treated with 3CI. The CVB3 3CP activity was monitored by the expression of GFP using fluorescence microscopy. 3CPI significantly reduced the expression of GFP in dose dependent manner, which was measured by the number of GFP positive cells in four different area (A), and western blot using anti-GFP polyclonal antibody 3CPI also showed the inhibited GFP expression (B).

In Vivo, Antiviral Activity of CVB3 3C Protease Inhibitor

Department of Medicine, Sungkyunkwan University School of Medicine,
Samsung Medical Center

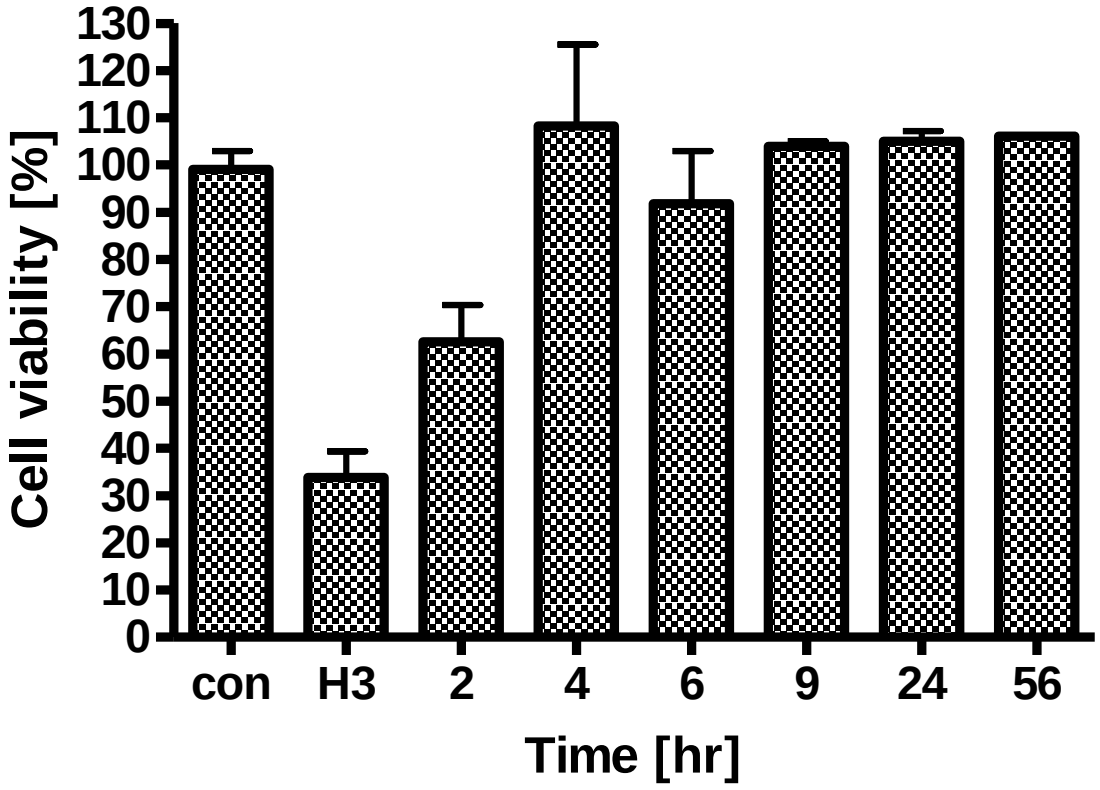
Soo-Hyun Yun, Eun-Seon Ju, **Eun-Seok Jeon, Yong-Chul Kim**



공동연구자:
광주과학기술원 약품화학 김용철

Yoon SH & Jeon ES. J Infect Dis 2011 in press

Serum concentration of 3CP inhibitor 9b

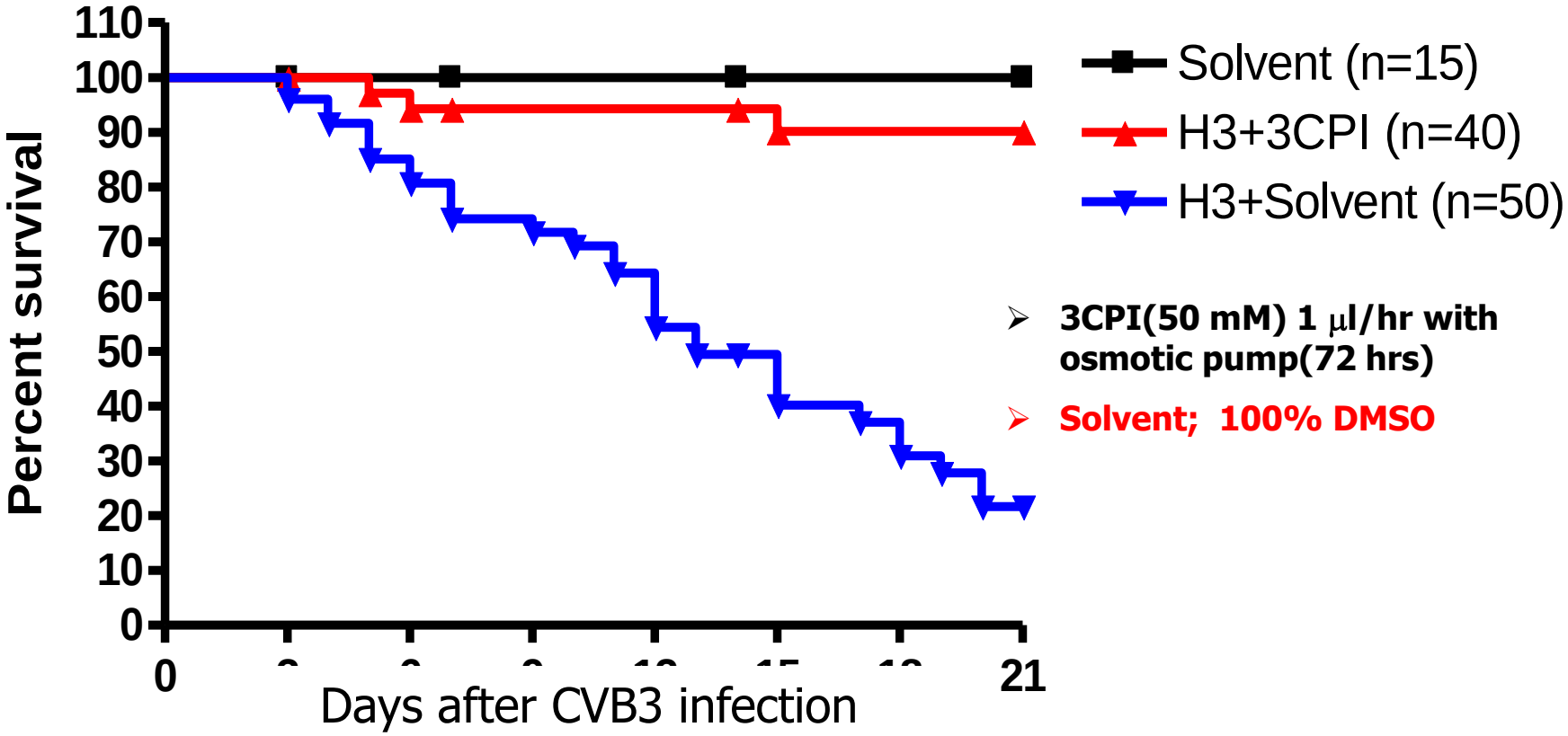


- 3CPI(50 μM) 1 μl/hr with osmotic pump(72 hrs)
- Solvent; 100% DMSO
- Serum dilution factor; 100

Serum concentration of 3C protease inhibitor. Serum concentrations of 3CPI were measured by *in vitro* neutralization test. Cell cytotoxicity was measured at 24 h post infection. **Antiviral activity was shown in sera of 4 hour post 3CI release .** Data are presented mean ±S.E.M. from three independent experiments.

Yoon SH & Jeon ES. J Infect Dis 2011 in press

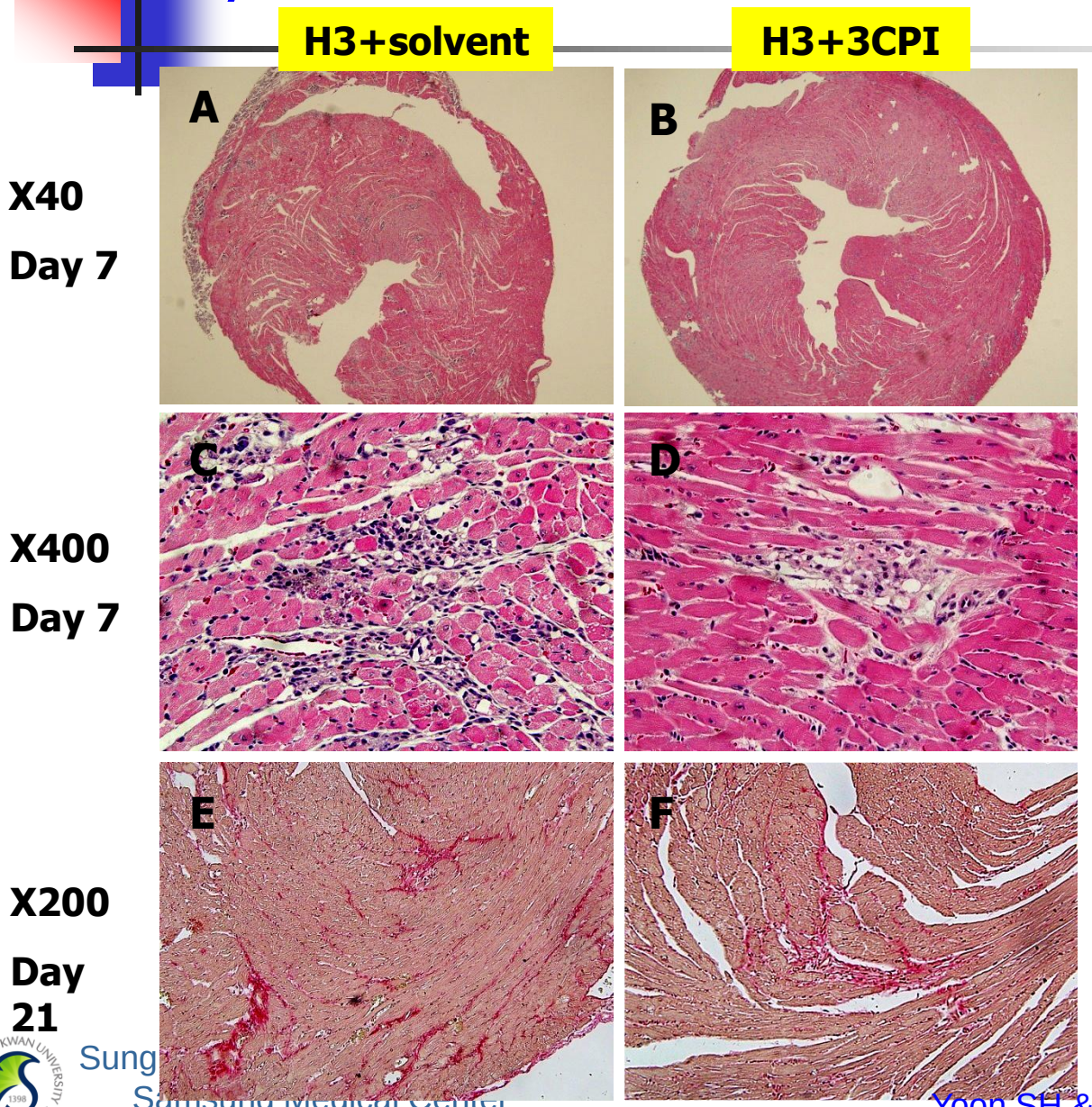
Released 3CP inhibitor by osmotic pump decreased Mortality in CVB3-H3 myocarditis



3-week-survival rate was higher in 3CP inhibitor-treated mice
(Solvent; 100%, H3+3CPI; 90.2%, H3+solvent; 21.6%, $p<0.0001$)

Yoon SH & Jeon ES. J Infect Dis 2011 in press

Myocardial inflammation and myocardial fibrosis



Myocardial inflammation

(A-D: hematoxylin-eosin staining)

Myocardial Fibrosis

(E,F: picrosirius red staining)

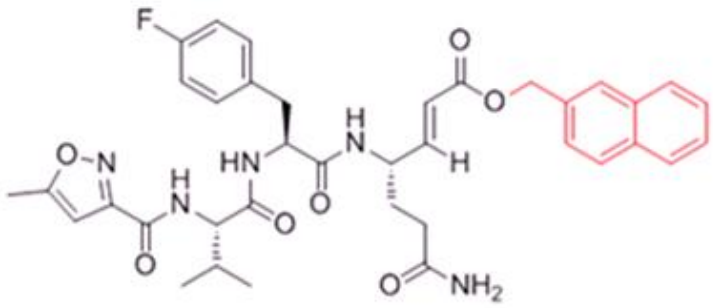
Yoon SH & Jeon ES. J Infect Dis 2011 in press

Unsolved Problems

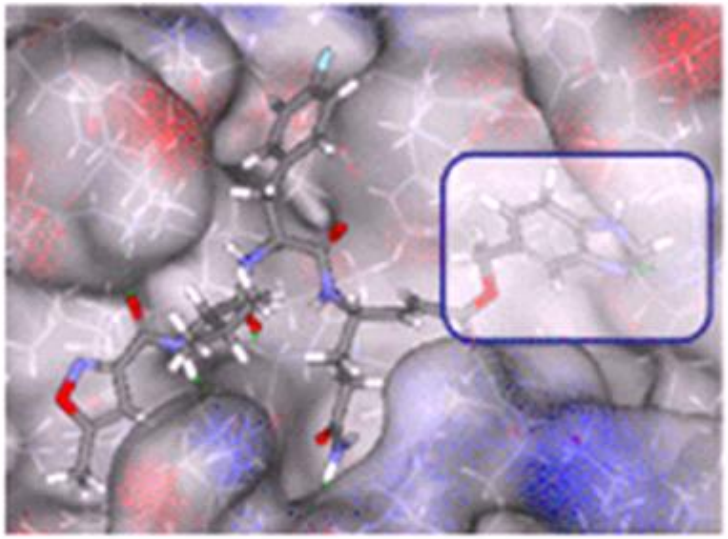
- Solubility: 3CPI of CVB3 only solved in DMSO, water –insoluble
  - How to deliver effectively *in vivo* ?
  - Adequate drug levels for complete CVB3 proliferation without toxicity
- How to measure drug levels?
- *In vitro* Quantification of anti-viral activity
- Antiviral spectrum versus 3CPI structure

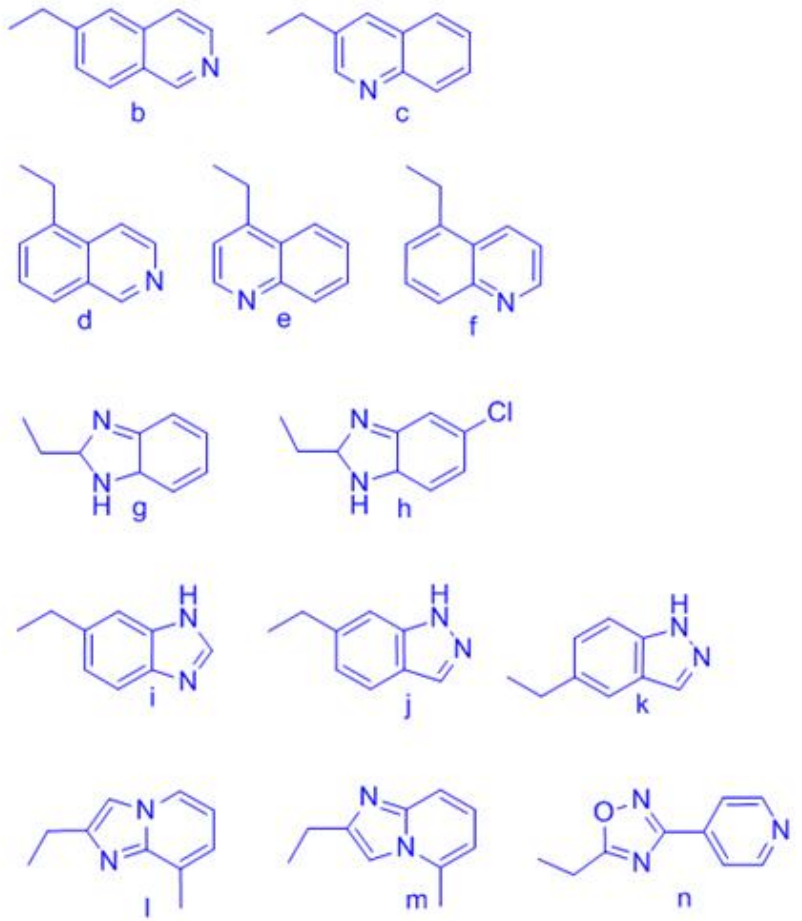
- 예비 실험 결과에 따른 한계점을 극복하기 위해 CVB3 3C protease 저해제의 용해도 개선을 위한 remodeling
- 비펩타이드성 3C protease 저해제를 구상

Water Soluble 3CPI's



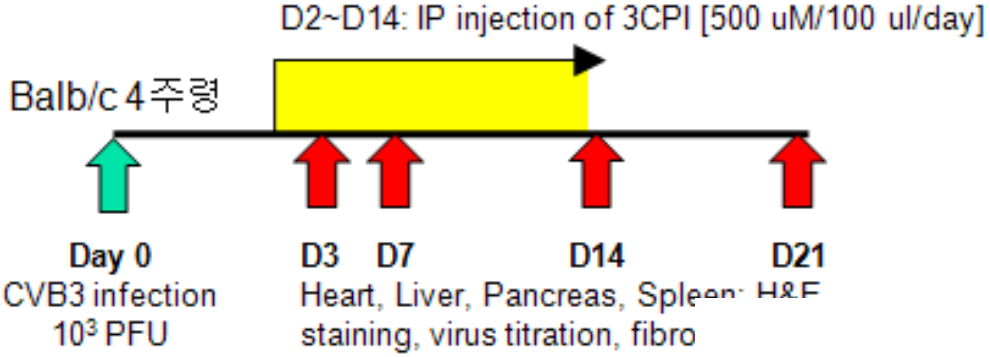
Coxscikie virus 3CP inhibitor



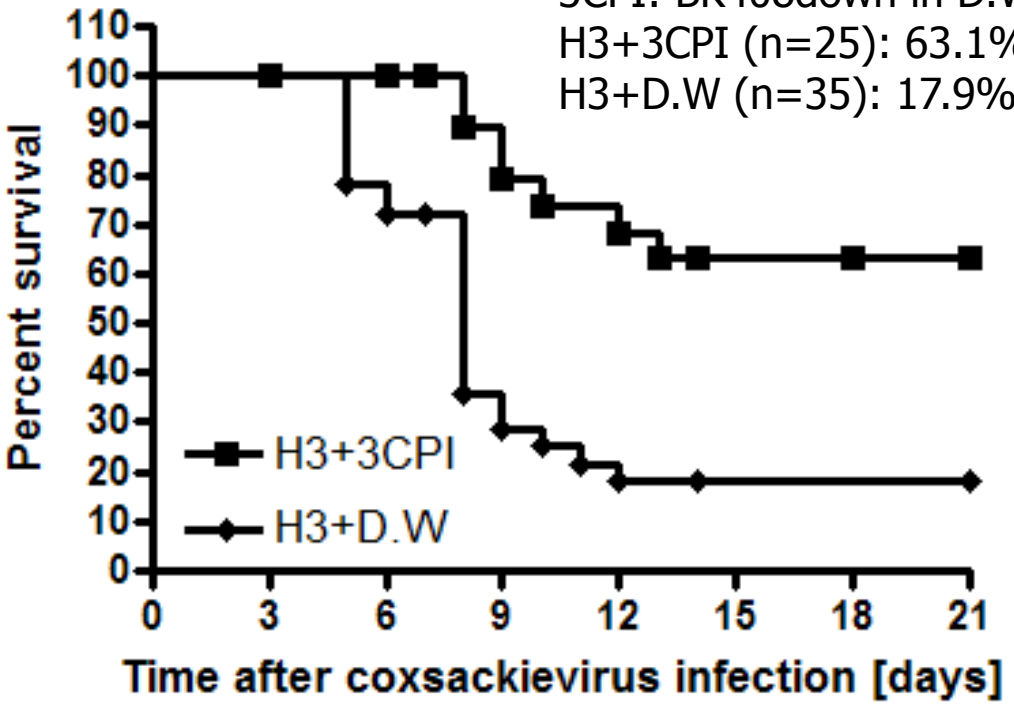


광주과기원 김용철 박사팀

Expressed Virus Trap by Electroporation decreased Mortality in CVB3 myocarditis

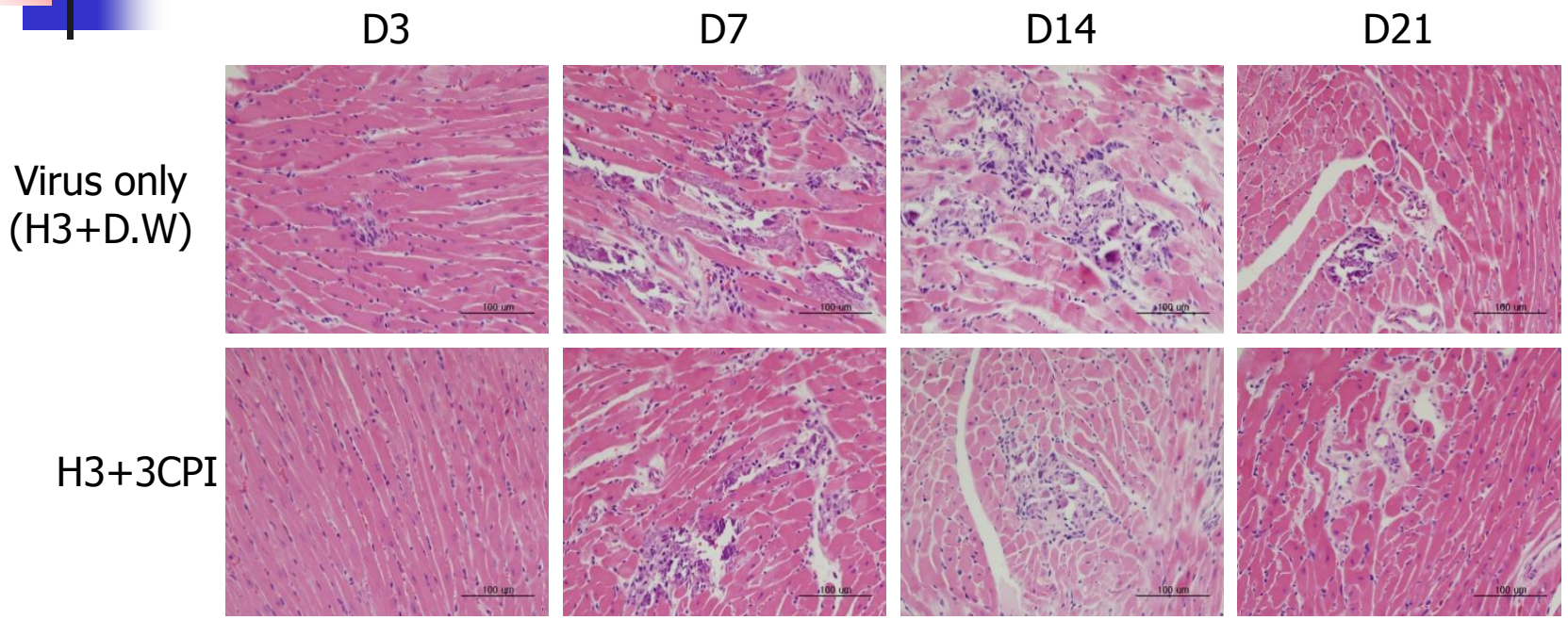


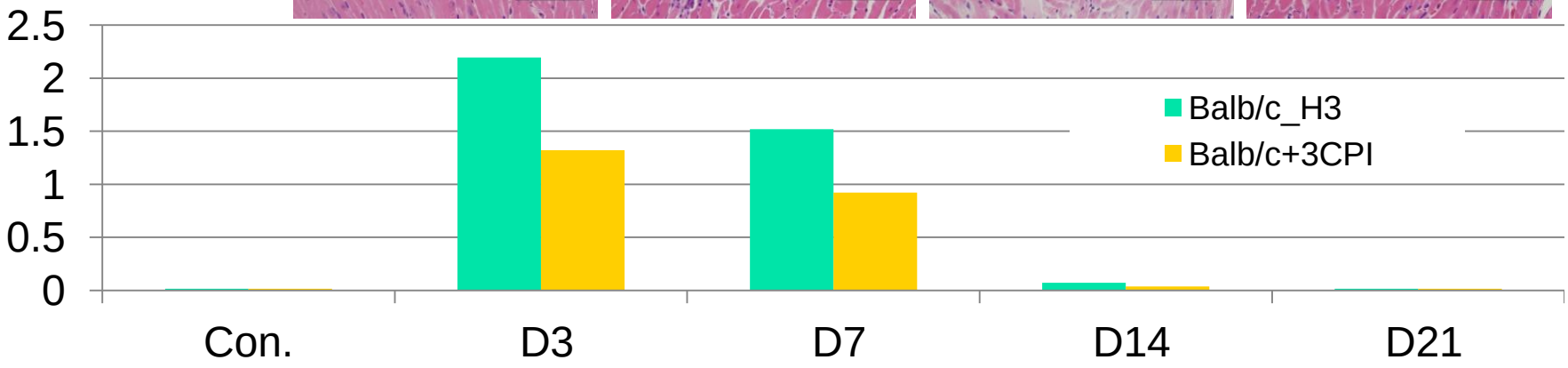
3CPI: BK408down in D.W
H3+3CPI (n=25): 63.1%
H3+D.W (n=35): 17.9%



Yun SH, Jeon ES et al. Unpublished

Heart Pathology and TnT levels





Yun SH, Jeon ES et al. Unpublished

Anti-CVB3-viral effects of KR chemicals

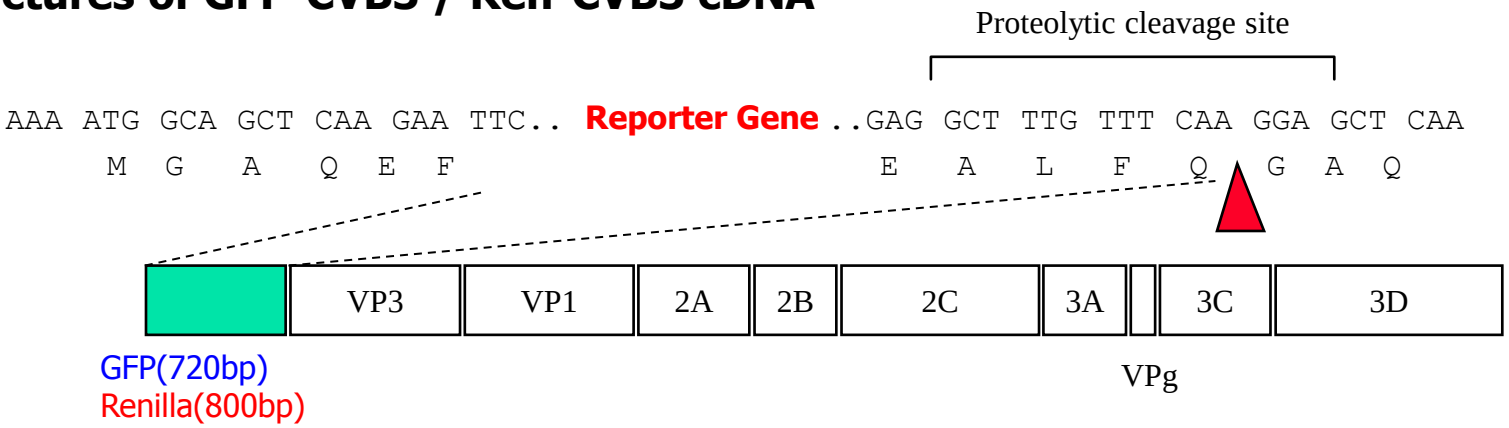
성균관의대 삼성서울병원 순환기내과
전은석, 임병관, 주은선, 이유정

셀트리온㈜ 윤수현

한국화학기술연구원 정영식

GFP 외 다양한 reporter를 가진 바이러스 제작

Structures of GFP-CVB3 / Ren-CVB3 cDNA



Renilla expression of renilla-CVB3

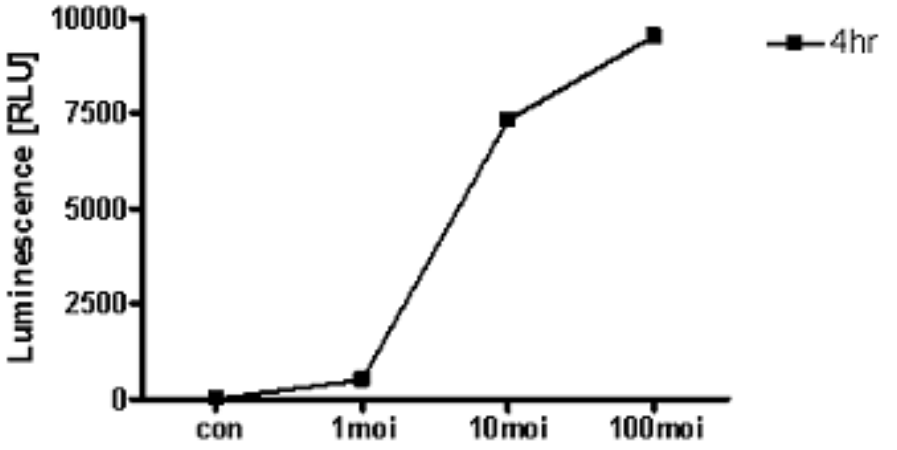
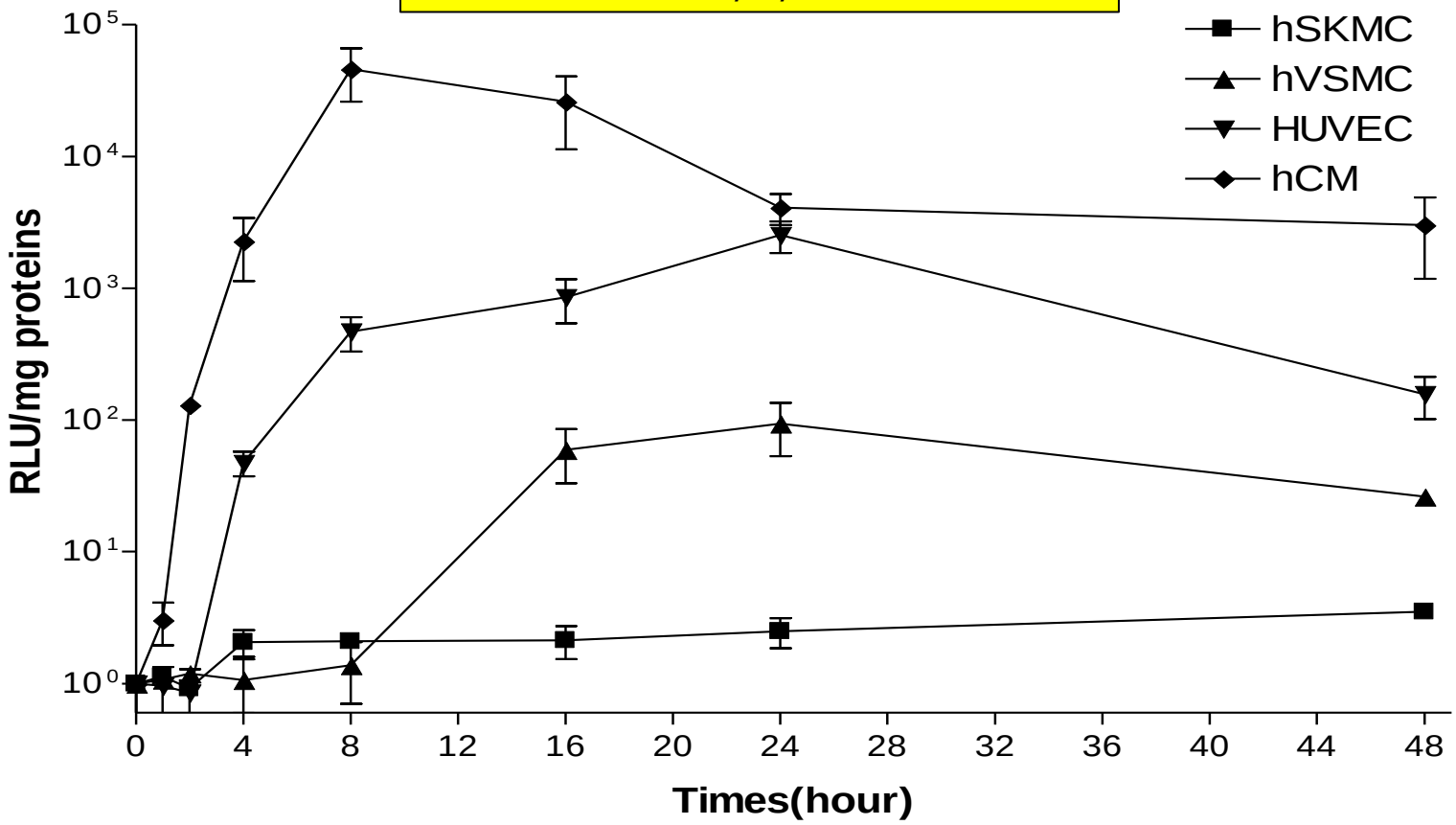


그림 1. Renilla-CVB3의 moi에 따른 renilla 발현

Reporter-rCVB3 virus infection

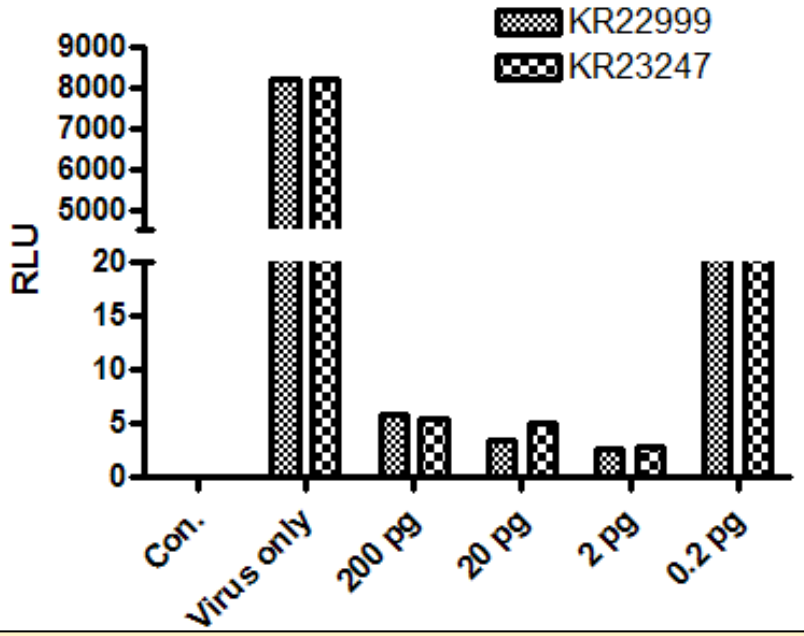
hSKMC: human skeletal myoblast cell
hVSMC: human vascular smooth muscle cell
HUVEC: human vascular endothelial cell
hCM: human cardiac myocyte cell line

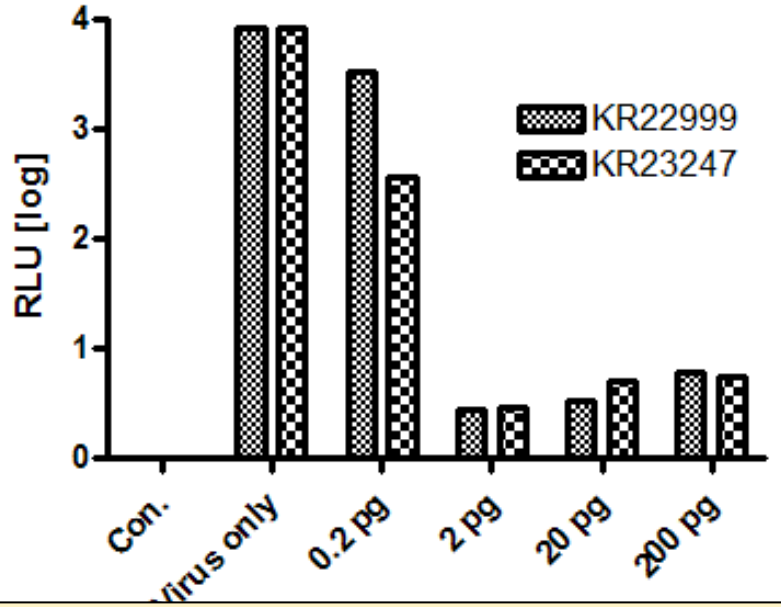


Renilla expression reaches the highest level

at 8 hour after infection of Renilla-CVB3 (100 moi) in HeLa cells.

Antiviral activity measured by Renilla expression after Renilla-CVB3 infection

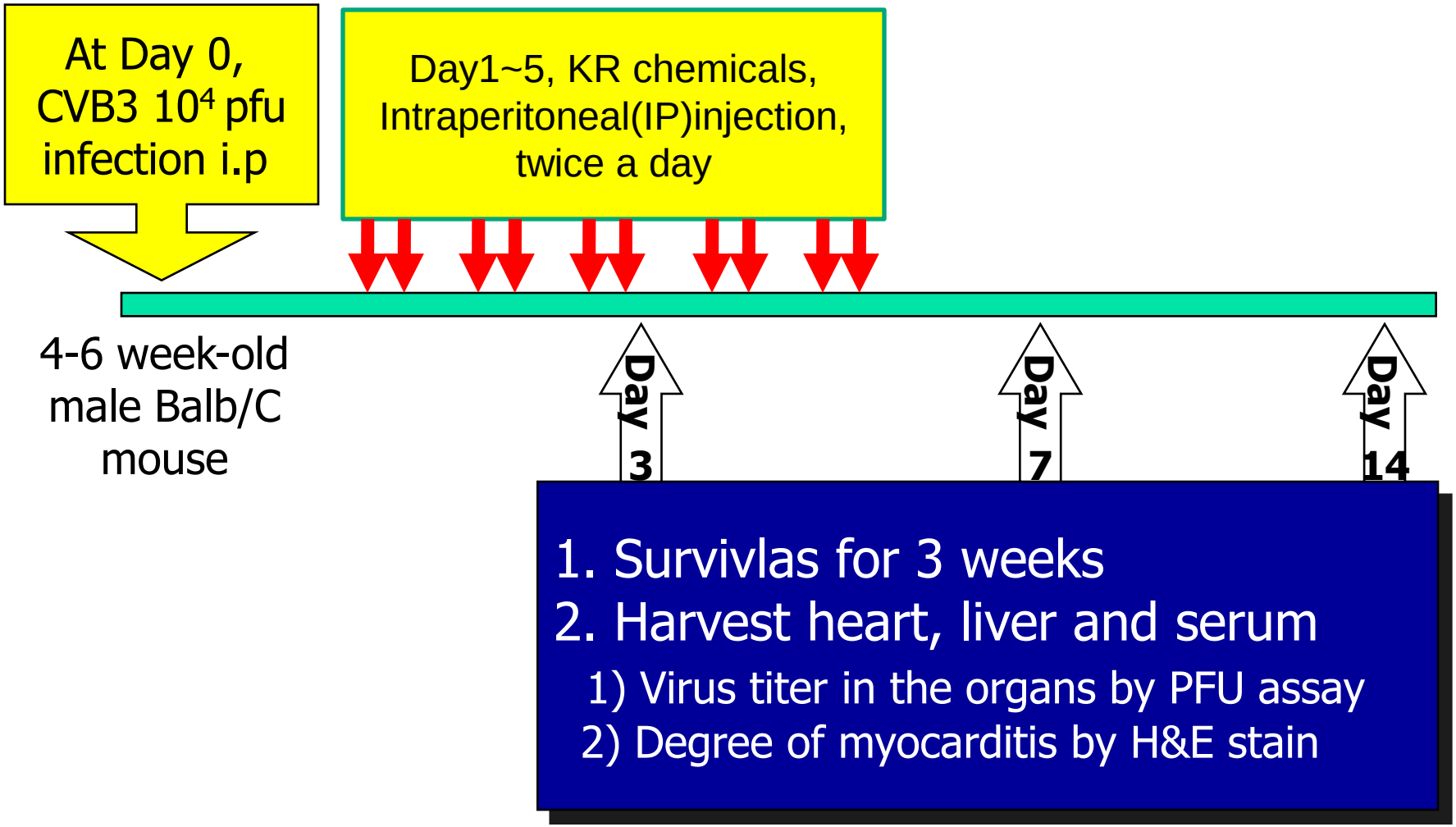




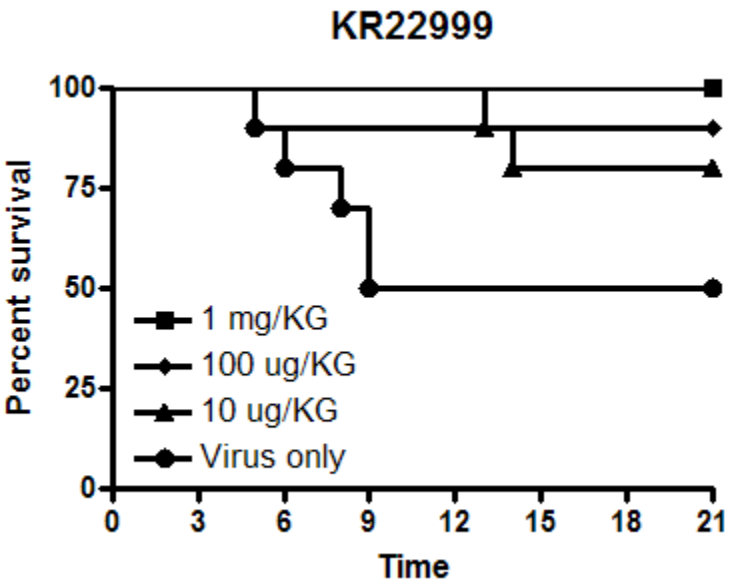
1) Hela cells were infected with Renilla-H3virus (100 M.O.I.)
2) At 1 hr post infection, infected Hela cells were treated with different concentrations of KR 22999 or KR 23247 in media.
3) Renilla expression was estimated at 6 hr post infection.

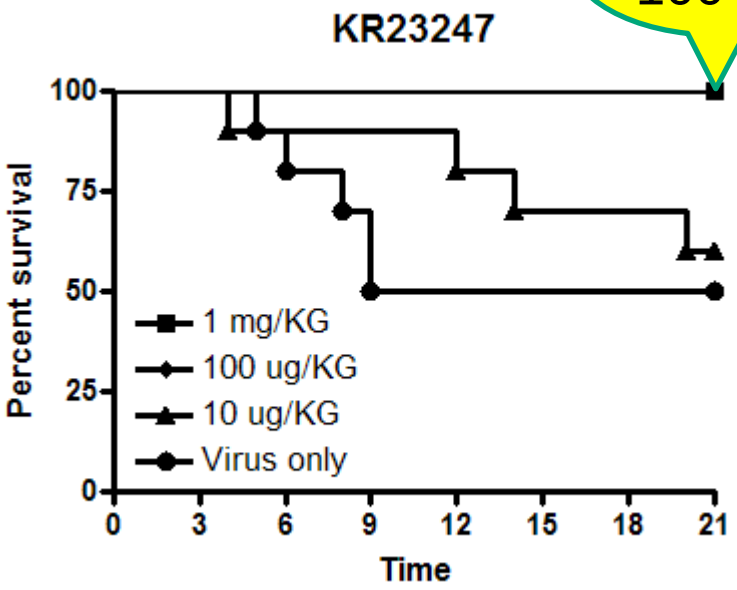
There was no cytotoxicity in control HeLa cells, which were treated > 200 pg of KR chemicals.

CVB3 myocarditis model



Survival curve

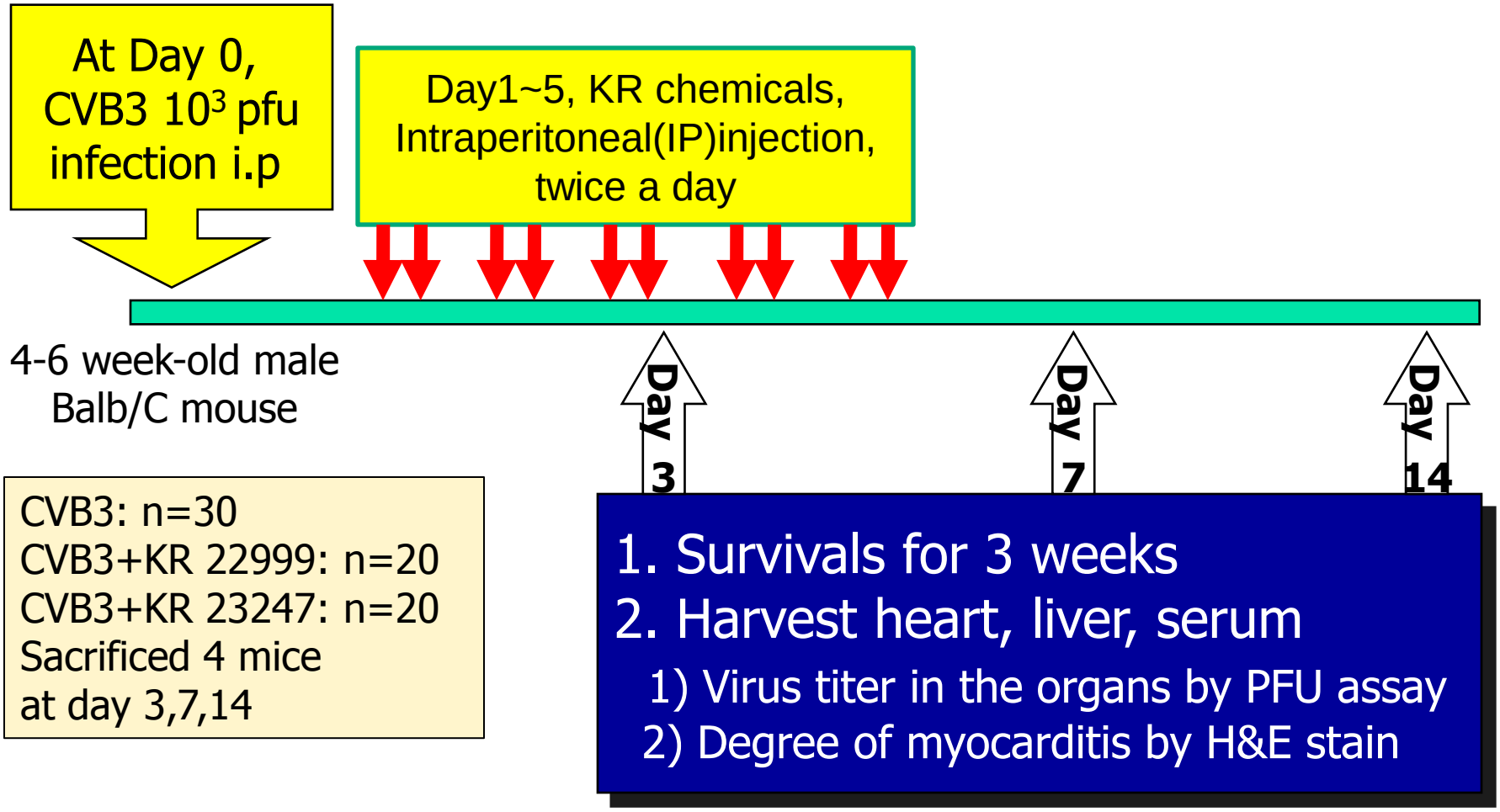




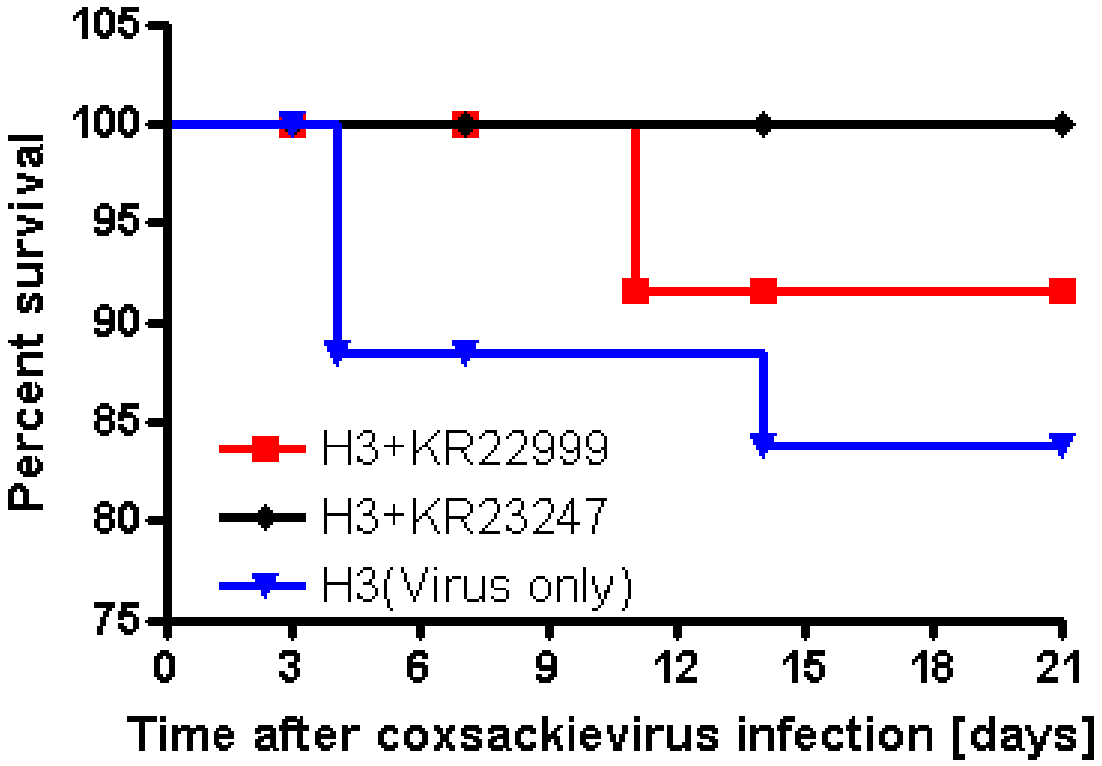
1mg/
100 μg

1) 10 μg/kg 에서는 KR22999 가 KR23247 보다 효과적
2) 100 μg/kg, 1 mg/Kg 에서는 두화합물 모두 mortality 는 거의 0%
3) Infected mice control 의 3 week-survival rate는 50%

CVB3 myocarditis model

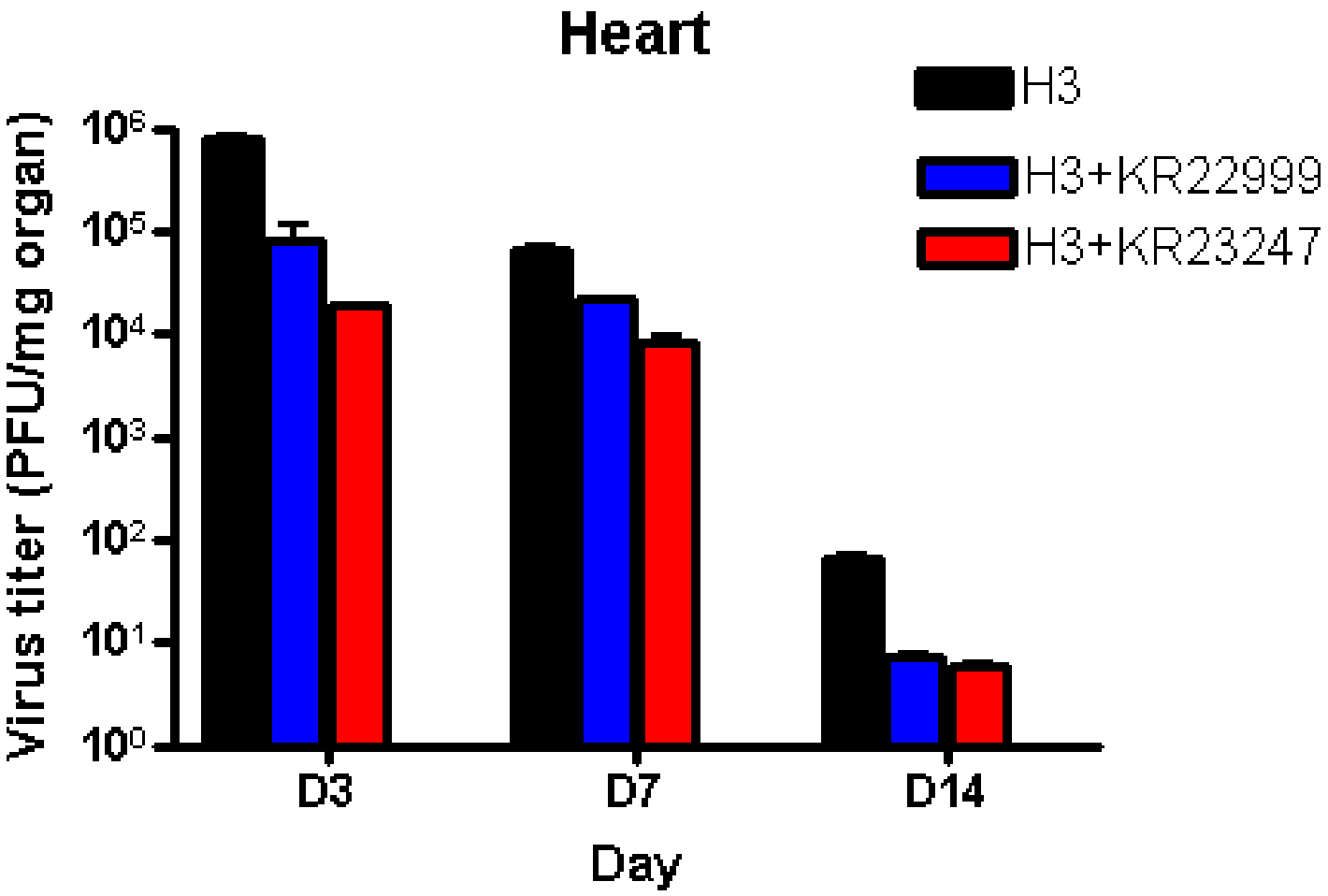


Survival curve

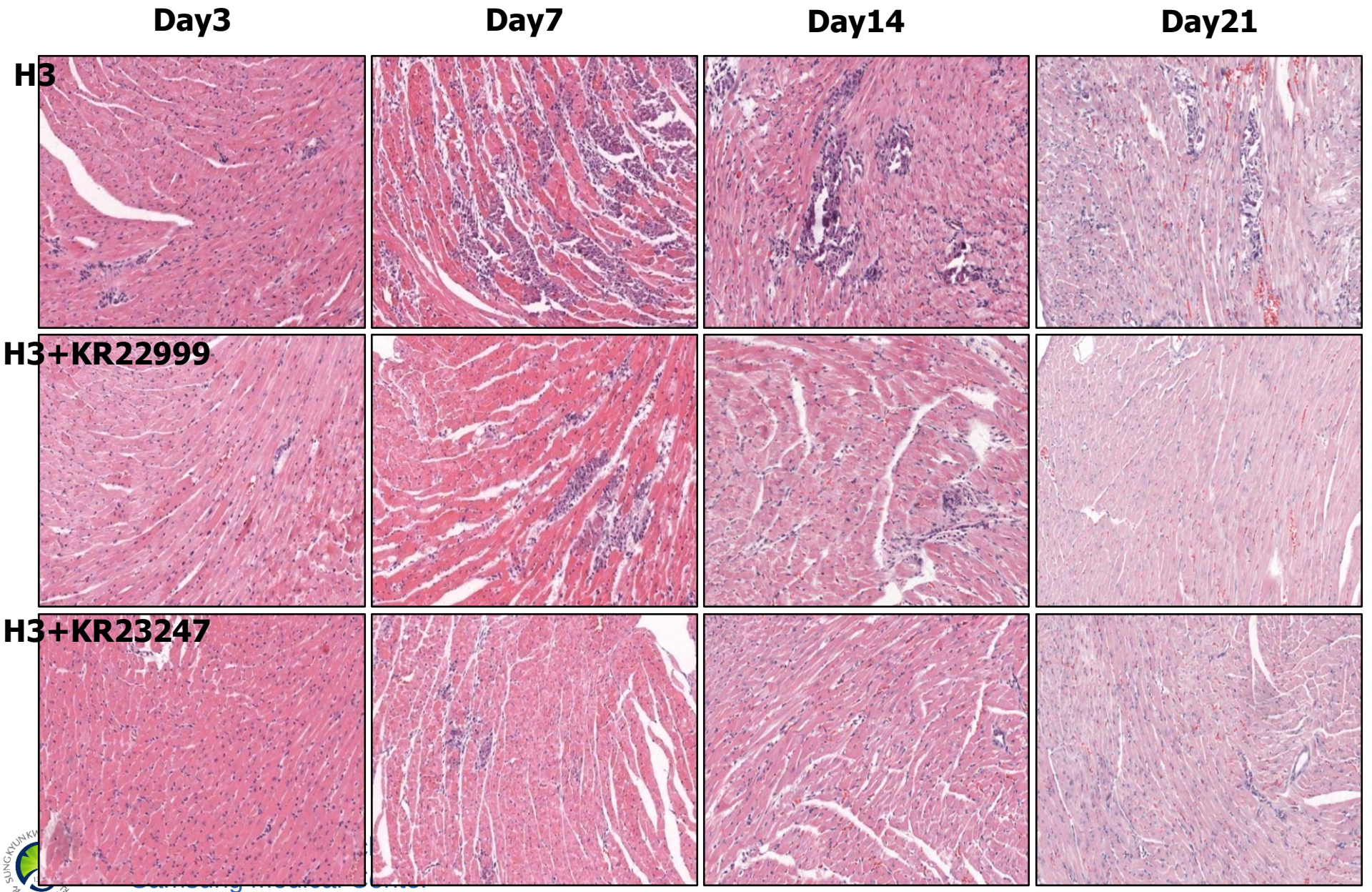


H3: 10^3 PFU/4 week-old female mouse
Duration of inoculation: KR22999,KR23247 (100ug/KG)
D1~5, twice per day (n=30 in CVB3, KR 투여군n=20)

Virus Titers in Heart

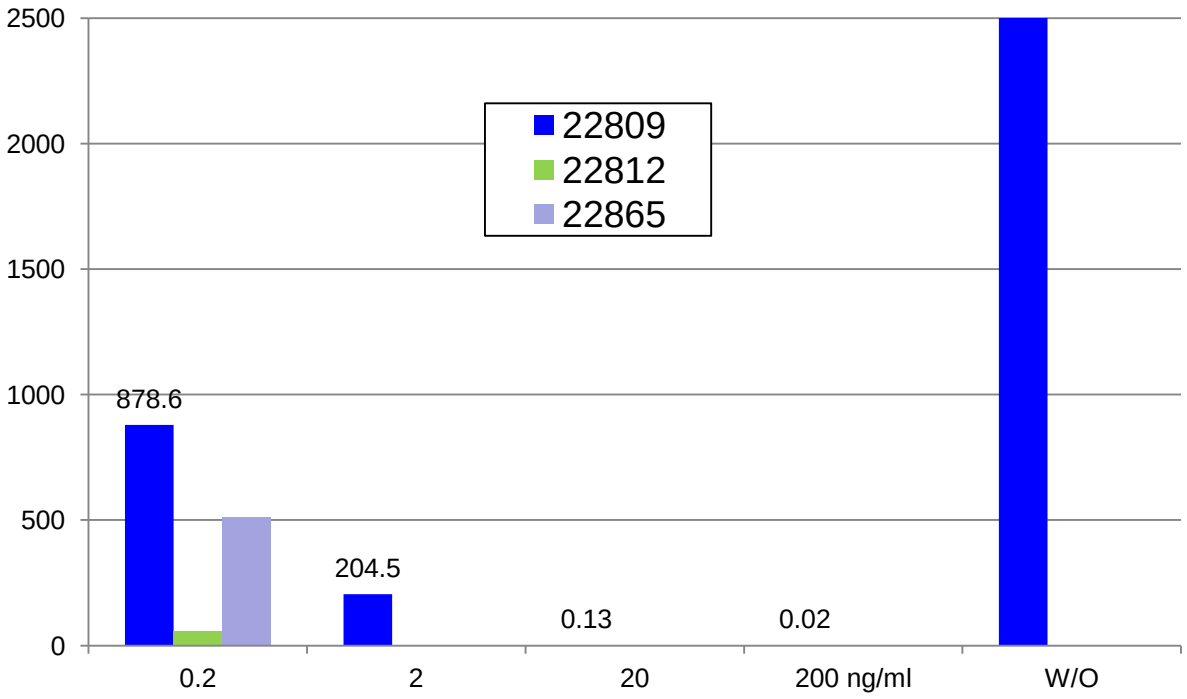


Heart Pathology at day 3-21



Antiviral activity measured by Renilla expression after Renilla-CVB3 infection

Long-term infection of HeLa cells for 60 hours

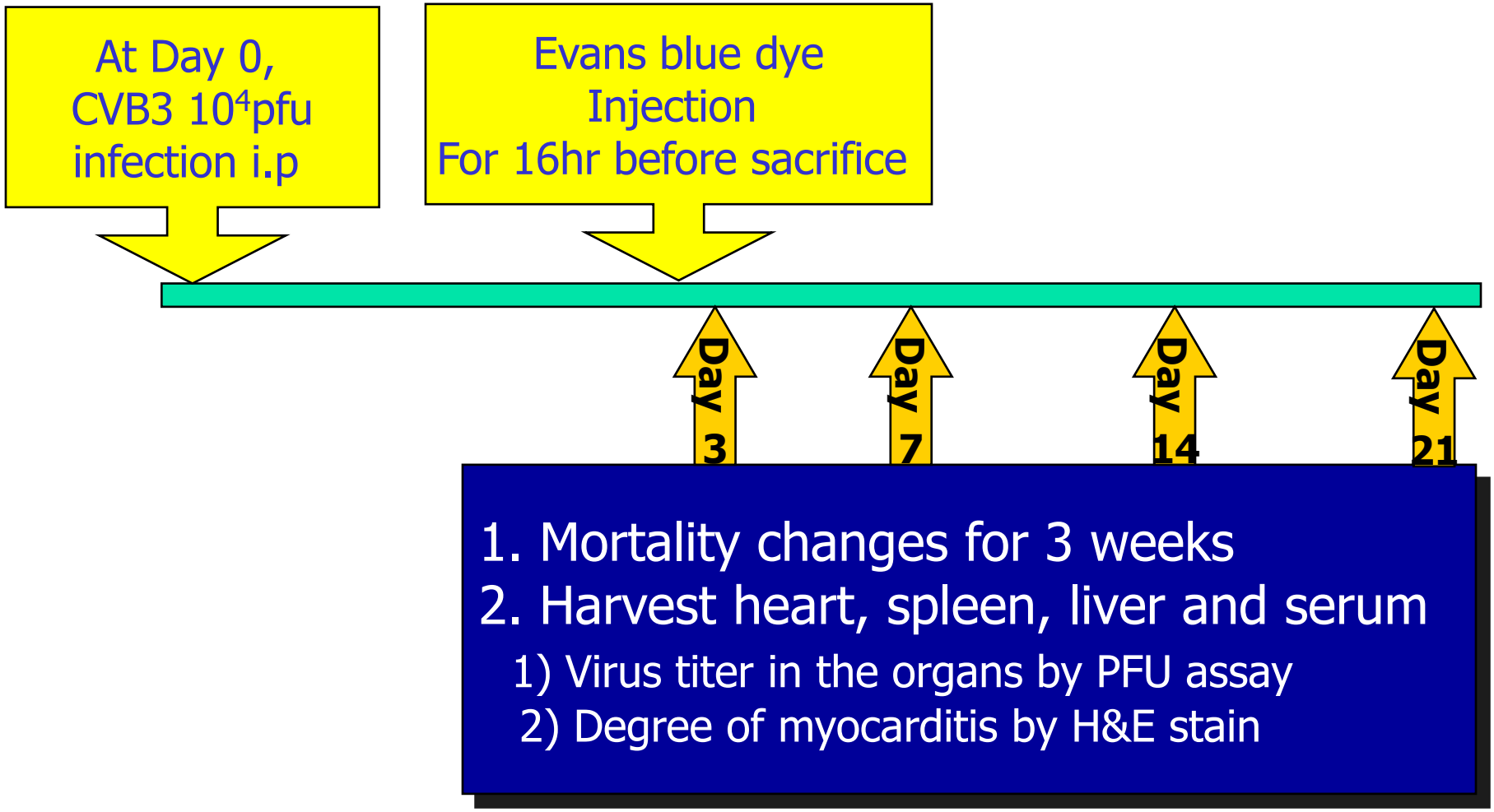


만성 심근염 모델 개발

- Balb/c 생쥐 심근염 모델의 문제점
  - Balb/c 심근염 생쥐 모델은 급성 심근염 모델
  - 급격한 염증과 심근세포의 파괴를 유발
  - 높은 생쥐의 사망률을 보여
  - 14일 내에 모든 실험이 종료되는 장점이 있으나
  - 실제 사람에서와 같이 바이러스 감염 후의 약물처리에 한계

→ 사람과 유사조건의 약물 투여 실험이 가능한 만성(chronic) 심근염 생쥐 모델 DBA, C57BL/6, C3H 생쥐에서 실험

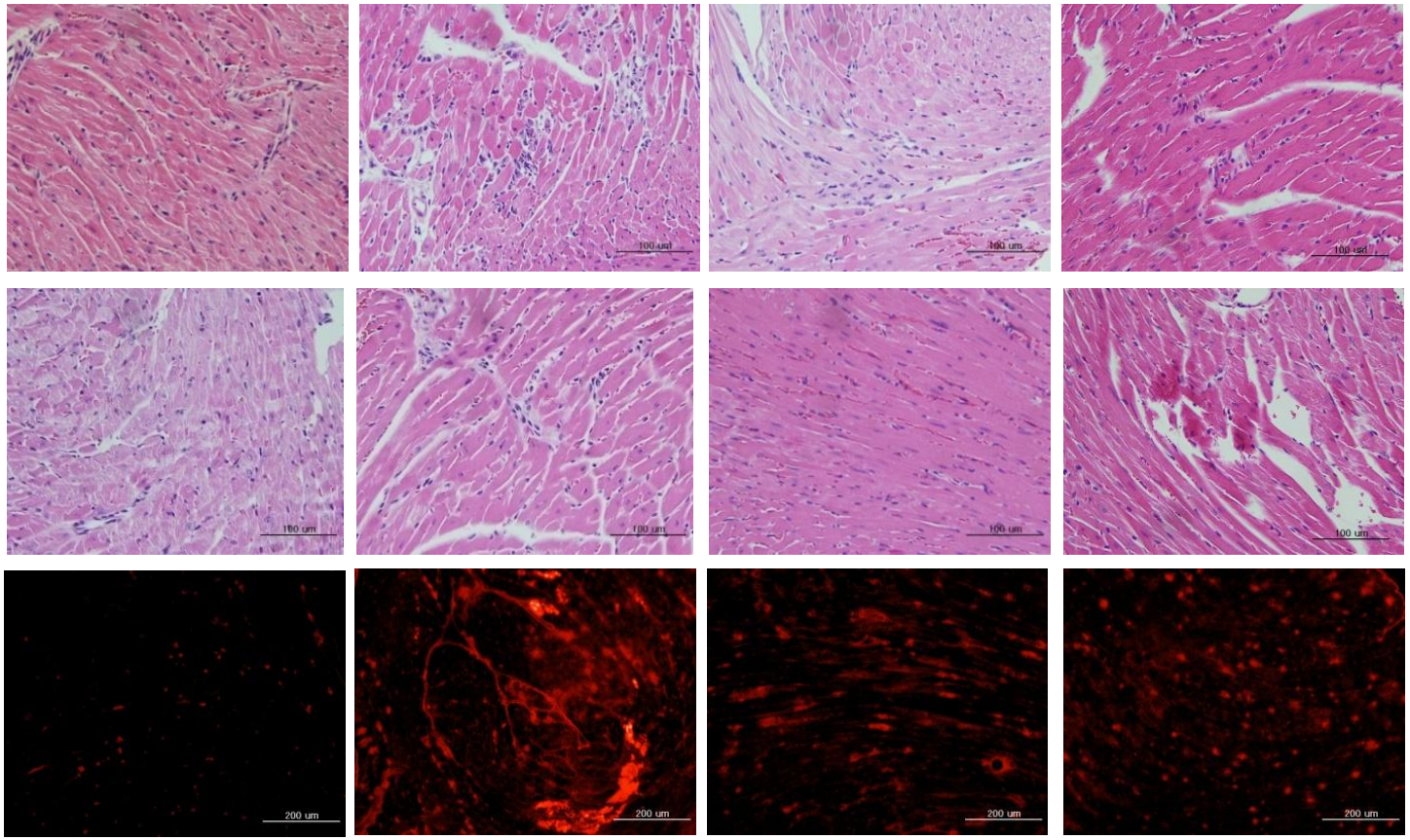
만성 심근염 모델 개발 방법



C57/BL6 Strain 심근염 모델(심장)

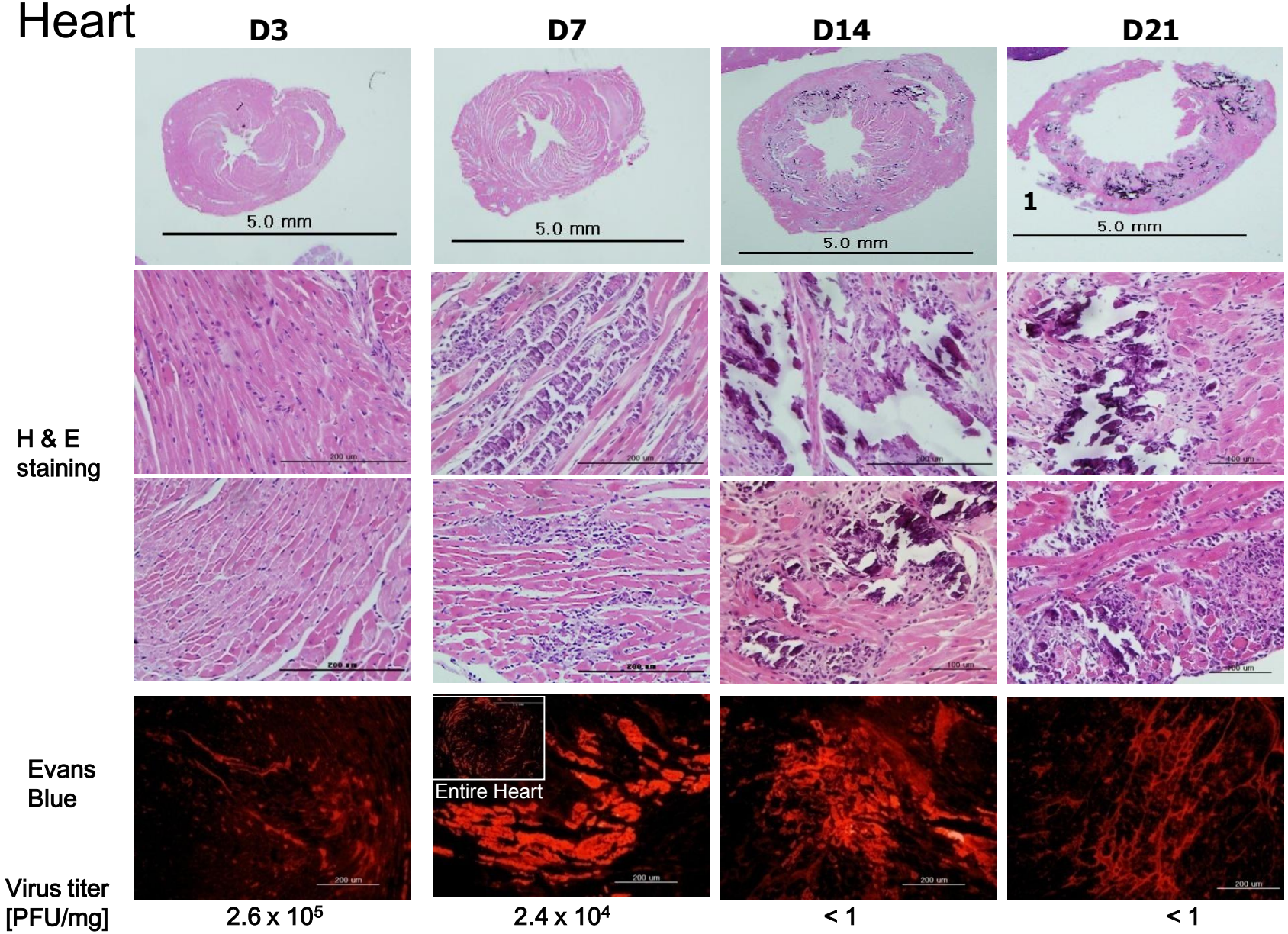
Inoculation: 10^2 PFU/mL
5-weeks survival rate: 93%

Heart

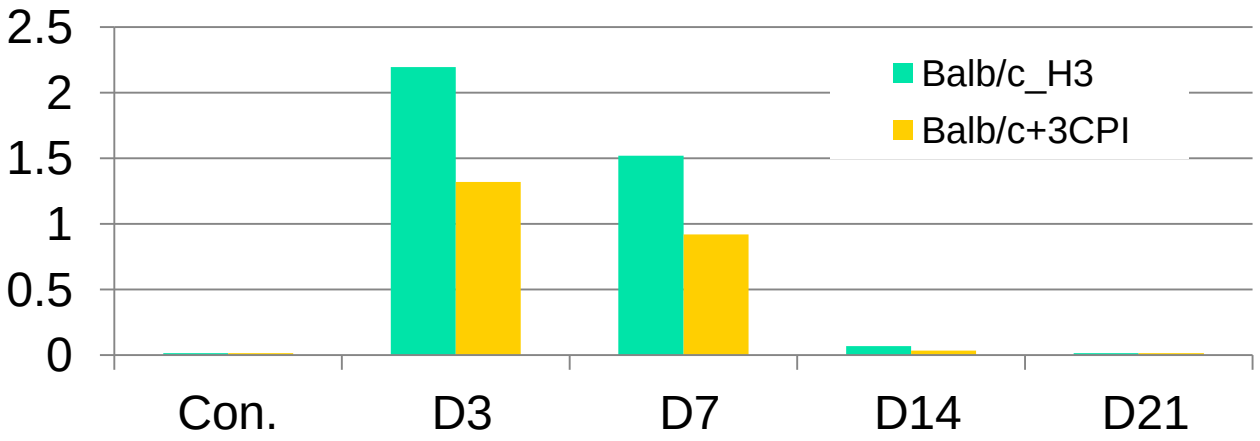


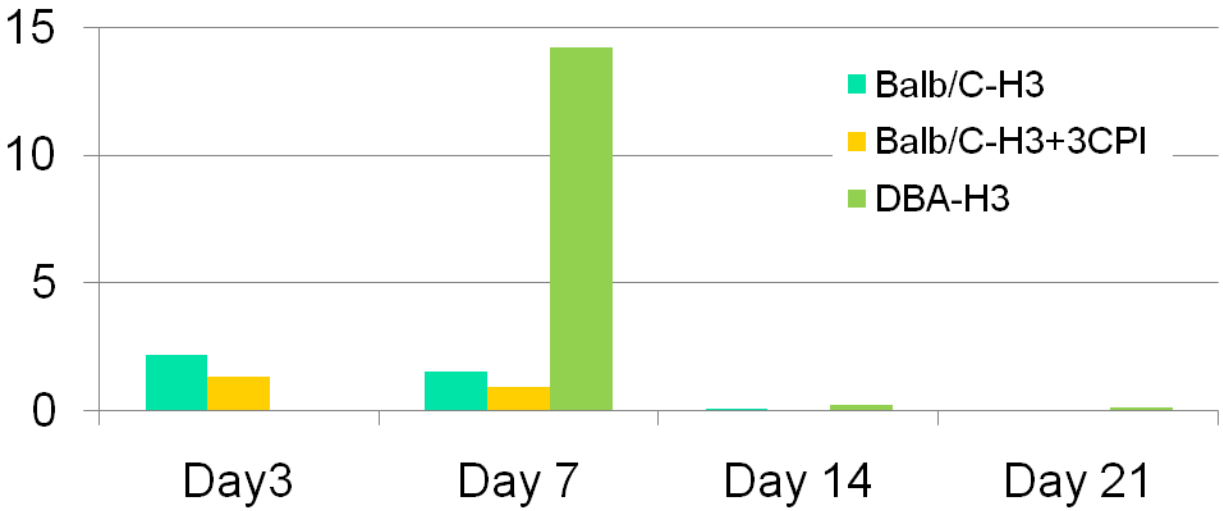
DBA Strain 심근염 모델(심장)

- Inoculation: 10^3 PFU/mL
- 3-weeks survival rate: 24.5%



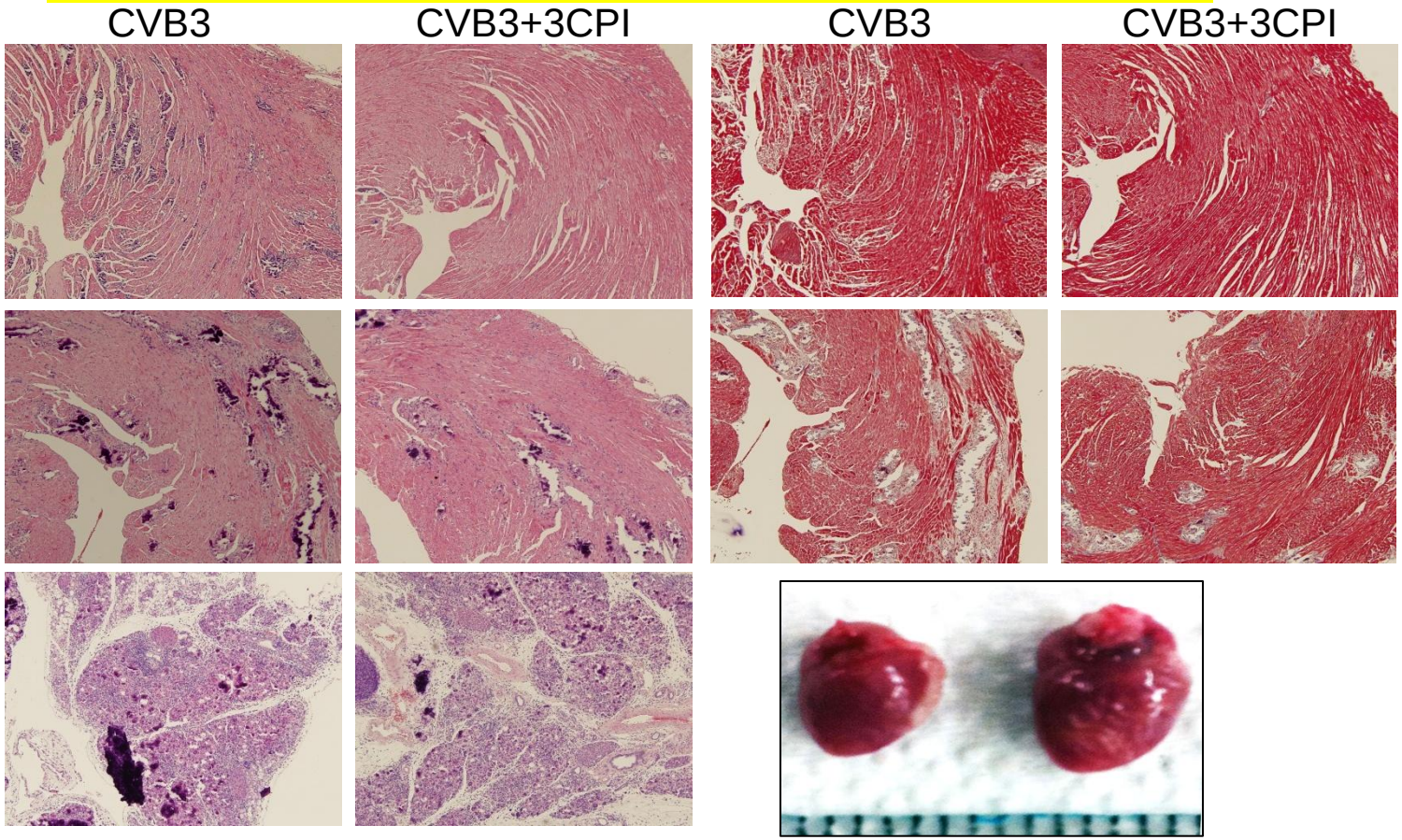
Troponin T changes in different strains





Administration of Soluble 3CP inhibitor

-10^3 PFU/ml CVB3 infect to **DBA mouse strain**
- Soluble 3CP inhibitor (50uM) injection at day3 post-infection
- Mouse was sacrificed at day7 and 21 post-infection
- 1 (5) died from no treated group, no mortality in treated group




El Capitan →
Enterovirus
If he doesn't know
where he is going,
he might not get there.

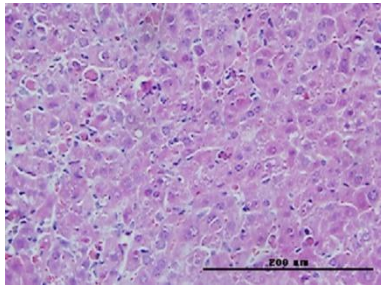
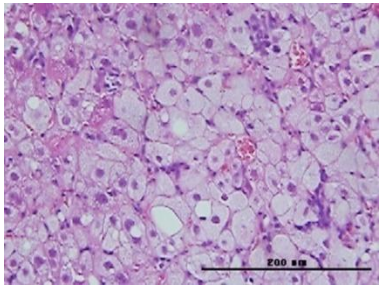
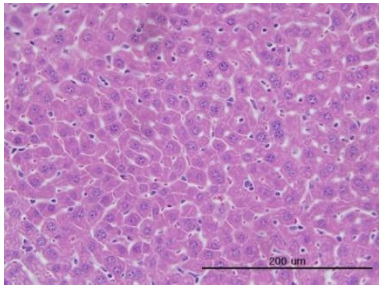
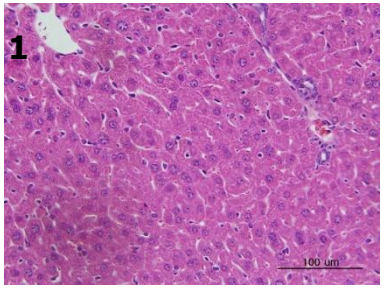
Acknowledgement

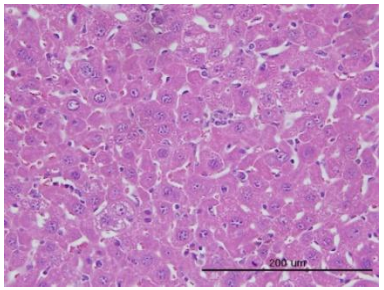
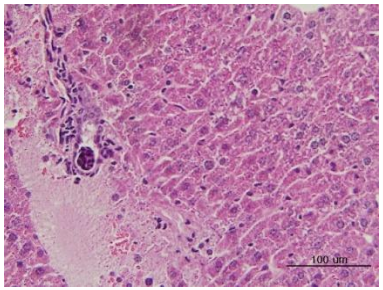
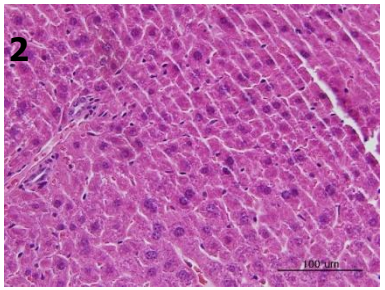
이유정, 주은선, 윤수현
신재옥, 길채옥, 임병관

광주과기원 김용철 박사팀
화학연구소 정영식 박사팀

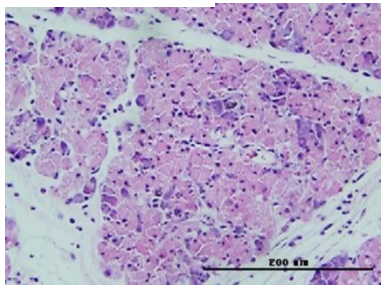
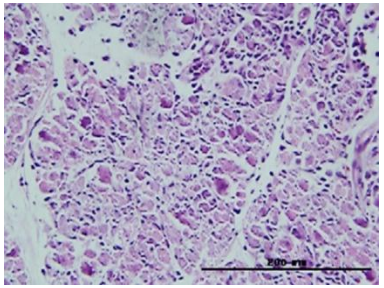
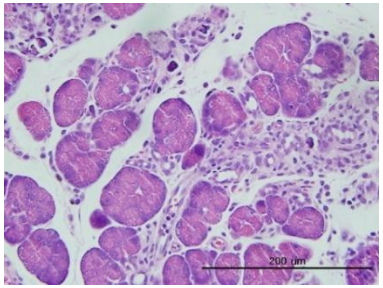
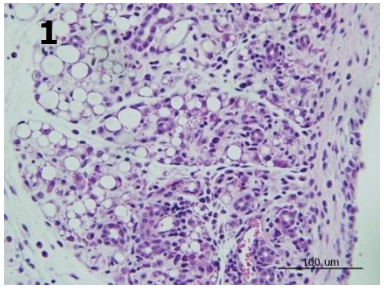
D3 D7 D14 D21

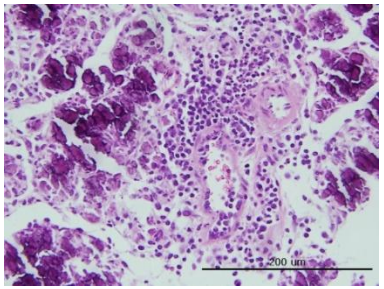
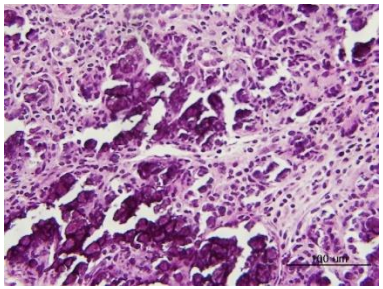
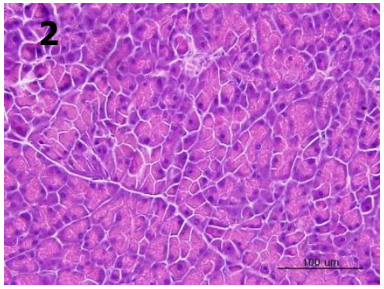
Liver

Pancreas

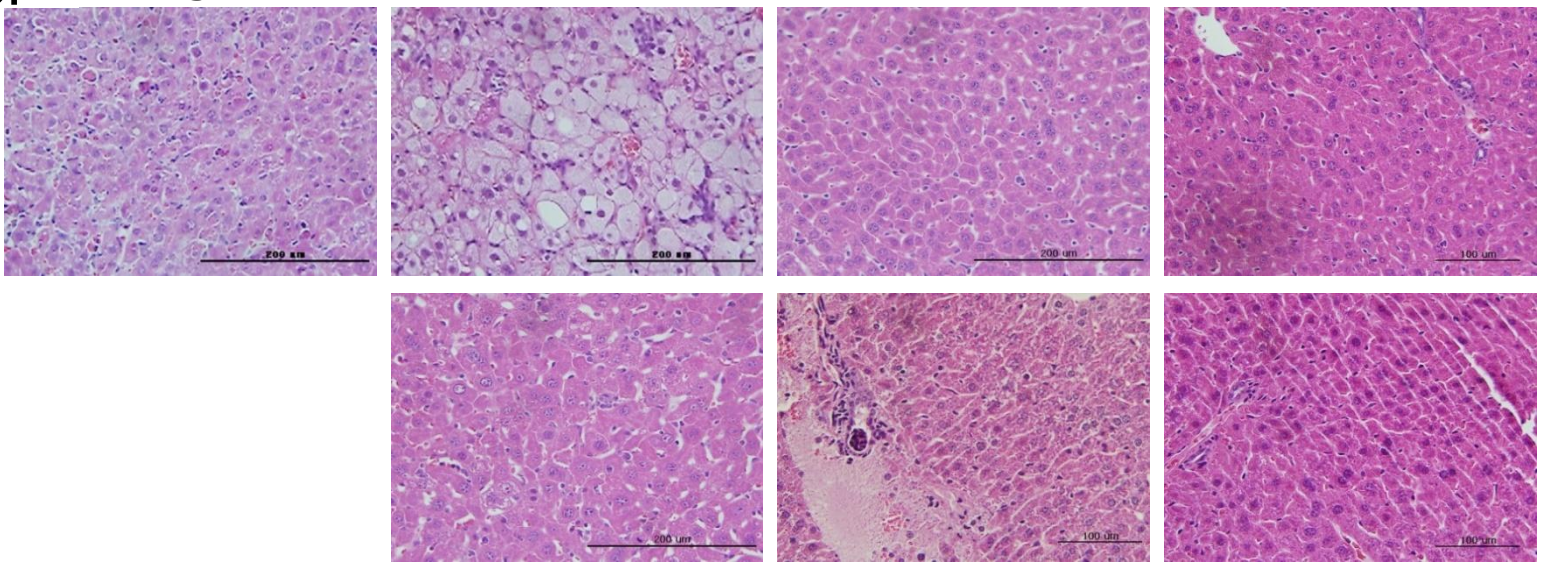





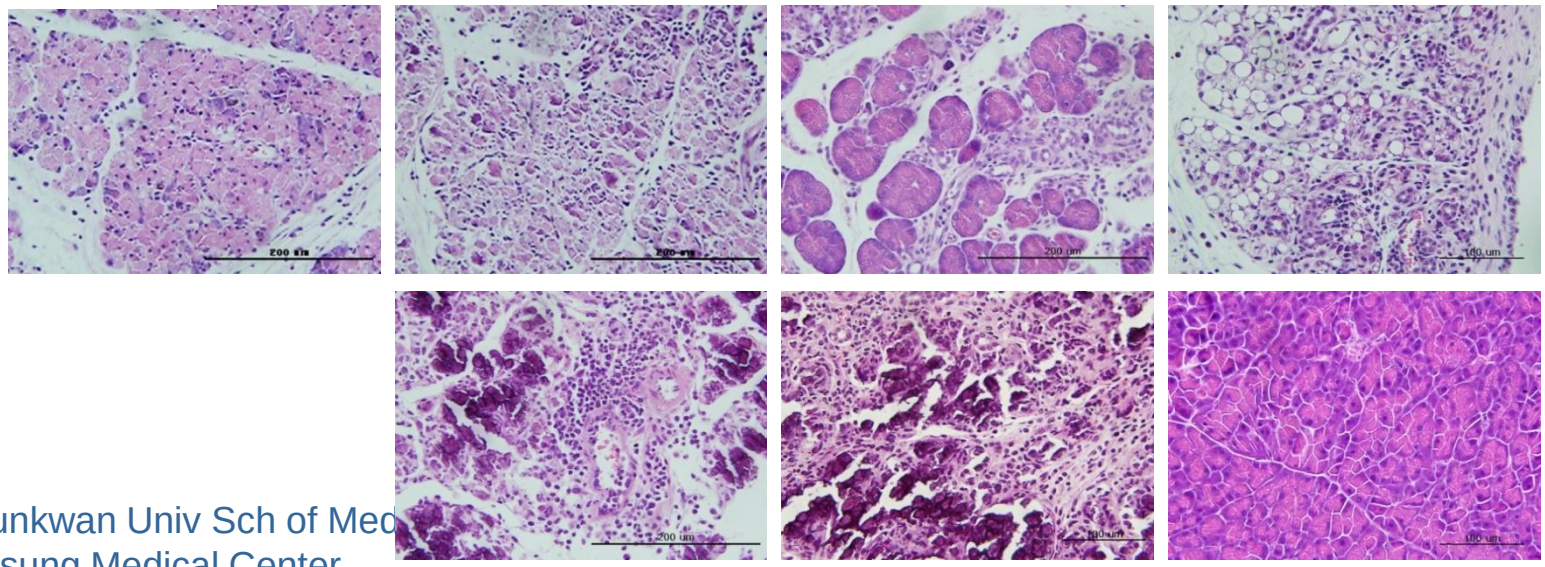
DBA Strain 심근염 모델(간, 췌장)

Liver

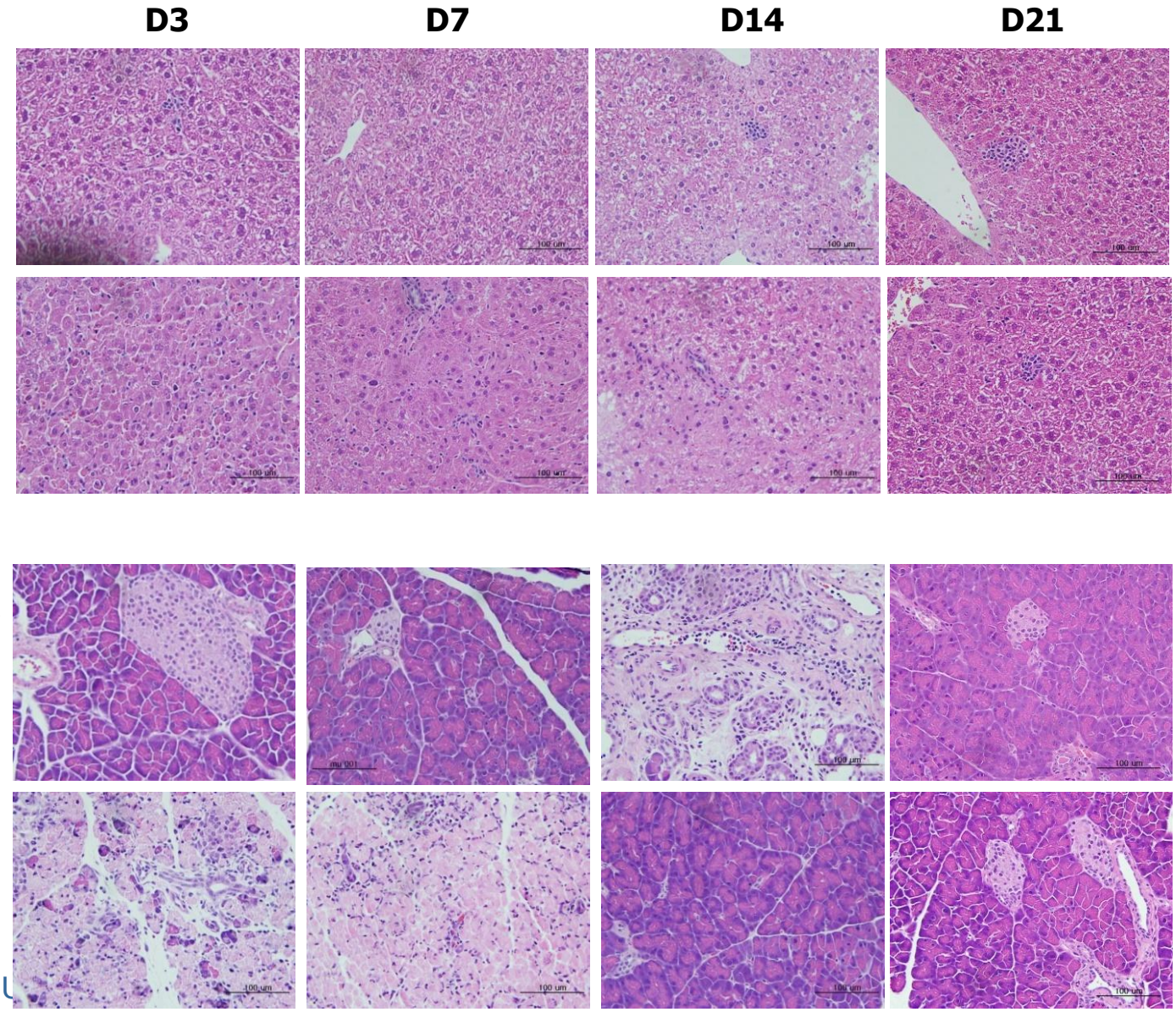
D3 D7 D14 D21



Pancreas



C57/BL6 Strain 심근염 모델(간, 췌장)



El Capitan, Yosemite National Park, CA, USA