

이질균 및 변종대장균 배출독소에 의한 생태환경오염과 교차에 의한 인체공중보건위협

1. 장출혈성 대장균배출 시가독소로 인한 병리
2. AB5 type 박테리아 독소의 세포내 이동과 tool로서의 유용성
3. 병원성 시가독소에 의한 숙주반응

2015. 5. 29

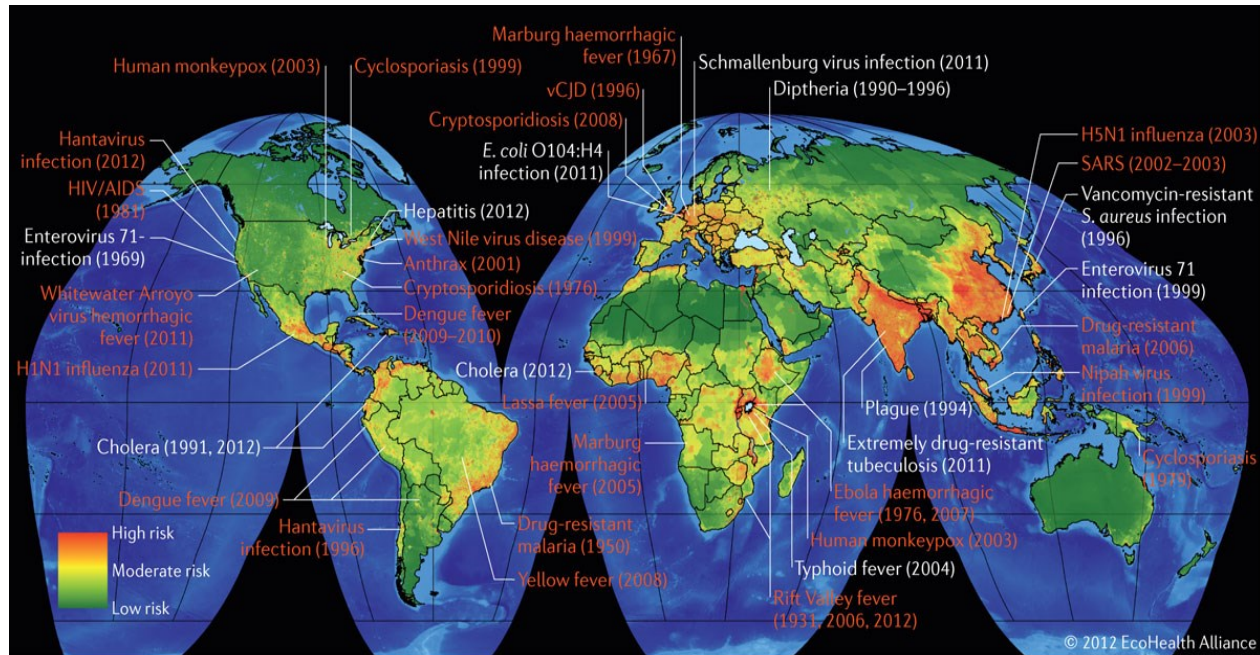
Moo-Seung Lee
Infection and Immunity Research Center



한국생명공학연구원
Korea Research Institute of Bioscience and Biotechnology

VLAOSTUDIO

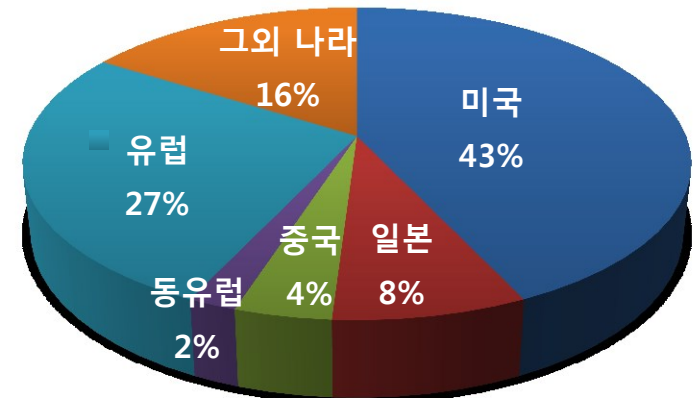
1. 연구 배경: 감염질환 심각성은 전세계적인 이슈



Nature Reviews | Microbiology

- 기후 온난화/환경 오염, 고령화, 각국의 교류에 따른 신/변종 감염병의 빠른 확산
- 감염 및 병원성 기전은 점점 복잡해지고 당뇨, 암, 염증성 질환 등의 질병과도 연계
- 감염/염증 질환 인구는 약 10억 명으로 추산
- 전세계적으로 치료 불가능한 고도 내성균 확산 추세
- 문제점 해결을 위한 지속적인 예방 및 치료제 개발 필수

전세계 감염/염증 치료제 시장 점유율



1-2. 연구 배경: 대내외 니즈 분석

5대 미래 환경변화

과학기술 비전 2040(교과부, '1010)

- 환경과 자원문제 심화
 - 에너지와 자원의 해외 의존 지속
 - 지구온난화 가속
 - 환경오염에 따른 건강 위협
- 지식기반 사회 진전과 글로벌화
 - BRICs(브라질, 러시아, 인도, 중국) 성장
 - 지식기반 경제의 강화
 - 생산 및 소비행태 변화
- 인구구조 변화
 - 선진국의 고령화와 개도국의 청년화
 - 도시화 지속/메가시티간 경쟁심화
 - 노동시장/가족개념 변화
- 과학기술 융합 가속화
 - IT기술 발전의 새로운 단계 진입
 - 바이오기술의 실용화 진전
 - 나노기술의 타 분야 발전 견인
- 새로운 안보이슈 등장
 - 새로운 전염병 위협
 - 테러위협 증가

정부니즈

국가 과학기술 중장기 발전전략(안)
(국과위, '1211)

- 지속가능한 청정사회
 - 미래 에너지원의 안정적 확보
 - 자원활용과 보존의 선순환 실현
 - 환경생태계의 자기치유기능 강화
 - 미래지구를 위한 기후변화대응
- 인간중심의 스마트사회
 - 지식정보자원 활용 고도화
 - 상호공감을 위한 감성 네트워크 구축
 - 생활공간의 지능화
 - 이용자중심의 사회 기반 시설 구축
- 창의적 성장사회
 - 미래 융합소프트 신시장 발굴
 - 미래 스마트소재프런티어 개척
 - 선도 산업의 신부가가치 창출
 - 미래 거대기술에 대한 도전 강화
- 활기찬 건강사회
 - 생명현상 규명을 통한 난치성 질병 극복
 - 환자 맞춤형 의료시대 실현
 - 스마트 유헬스시스템 구현
 - 보편적 공공사회 보건안전망 구축
- 창의적 성장사회
 - 자연재해 대응력 강화
 - 사회적 재난 대응체계 확보
 - 신기술 위험관리 체계 구축
 - 식량 안보력 및 안전성 향상

경영목표

- 바이오 기반 융복합 헬스케어
 - [1-1] 바이오 기반 BNT 융복합 진단·치료 기술 개발
 - [1-2] 천연물 신약 소재 개발
 - [1-3] 줄기세포/항체 바이오 신약 후보물질 개발
- 바이오경제 창출
 - [2-1] 스마트 세포공장/세포제어 기술혁신
 - [2-2] 바이오 기반 석유화학 대체 소재 개발
- 국가임무형 아젠다 해결(NA)
 - [3-1] 국가재난형 감염병 극복기술 개발
 - [3-2] 노화성질환 극복기술 개발
 - [3-3] 차세대 바이오매스/에너지 기술 개발
- 바이오인프라 활용 촉진
 - [4-1] 생명연구자원/정보 활용성 강화
 - [4-2] 바이오 평가 및 상용화 지원

연구그룹 목표

생체방어시스템 연구센터

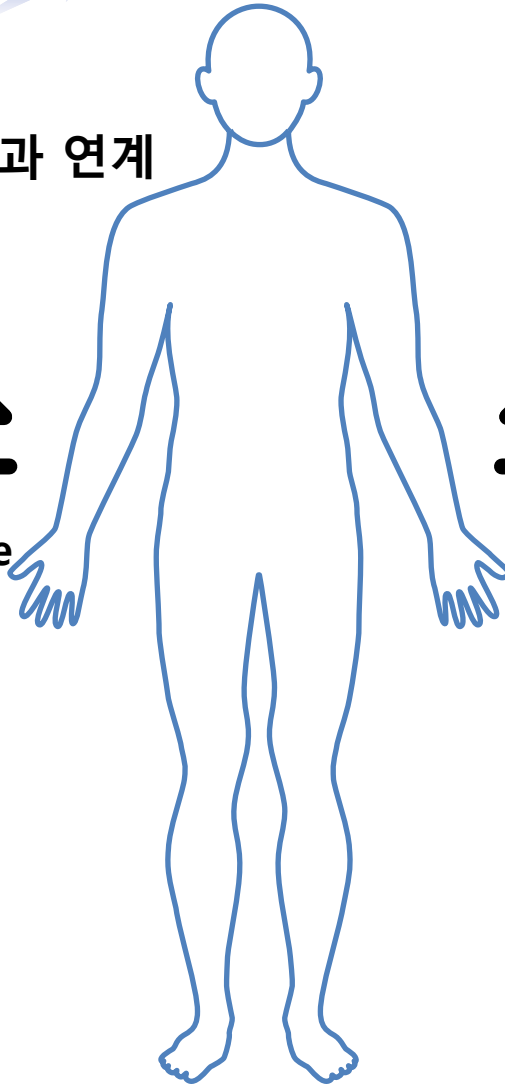
융합적 연구 기반
감염 및 면역
생체 방어 기전
연구를 통한
제어/치료제 개발
원천 기반 기술
확보

2. Theme : 병원균-숙주 상호작용

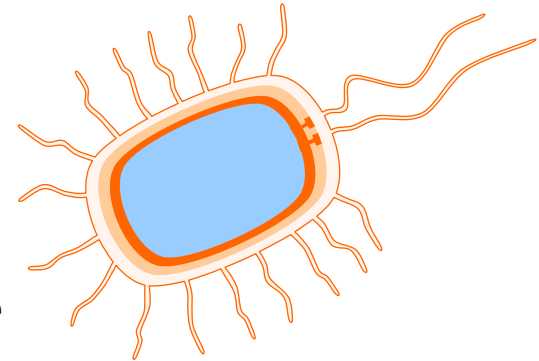
- 고령화 2016년 13.4% → 2026년 20.8%
- 암, 당뇨, 감염 등 각종 질환과 연계

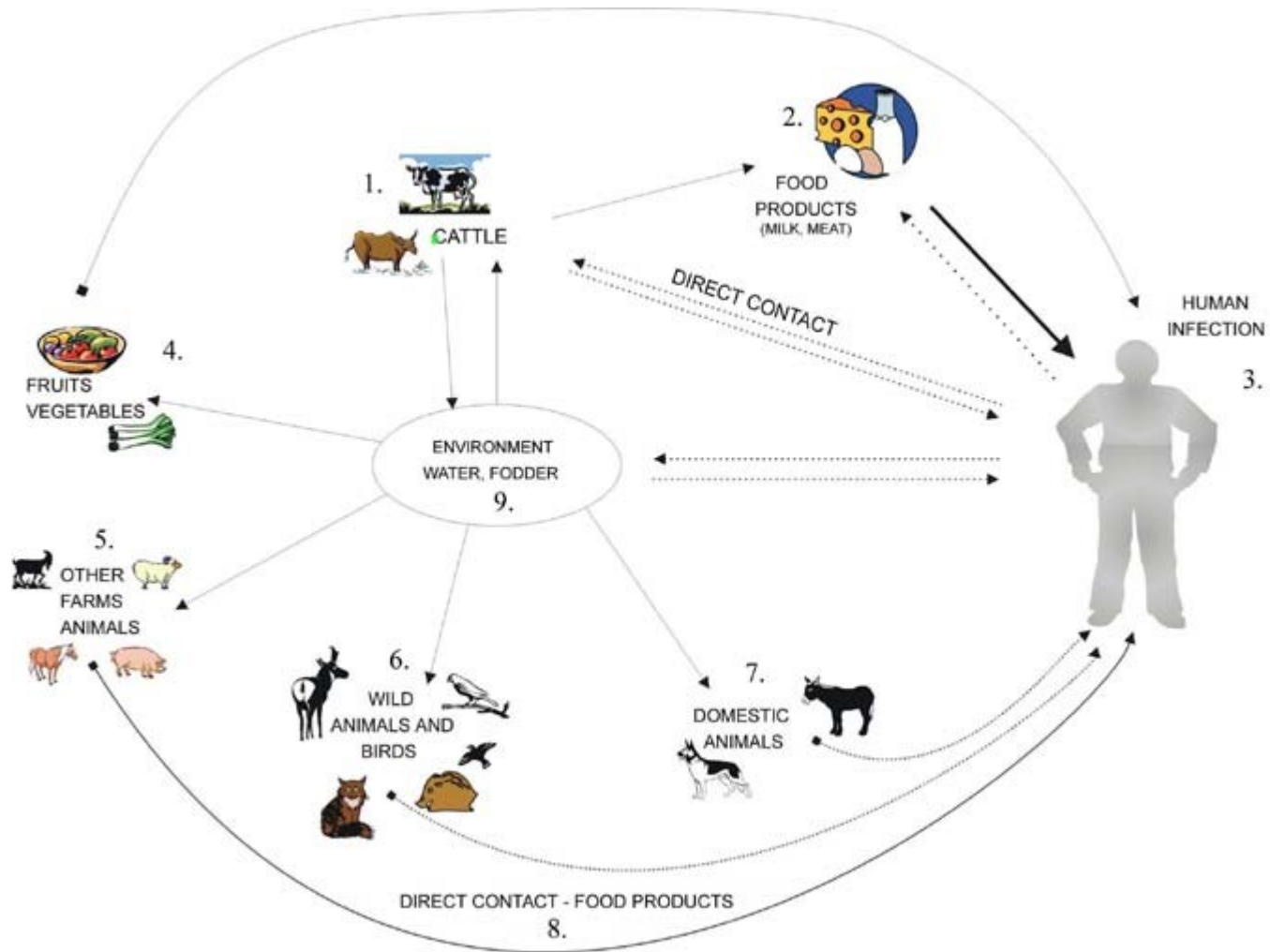


Survival
↓
Defense

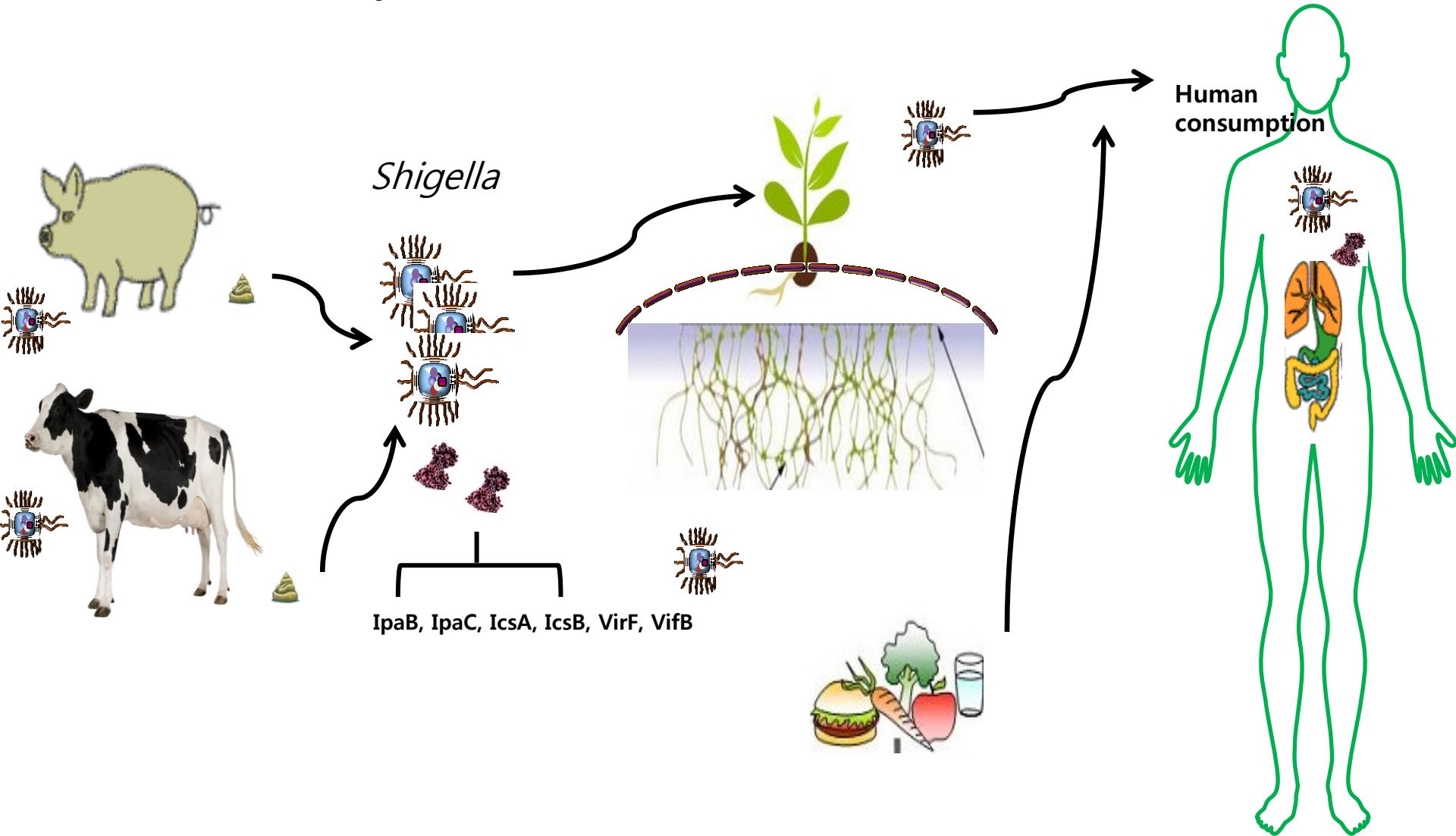


Survival
↓
Defense





Shigella transmission to human via alternative hosts in environmental system



국내외 양돈 사육규모 및 육류 소비 패턴

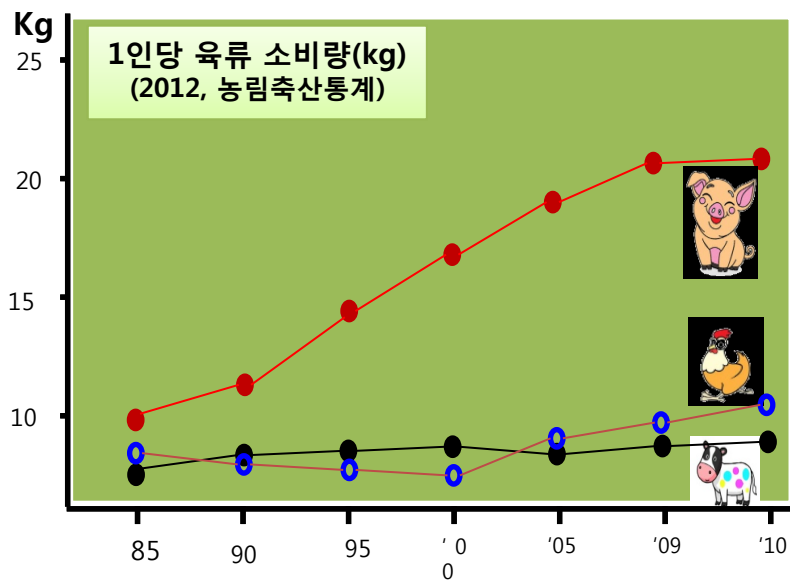


	2004	2005	2006	2007	2008
중국	466,017	481,891	503,348	494,407	469,758
EU-27	158,970	156,973	157,364	159,887	159,900
브라질	32,081	32,323	32,938	33,147	33,877
러시아	17,200	16,500	16,550	17,180	18,580
캐나다	14,725	14,810	15,110	14,907	14,400
멕시코	10,668	10,303	10,125	10,250	10,410
일본	9,724	9,600	9,620	9,759	9,725
한국	8,367	8,044	8,098	8,518	8,754
우크라이나	7,321	6,466	7,052	8,055	8,650
호주	2,658	2,530	2,490	2,471	2,410
기타	46,348	47,416	50,013	51,522	52,398
미국	60,444	60,975	61,449	62,489	64,400
총 합계	834,523	847,831	874,157	872,592	803,262

※ 자료 출처 : 미국 농무성 (단위 :천두)

가 구 수		마 리 수
5,441		9,697,709
1000두 미만	2,611	991,210
1000-5000두	2,496	5,392,899
5000-10,000두	229	1,513,454
10,000두 이상	105	1,800,144

(국가 통계포털, 2014)

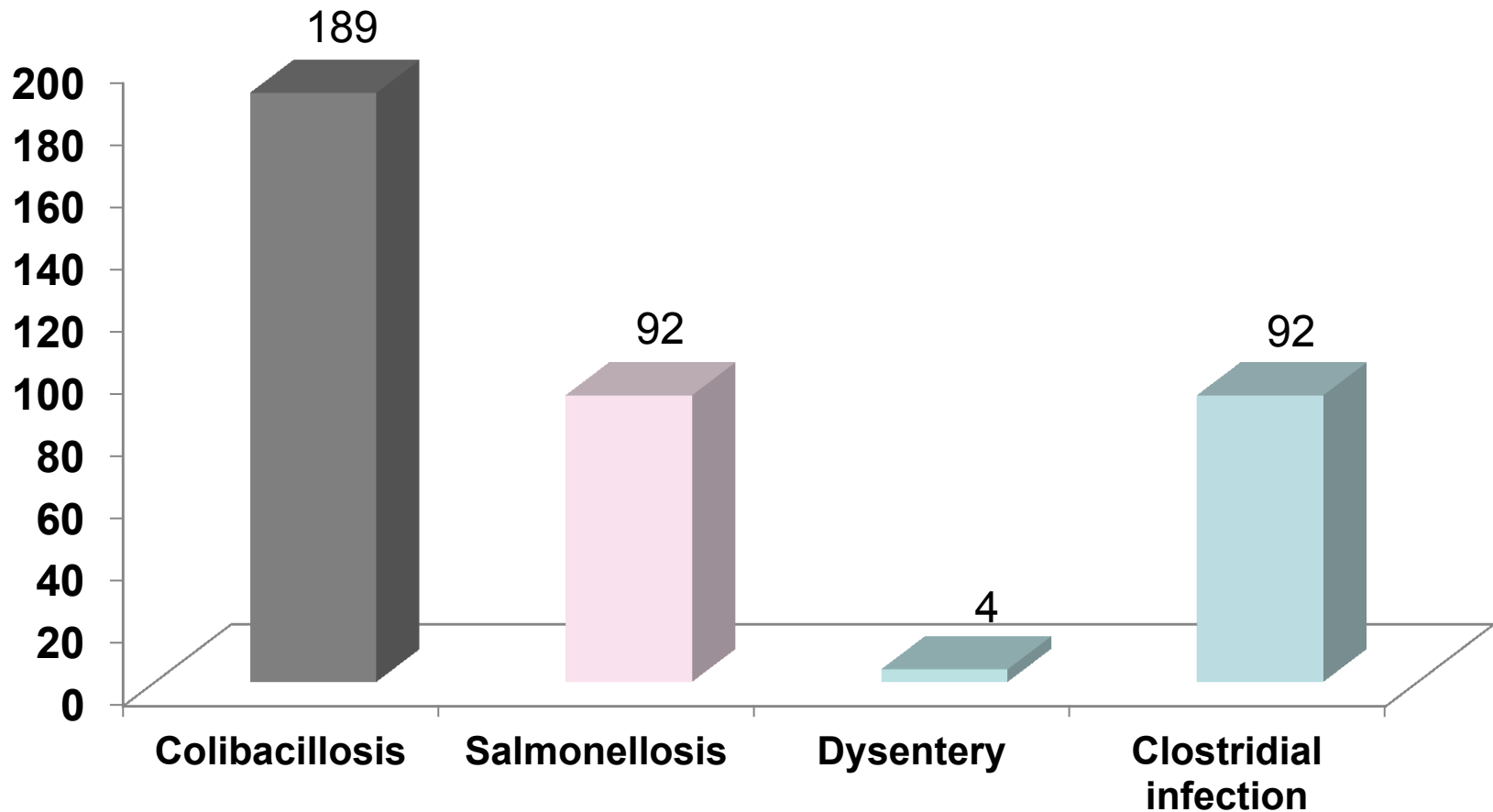


국내 양돈질병 현황(대한양돈협회, 2011)

출처: 2011년도 전국 양돈장 질병실태 조사 결과, (사)대한양돈협회

	질병명	2011	2010	2009	2007
지속발병 질병	피알알에스	71.1%	77(30.1)	72(54.1)	3(6.0)
순위 상승질병 ➡	홍막폐렴	54.7%	16(6.3)	24(63.2)	7(20.1)
순위 상승질병 ➡	대장균증	31.1%	0	0	0
지속발병 질병	씨코바이러스	28.9%	100(39.1)	47(35.3)	26(52.0)
순위 상승질병 ➡	살모넬라증	25.3%	11(4.3)	16(21.3)	17(40.5)
순위 상승질병 ➡	글래서씨병	15.3%	1(0.4)	6(15.8)	3(8.7)
순위 상승질병 ➡	회장염	11.1%	1(0.4)	2(2.7)	3(7.2)
	파스튜렐라성 폐렴	8.4%	0	0	0
	PED	5.8%	13(5.1)	18(24.0)	9(21.4)

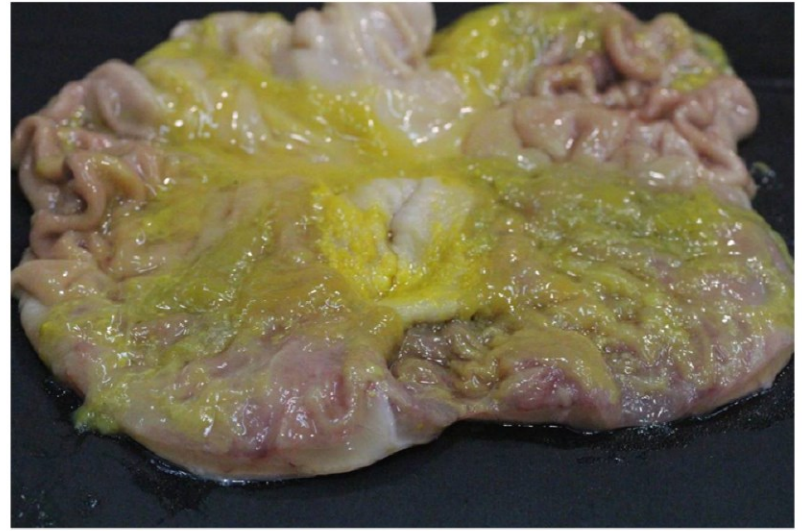
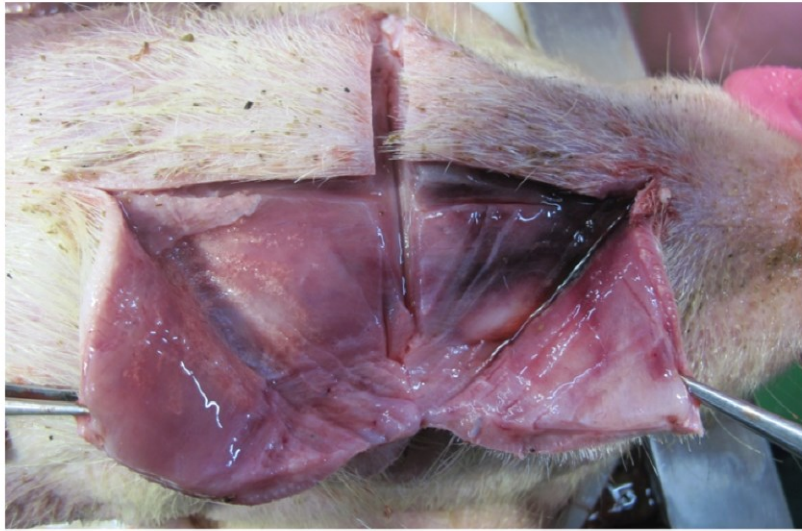
돼지 대장균성 설사병 진단건수(2012, KAHIS)



국내의 부종병 발생 농장상황 (2013)



부종병 발생돼지의 부검소견



돼지 대장균설사병 및 부종병의 경제적 피해

■ 국가별 대장균 설사병 및 부종병 손실액

Author	Date	Publication	Country	Type	Economic Impact
Tacha et.al	Jan 2000	Swine Health production	US	이유	\$ 5.88/pig
Marquardt et.al	Mar 2001	The Leman swine conference	US/Canada	포유	\$ 0.2~1 billion/year
Ngyuen et.al	Aug 2013	Pig progress	EU	이유	10% of herd /outbreak

■ 국내 대장균 부종병 손실액(추정)

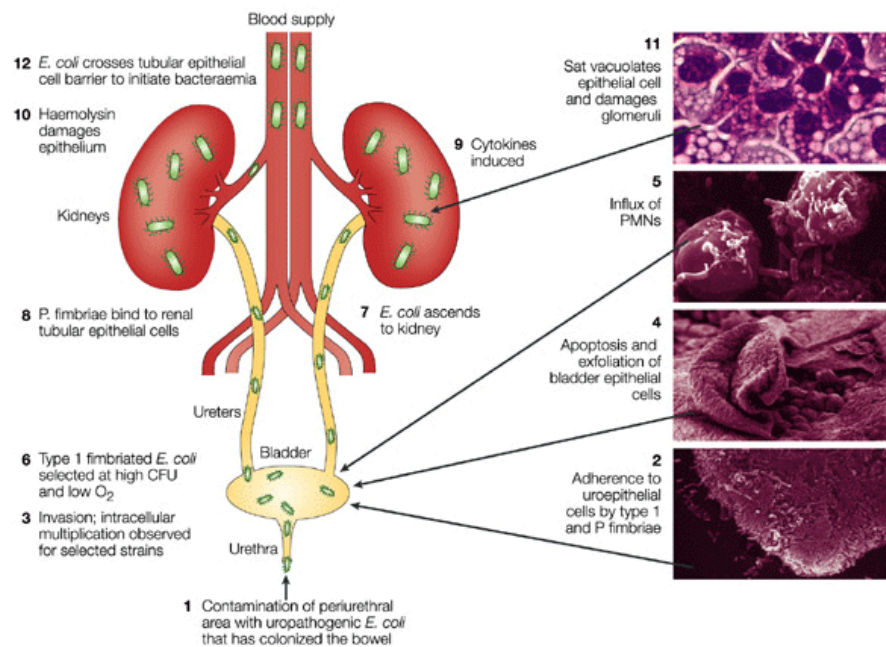
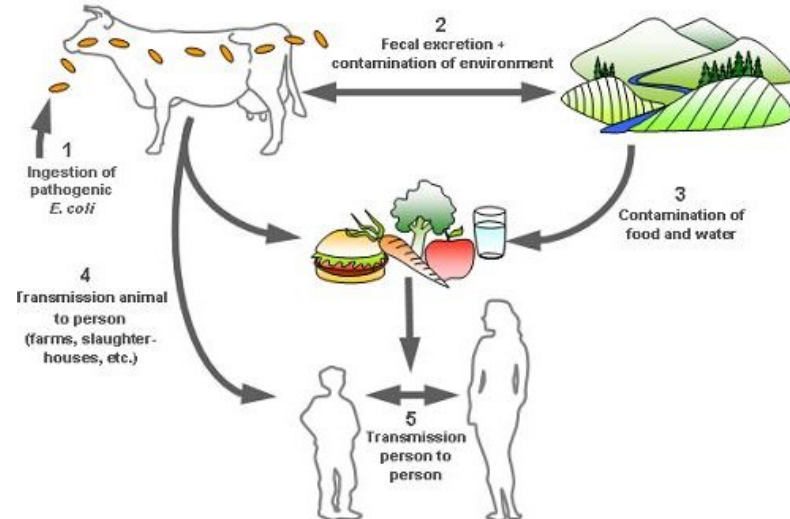
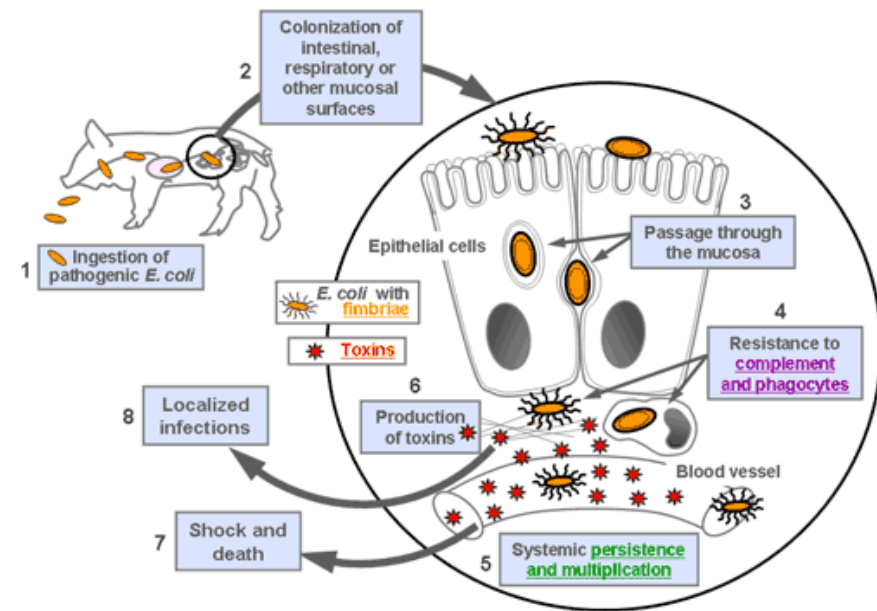
피해내용	내 용	금액
직접피해	$\text{폐사수} = 100\text{두(모돈)} \times 2.3(\text{회전수}) \times 10\text{두(평균산자수)} \times 0.3(30\% \text{ 폐사율 적용}) = 600\text{두/년} \rightarrow 600\text{두} \times 40\text{만원(정상 출하시)}$	2억4천만원
간접비용	처리비용(인건비 + 랜더링 및 소각+매물)	1천만원
추정비용		2억5천만원

돼지 부종병의 경제적 피해 (현장 사례1)

부종병 진단 현황

검사기관 : 부경양돈농협 질병진단센터

순번	지역	농장주	최초진단일	발생일령	비고	피해발생	추정 폐사율	조기 진정 여부
1	양산	GS농장	10년 7월	30~40일령		미발생중	30%	부
2	김해	KW농장	10년 9월	70~90일령	자가백신접종(13년 8월~11월)	미발생중	40%	부
3	사천	SC농장	11년 4월	50~60일령		미발생중	20%	부
4	김해	JS농장	12년 7월	70일령	13년 5월 재발	최근 재발생	10%	부
5	진주	KW농장	12년 9월	70일령	타진단기관 진단	발생중	30%	부
6	진주	YS농장	12년 9월	70일령		미발생중	30%	부
7	함안	KH농장	12년 9월	40~90일령	자가백신접종(13년 8월~10월?)	미발생중	30%	부
8	경주	ND농장	13년 3월	40~50일령	타진단기관 진단	미발생중	15%	여
9	함안	MR농장	13년 4월	40~50일령	타진단기관 진단	미발생중		여
10	의령	SB농장	13년 8월	30~40일령	타진단기관 진단	미발생중	10%	여
11	함천	HB농장	13년 9월	70일령		미발생중	20%	여
12	함안	YS농장	13년 9월	30~40일령		미발생중		여
13	김해	JH농장	14년 2월		40일령에서 검출	잠재농가	설사만 다발	
14	김해	TH농장	14년 2월		40일령에서 검출	잠재농가		



Research Significance/Novelty

Goal & Impact on the field

To provide fundamental insights into cellular mechanisms (inflammasome) of human host defense against EHEC Shiga toxin-mediated pathogenesis.

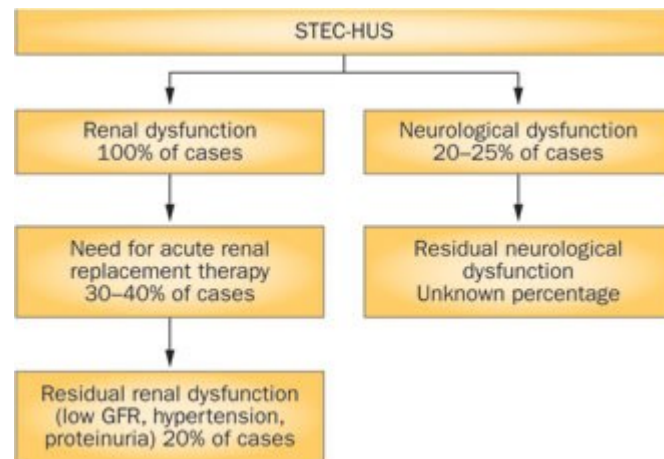
There is no satisfactory vaccine to treat the patient with Stxs-intoxication

- Shiga toxin-producing *E.coli* still public health concerns (110,000 cases/year)
- Complex modulatory immune system disrupted by STEC, thus hard to mitigate disease progression

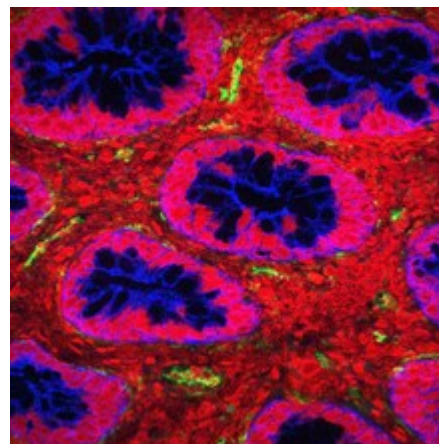
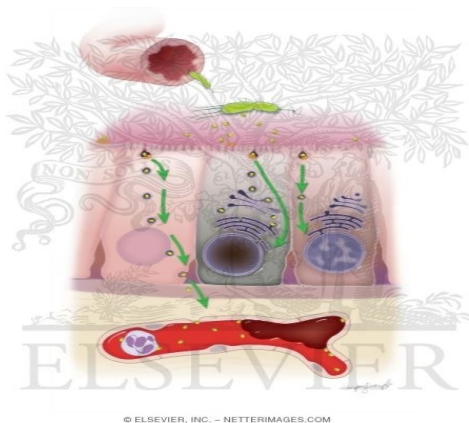
Innovations:

- From this study, improved understanding differences in the innate immune response to , and death caused by Stxs in the enhanced potential of Shiga toxin type 2 resulting in extra-intestinal disease
- Regulatory immunity to the intoxication at multiple steps in the signaling process so as to minimize tissue damage.
- Elucidating fundamental cellular mechanism by which platform for resolution of the inflammatory cytokines may be built into the induction phase
- Resolve the mis-link between apoptosis through ER-stress and pro-inflammatory response
- Resolving the mechanism by which Stxs mediate functional NLRP3 inflammasomes to induce response.

Outcomes of renal and neurological involvement in patients with STEC-HUS.



Howard et al., Nature Reviews Nephrology 2012



Shiga toxin in intestine
S. Shuller

Where did Shiga toxin-producing bug come from?

The Shiga toxin family

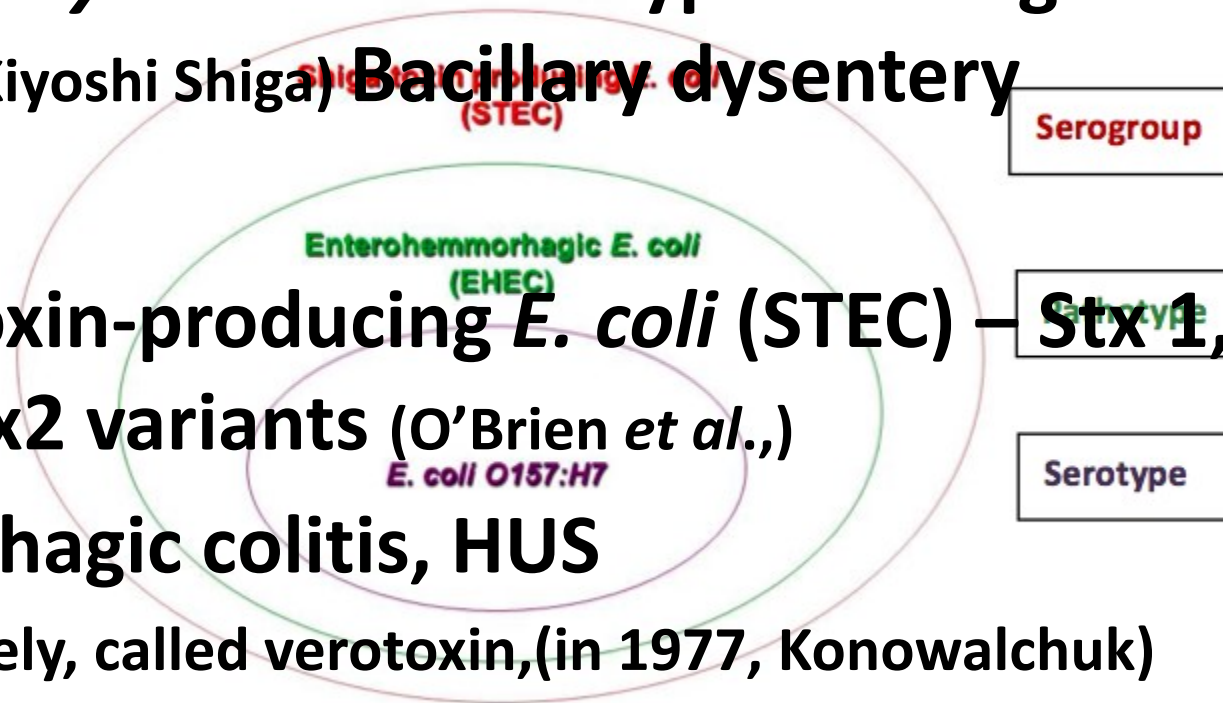
***Shigella dysenteriae* serotype 1 – Shiga toxin (Stx)**

(in 1897, Kiyoshi Shiga) **Bacillary dysentery**

Shiga toxin-producing *E. coli* (STEC) – Stx1, Stx2, Stx1/Stx2 variants (O'Brien *et al.*,)

Hemorrhagic colitis, HUS

Alternatively, called verotoxin, (in 1977, Konowalchuk)



Bacterial Shiga toxin secretion system.

Shiga toxin genetics

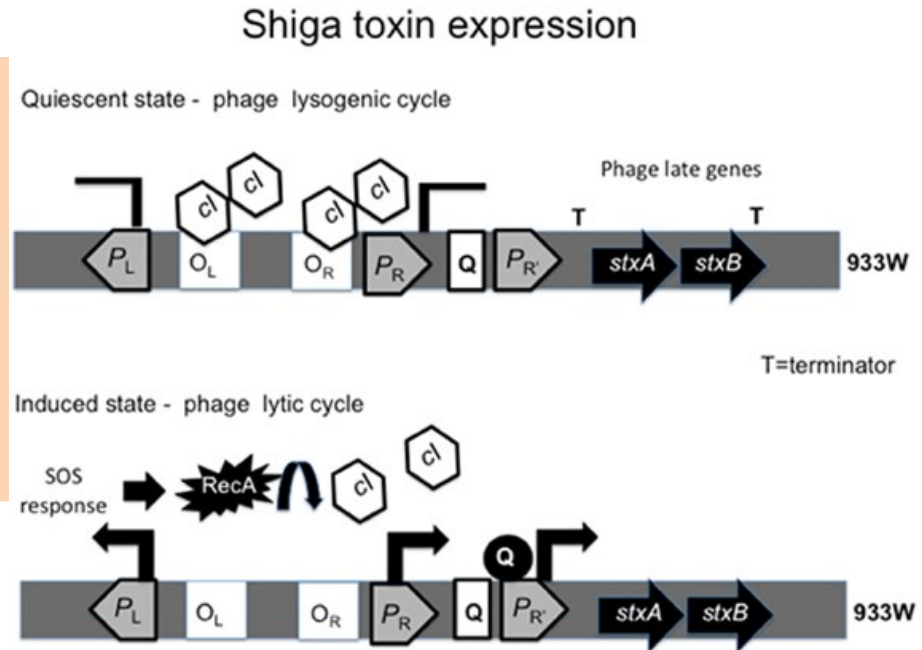
1. Lambdoid bacteriophage encoded
2. Induced on lysis
3. Ion-regulated expression, via Fur
4. Variants : Shiga toxin type 1 and 2



Phage regulates the production of Stx through phage promoter.



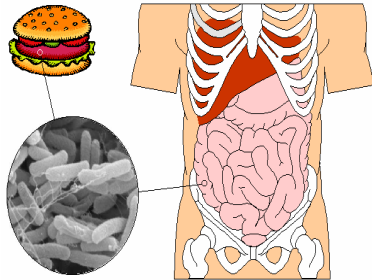
Shiga toxin assembled in the unique periplasm of gram-negative bacteria



Sources of *S. dysenteriae* and STEC

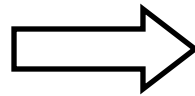
Shigella dysenteriae

- Developing countries
- Contaminated



STEC

- Developed countries
- Hamburger
- Unpasteurized fruit juices
- Unwashed vegetables
- Well water
- Underchlorinated swimming pool water
- Petting zoos



- Bloody diarrheal diseases
- Acute renal failure
- Hemolytic uremic syndrome
- Central nervous system complication

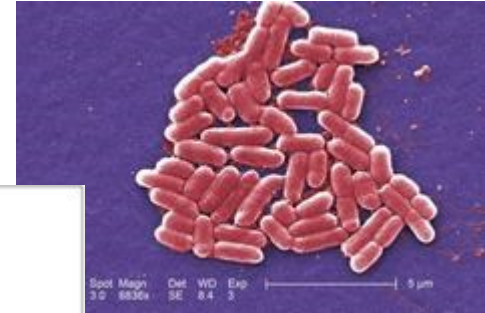
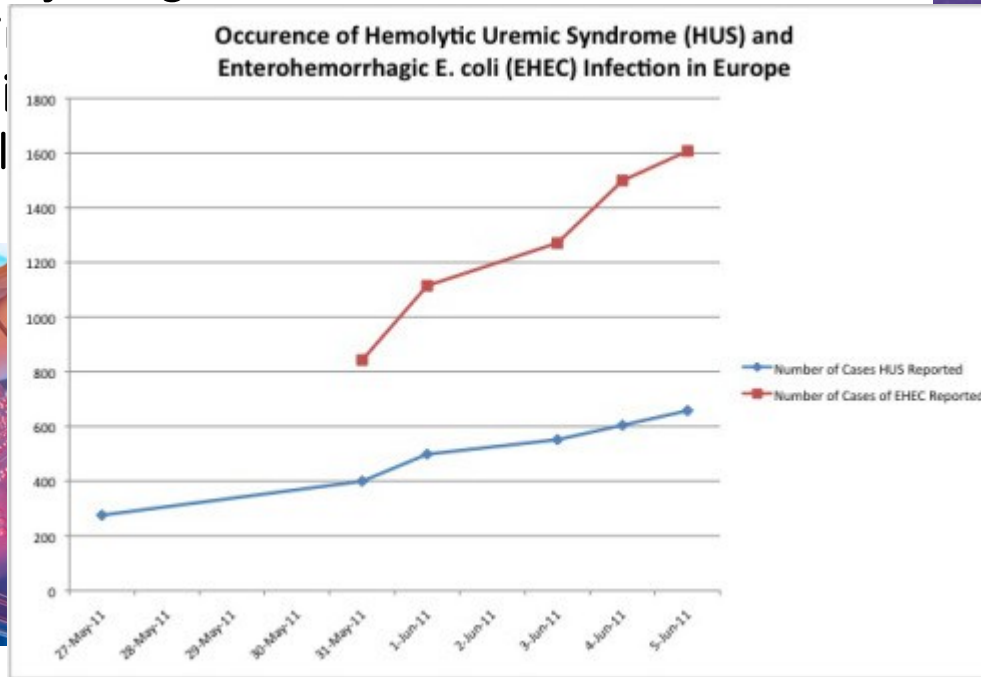
“6 Superbugs to Watch Out For”

1. Shiga toxin-producing *Escherichia coli*
2. Drug-resistant Gonorrhea
3. Extensively Drug Resistant Tuberculosis
4. Clostridium
5. Methicillin-resistant *Staphylococcus aureus*
6. Klebsiella



by Rachael Rettner

Published December 14, 2012 MyHealthNewsDaily



ation (WHO) [initially reported](#) an [enterohemorrhagic *E. coli* \(EHEC\)](#) secondary to [hemolytic uremic syndrome \(HUS\)](#); preliminary cases of HUS were reported to have occurred as of May 2011,.....

Specific effects of this particular *E. coli* strain **presence of Shiga toxins.**



Breaking News on 4/17/2013

Letter sent to US senator tests positive for **ricin**

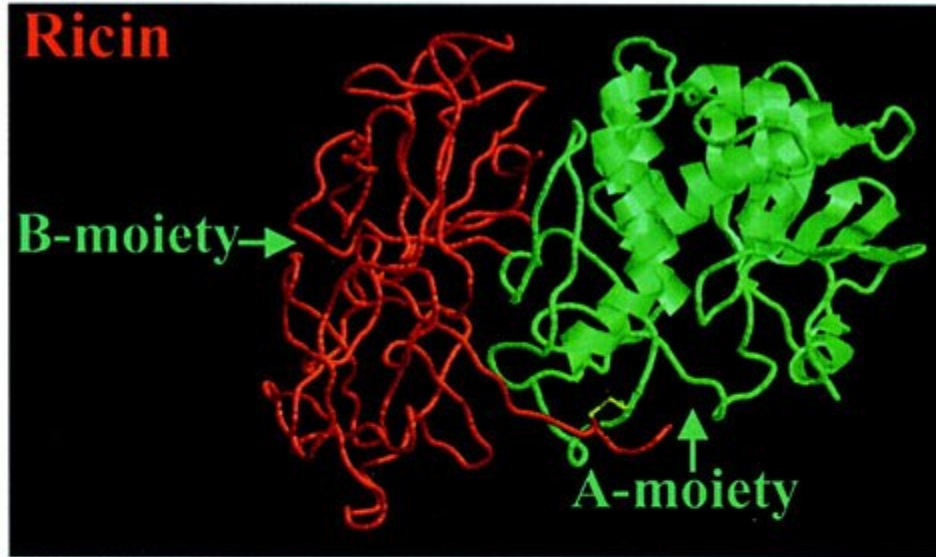


AB t^A

species

◆ **Ricin (Pla**

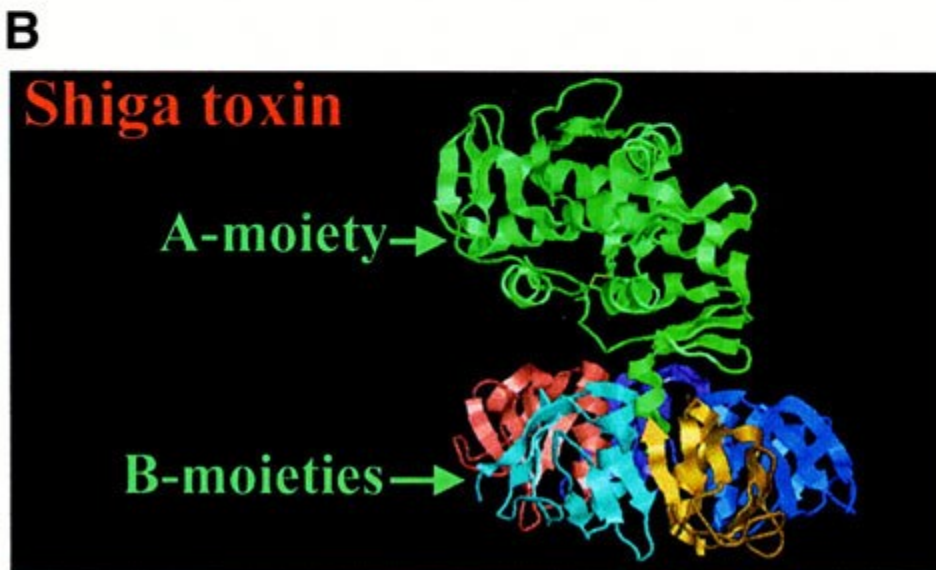
- AB toxin
- Endospe
- RNA N-g
- Retro-tra
- Potential



us communis

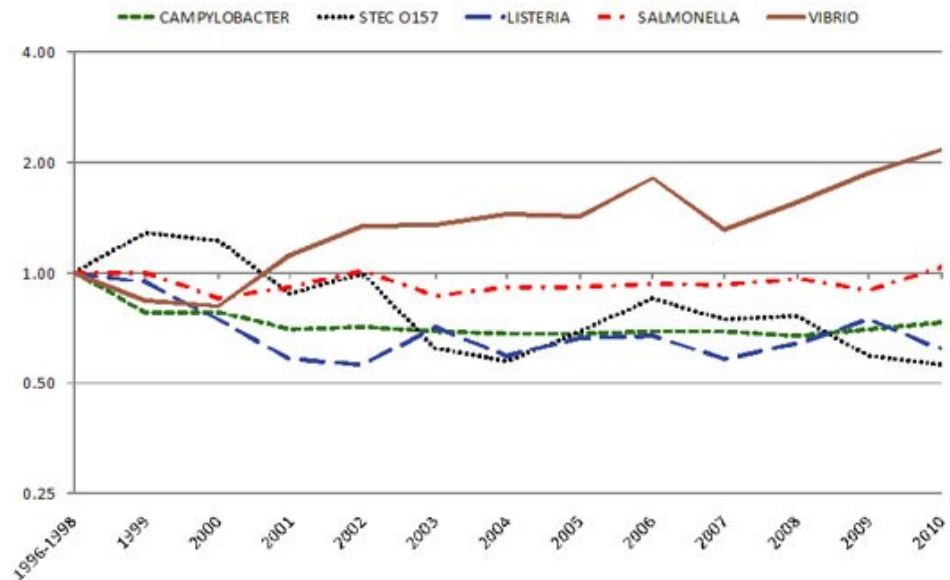
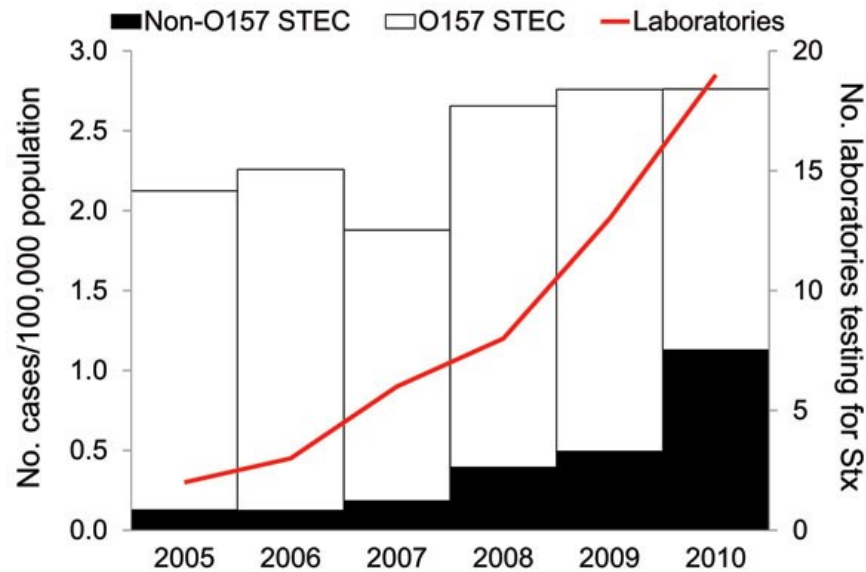
◆ **Shiga toxin**

- AB5 toxin
- EHEC, S
- Retro-tra
- Apoptosi
- Potent b



d endocytosis.
y II by CDC)

Shigella dysenteriae type 1 or Shiga toxin-producing *E. coli* serotypes are major health problems



Sero – pathotype1	Serotype	Frequency of Association with Disease	Involvement in outbreaks	Association with HUS and HC*
A	O157:H7, O157:NM	High	Common	+
B	O26:H11, O103:H2, O111:NM, O121:H19, O145:NM	Moderate	Uncommon	+
C	O5:NM: O91:H21, O104:H21; O113:H21; O121:NM: O165:H25 and others	Low	Rare	+
D	O7:H4: O69:H11; O103:H25: O113:H4; O117:H7: 119:H25: O132:NM; O146:H21: O171:H2: O172:NM: O174:H8 and others	Low	Rare	-
E	O6:H34; O8:H19: O39:H49: O46:H38: O76:H7; O84:NM: O88:H25; O98:H25: O113:NM: O136:NM: O143:H31: O156:NM: O163:NM and others	Not implicated	Not implicated	-

M.S. Lee *et al.*, 2014
M.S Lee, 2015

Bloody Diarrhea, HUS (Hyper Uremic Syndrome) → 1.1million fatal cases per year

진단기술 동향

- Optical immunoassay
- Sensitivity/Specificity
- Shiga toxin Quick Check

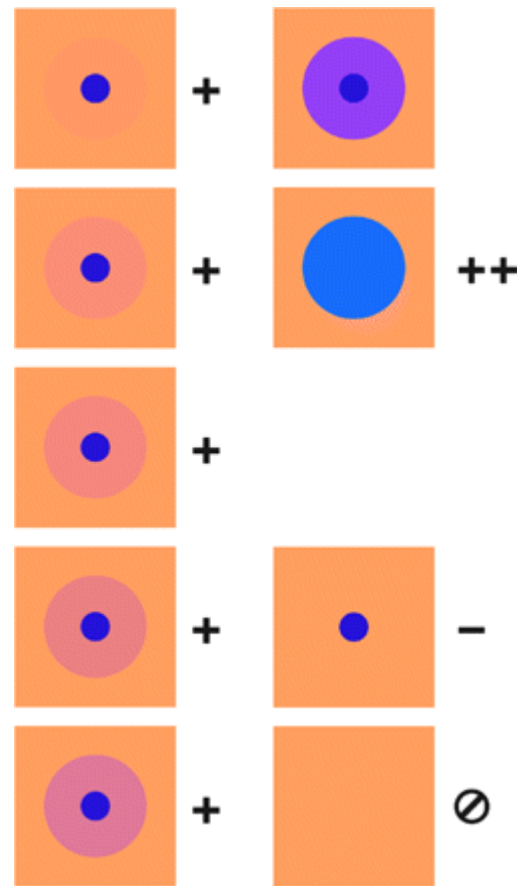


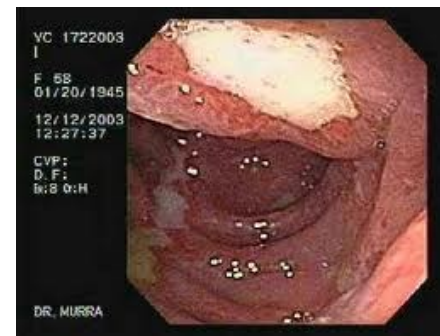
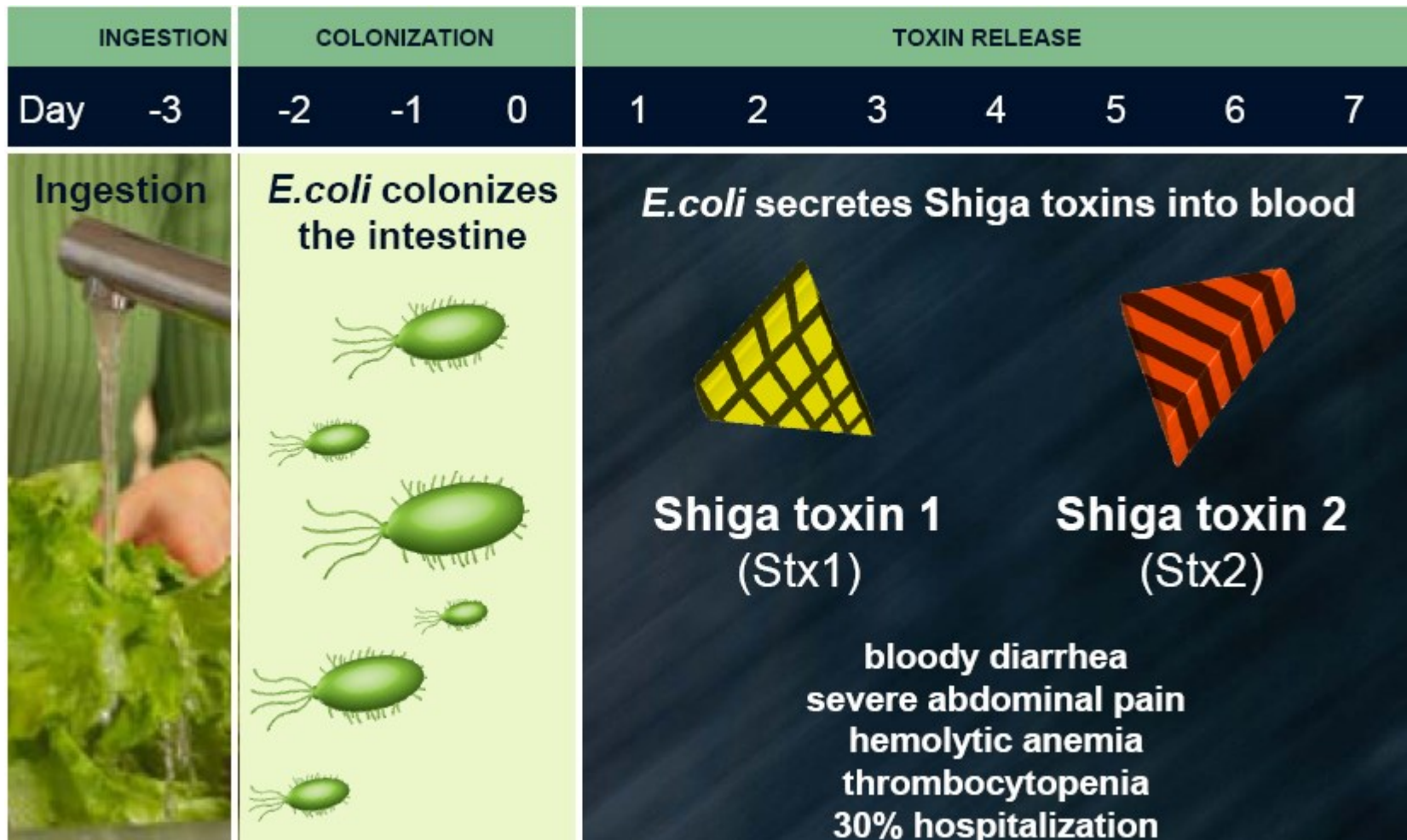
TABLE 2. Detection of Shiga toxins in frozen stool specimens tested directly after thawing and following broth enrichment with the BioStar OIA SHIGATOX and Premier EHEC kits^a

Test	No. of results				Sensitivity (%)	Specificity (%)
	True positive	True negative	False positive	False negative		
Direct stool						
OIA	46	33	0	6	88.5	100
EIA	44	33	0	8	84.6	100
Broth enrichment ^b						
OIA	44	24	0	6	88.0	100
EIA	43	24	0	7	86.0	100

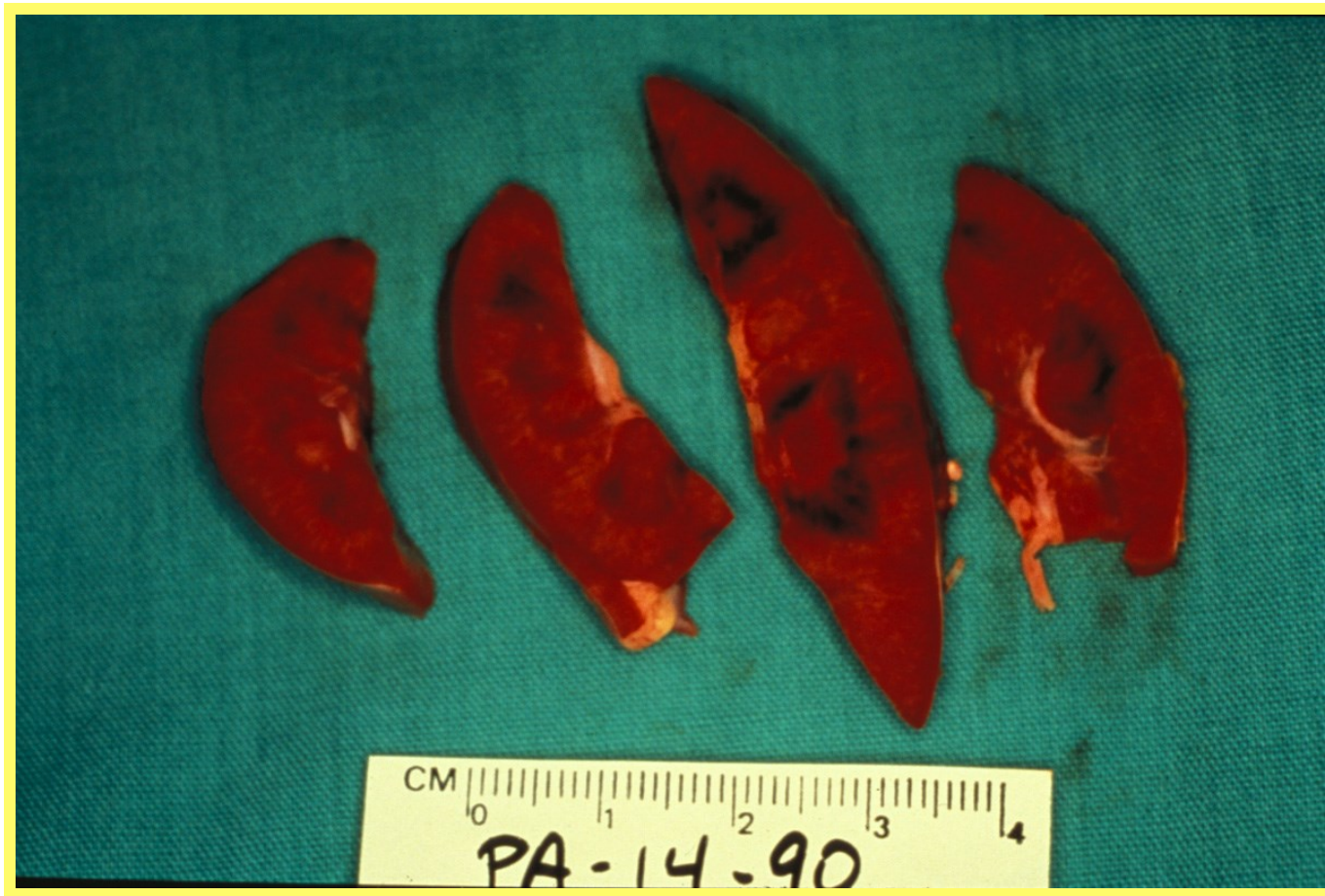
^a A total of 85 samples were tested. Positive results were confirmed by the Vero cytotoxicity assay.
^b Eleven previously frozen samples failed to grow when subcultured in MacConkey broth for enrichment.



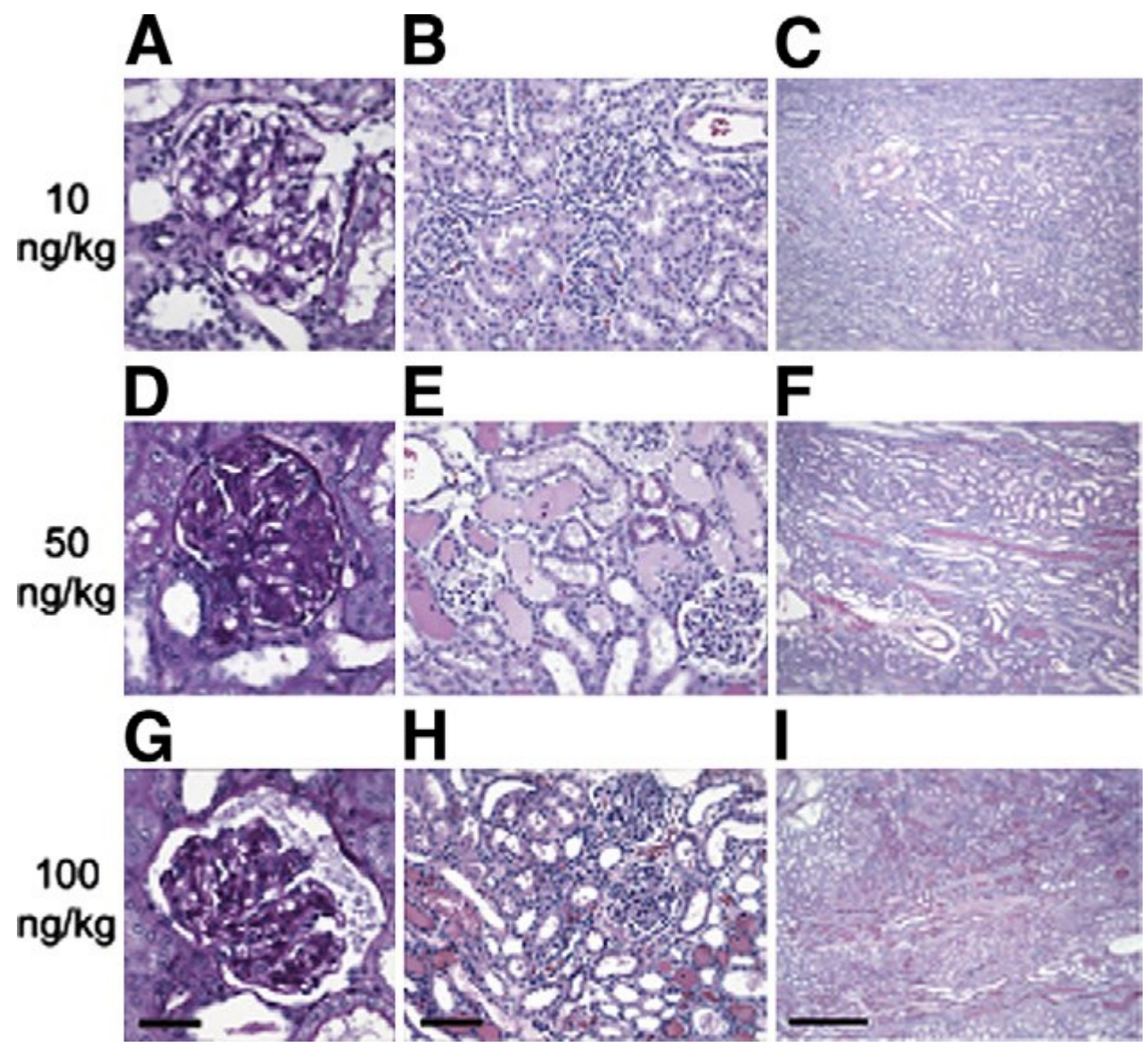
Progression of Shiga toxin-producing *E.coli* (STEC) infection



Hemolytic Uremic Syndrome (HUS)



Kidneys from **Stx1**-challenged non-human primates; glomerular filtration

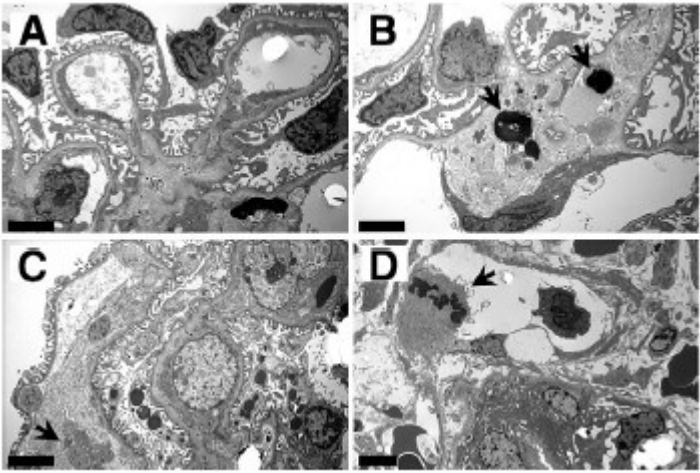
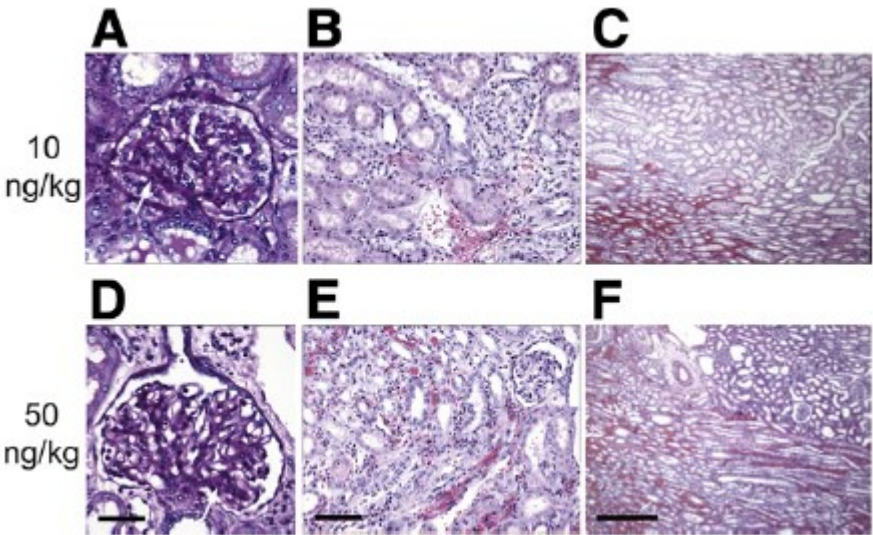
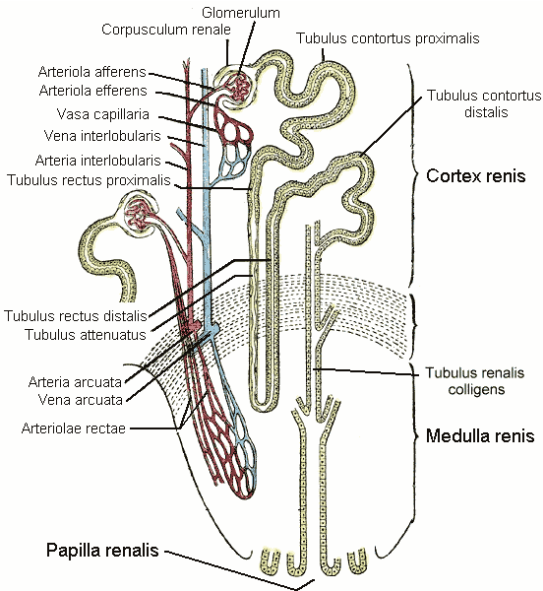
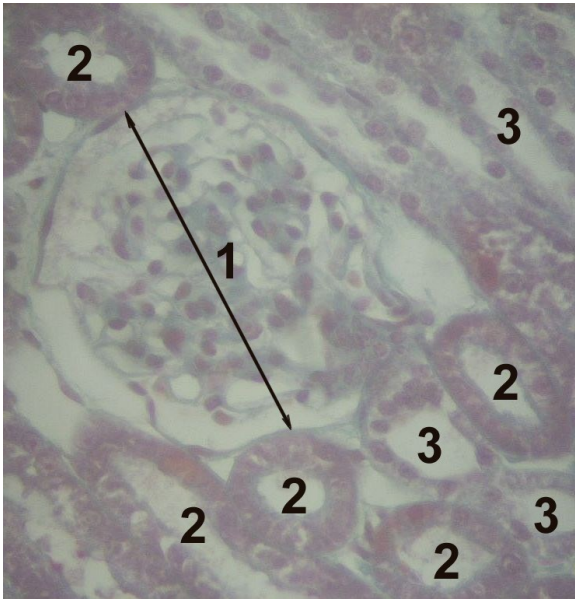


American Journal of Pathology 182(4) 2013

공동연구(보스턴의과대학)

Kidneys from **Stx2**-challenged non-human primates; Hemorrhage!

정상



Stx-병리검사(영장류)_Kurosawa,이무승&보스턴영장류센터

Stx, Renal Pathology, and Chemokines

Table 2 Light Microscopic Findings after Challenge with Stx1 or Stx2

Glomeruli			Tubulointerstitium							
Toxin	Dose (ng/kg)	Survival (h)	Endocap infil	Mesangial apoptosis	Mesangial mitosis	Distension	Epithelial flattening	Tubular pyelo. (poly)	Interstit hemor	Tubular epithelial prolif./mitoses
Stx1	10	168*	0	0	0	0	0	0	0	0
	10	168*	0	0	0	0	0	0	0	0
	10	168*	0	0	0	0	0	0	0	0
	50	58	0	0	0	1	1	0	0	0
	50	72	0	0	0	1	1	0	0	0
	50	72	0	1	0	1	1	0	0	0
	50	74	0	0	0	1	1	2F	0	0
	50	168*	0	0	0	0	0	0	0	0
	100	48	0	0	0	1	1	0	0	0
	100	49.5	0	0	0	1	1	0	0	0
	100	57.5	0	0	0	2	2	0	0	0
	100	60	0	0	0	1	1	0	0	0
	100	72	0	0	0	2	2	0	0	0
Stx2	10	111.2	0	3	0	1	1	2F	3	0
	10	288*	0	0	0	0	0	0	0	0
	10	672*	0	0	0	0	0	0	0	0
	10	720*	1F(1)	0	0	0	0	0	0	0
	50	84	0	1FS	0	1	1	0	2 [†]	+/-
	50	96	0	2	1	1	0	0	2 [†]	0
	50	96	0	0	0	0	0	1F	0	0
	50	112	0	4	0	2	2	0	4+	3
	50	121.6	0	4	0	2	2	1F	4+	2
	50	121.3	0	4	0	2	3	0	4+	4+
	50	128	0	3	0	2	2	1F	1F	2

Categories scored: +/- (occasional), 0 to 4+ where 0 = not observed and 4+ = most severe.

*Time at necropsy of survivor (survivor is ≥168 hours).

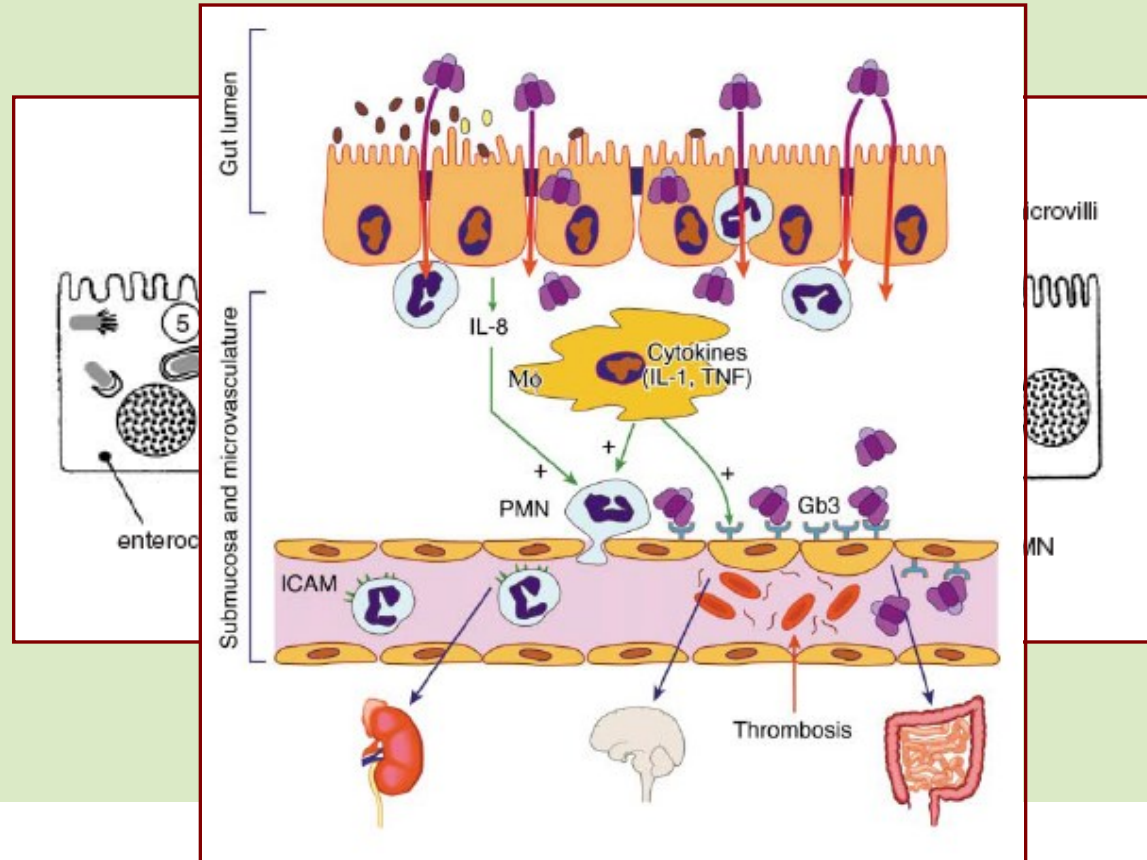
[†]Medulla only.

F, focal (otherwise diffuse); S, segmental (otherwise global).



INTRODUCTION

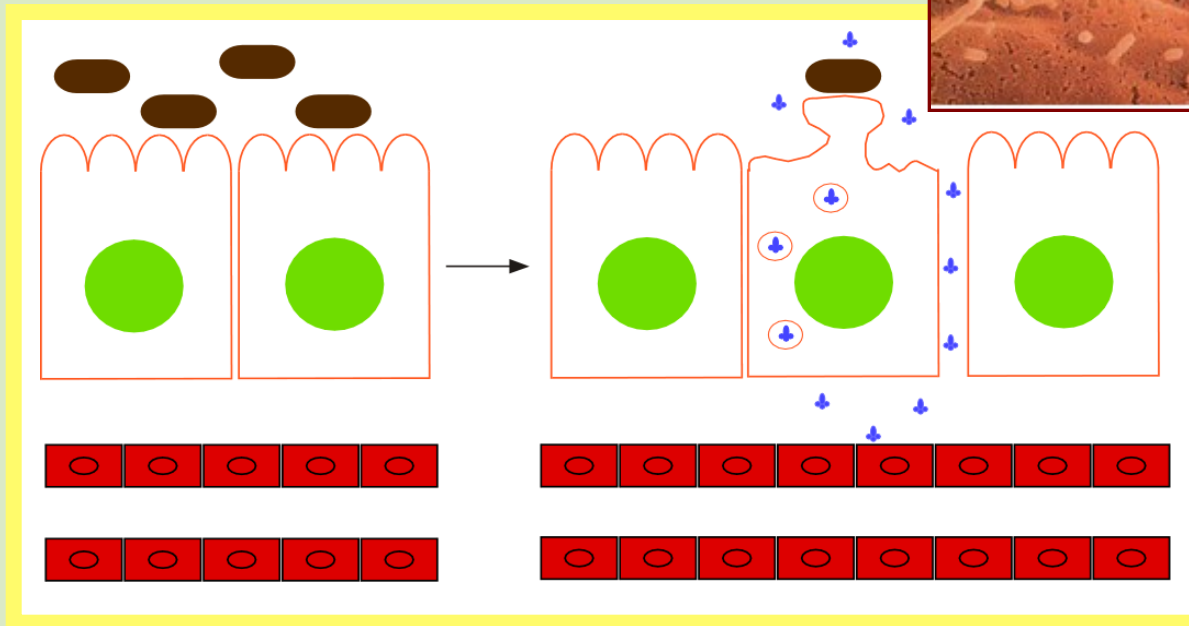
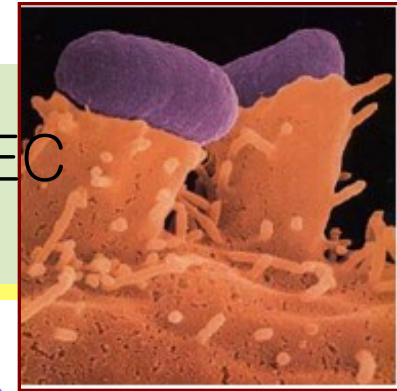
Pathogenesis of *S. dysenteriae*





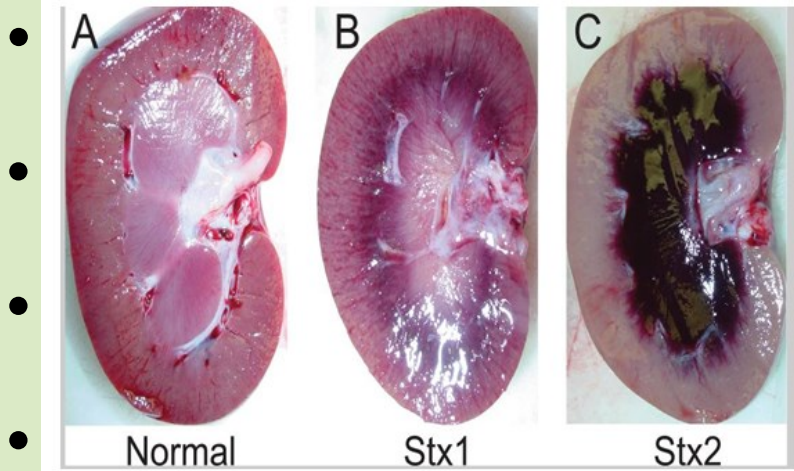
INTRODUCTION

Pathogenesis of STEC





INTRODUCTION



Kurosawa et al., 2011 IAI

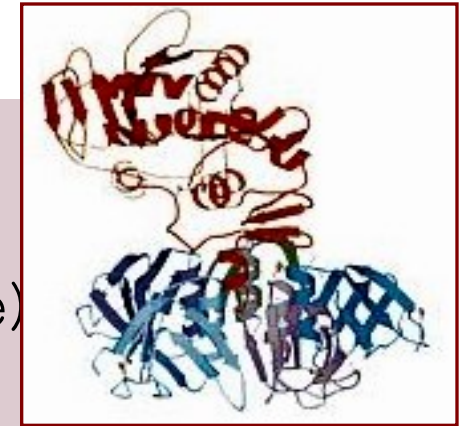
n



INTRODUCTION

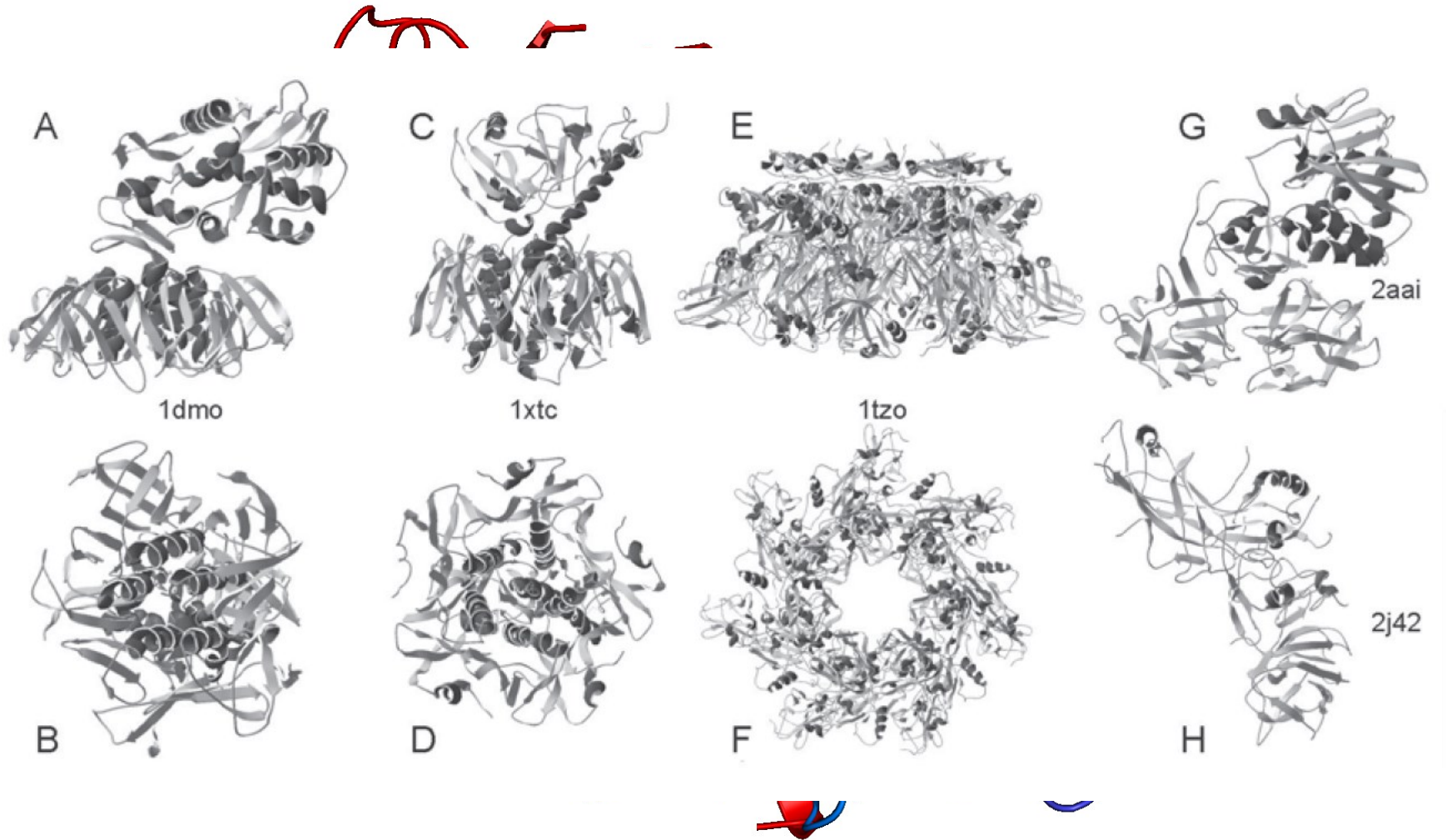
- AB₅ toxin

One A subunit (N-glycosidase)
5 B Subunits

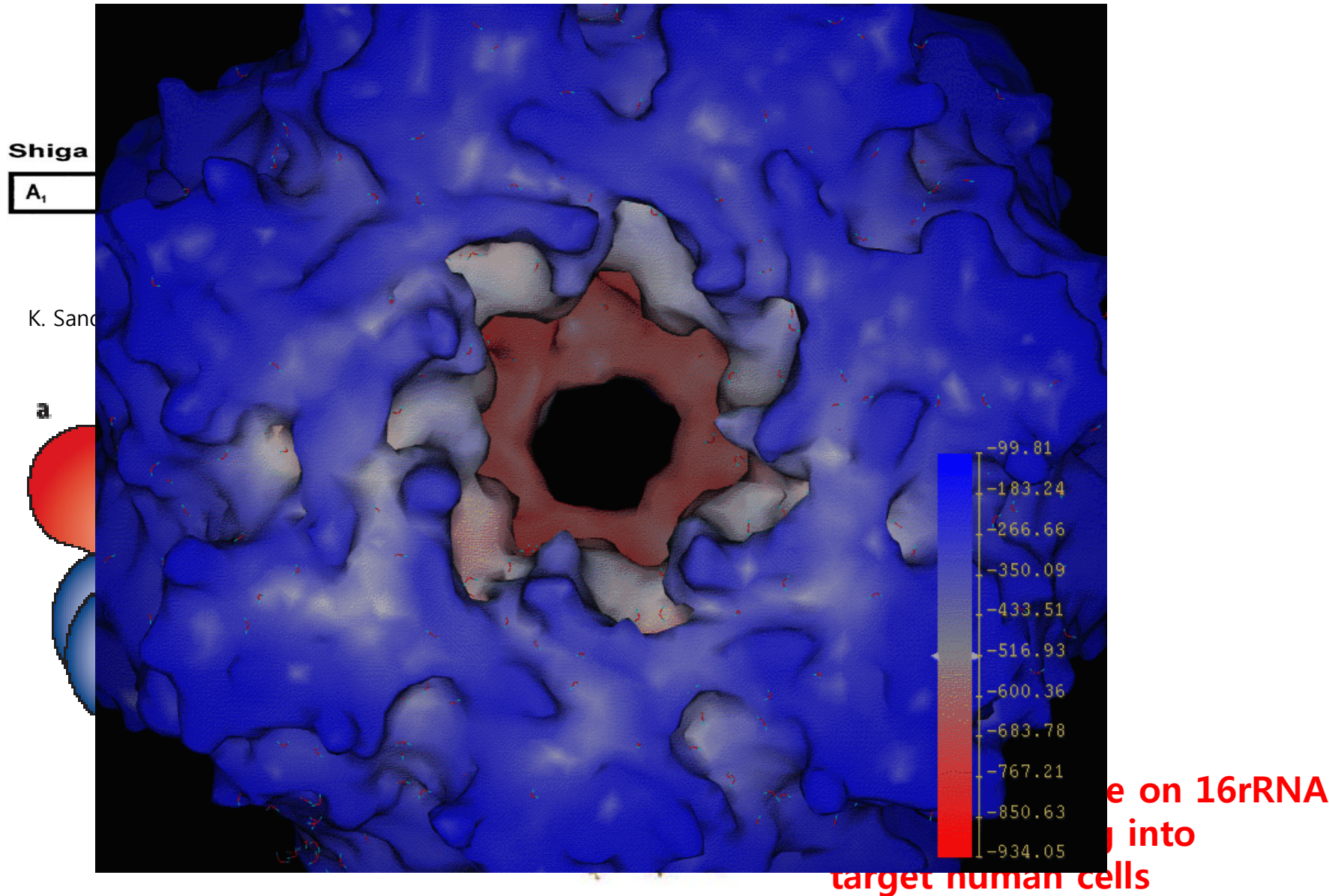


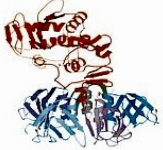
- Binds to Gb3 (globotriaosylceramide)
on target cell
- Inhibit protein synthesis
- Associated with HUS and neurological
complication

Enterohemorrhagic E.coli (장출혈성 대장균) Shiga Toxin



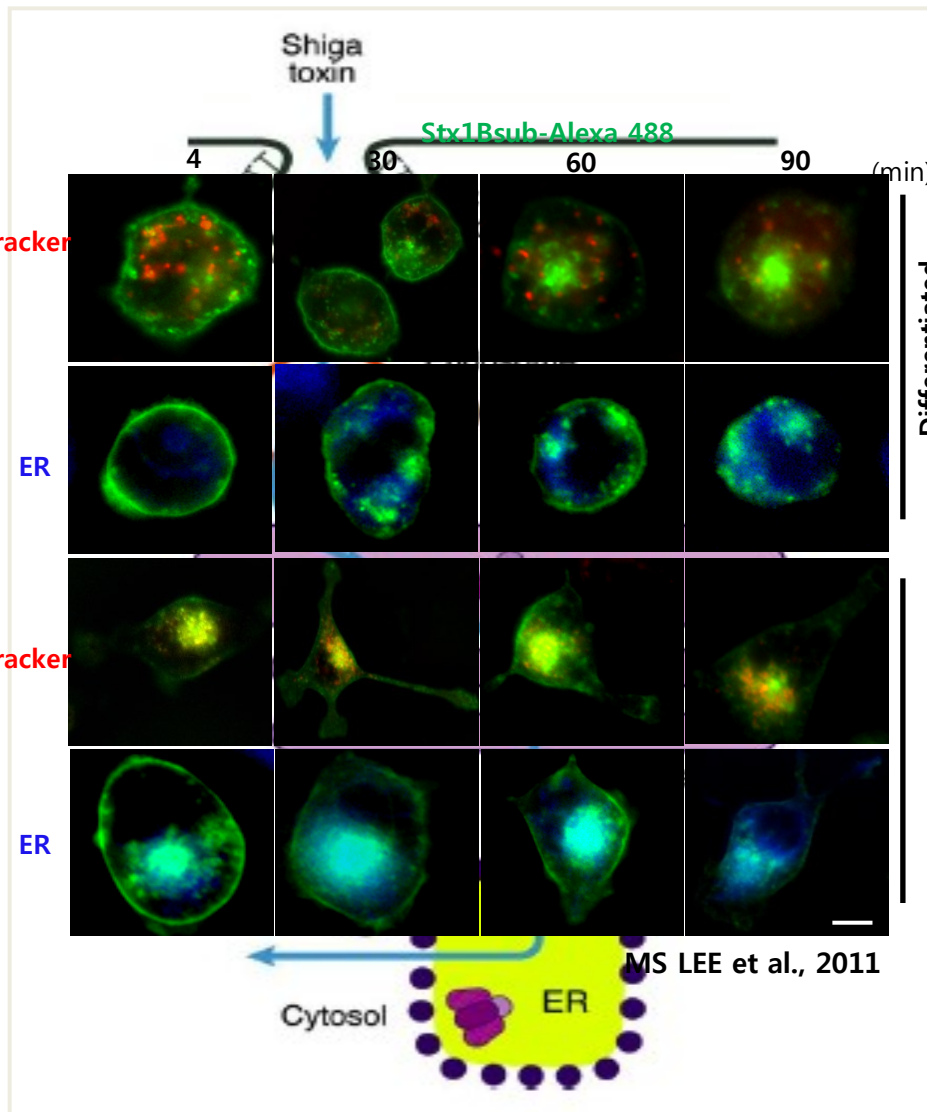
Structure of the Toxin





INTRODUCTION

Shiga Toxin



Internalization

Retrograde transport
(Golgi to ER)

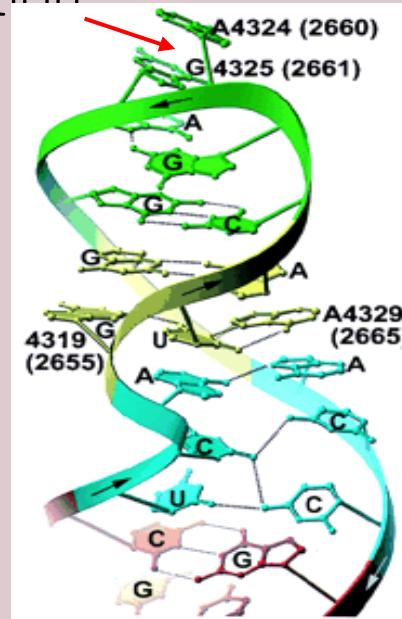
Cleave single adenine
residue
(From 28rRNA)



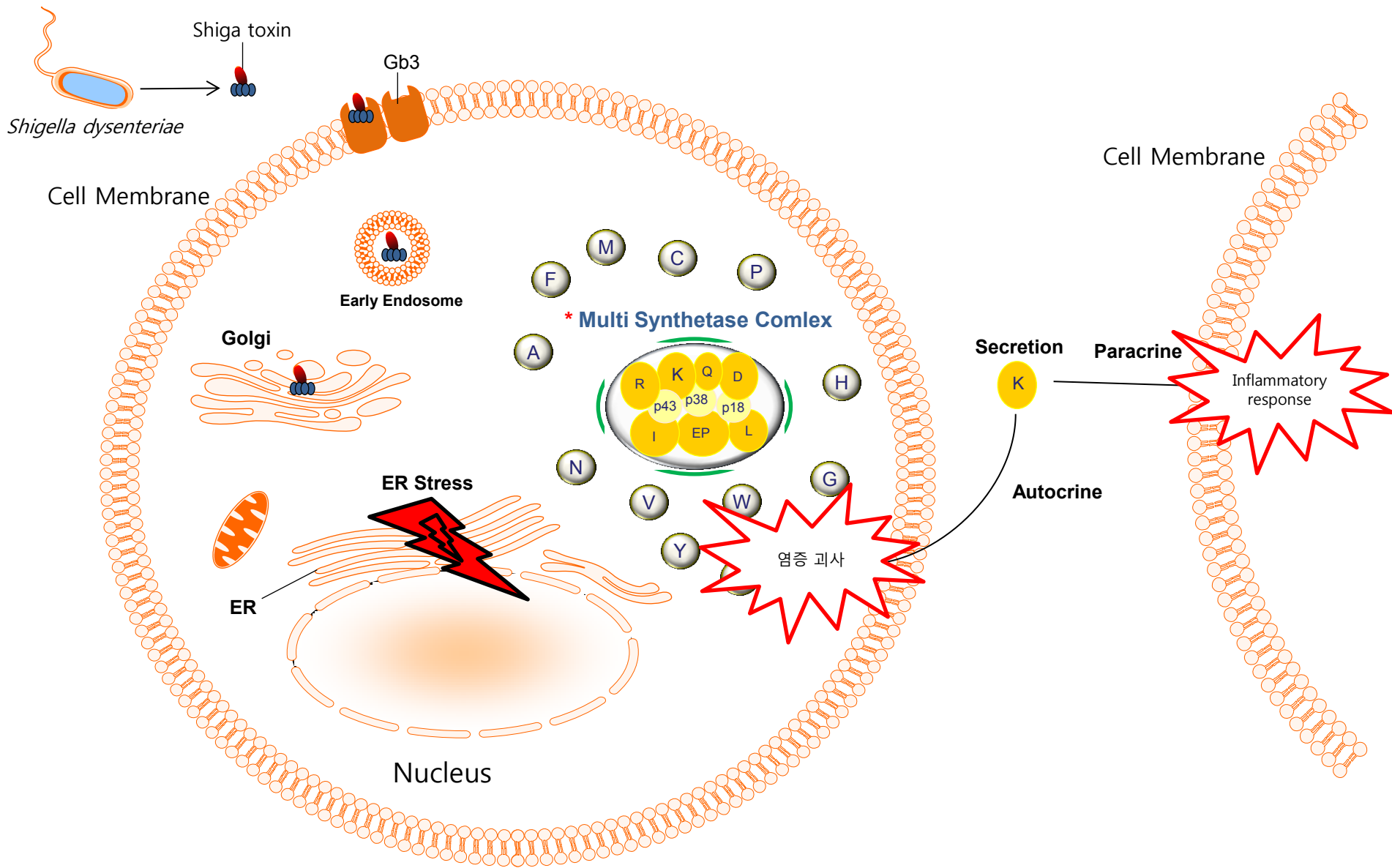
INTRODUCTION

- Protein synthesis inhibitors

Remove single adenine from ribosomes to block peptide elongation



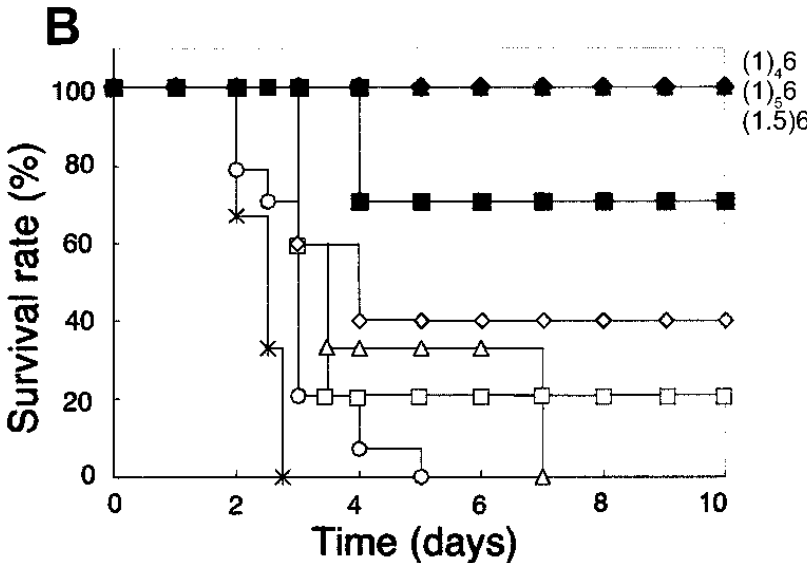
병원성독소의 인체세포내 침투경로



Overview of Therapeutic interventions against Stxs

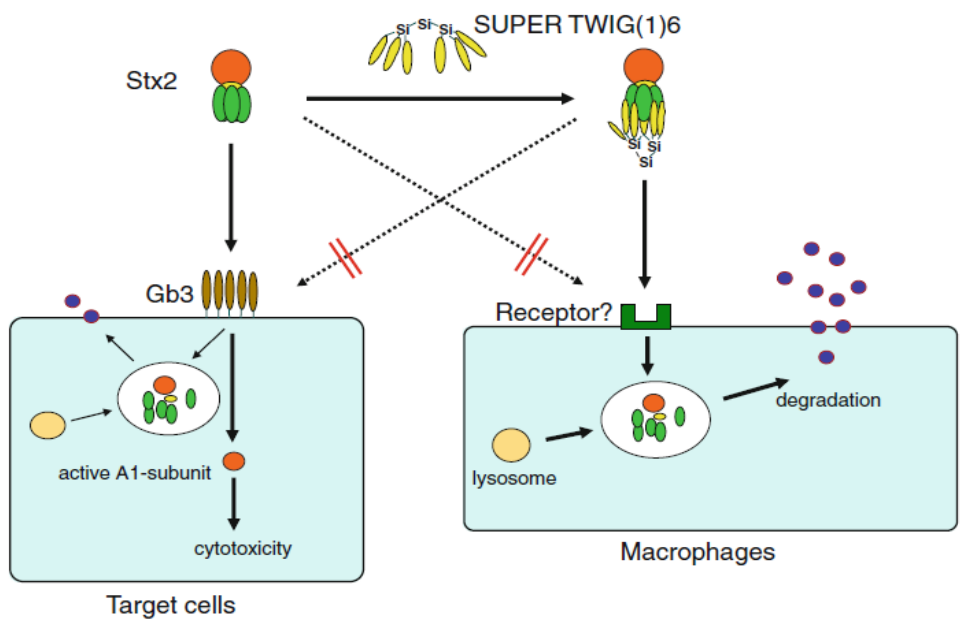
- **No toxin-specific therapy available**
 - **Azithromycin, Ceftriaxone, Ciprofloxacin** may reduce shigelosis severity.
1. **Synsorb-Pk : analogue Gb3 membrane bound synthetic material, Phase I**
 2. **STARFISH**
 3. **Diuretics**
 4. **Peritoneal dialysis or Hemodialysis (kids)**
 5. **Exo2 : blocker of the toxin-trafficking**
 6. **Cell-permable peptides**

마우스실험 : Stx-neutralizer

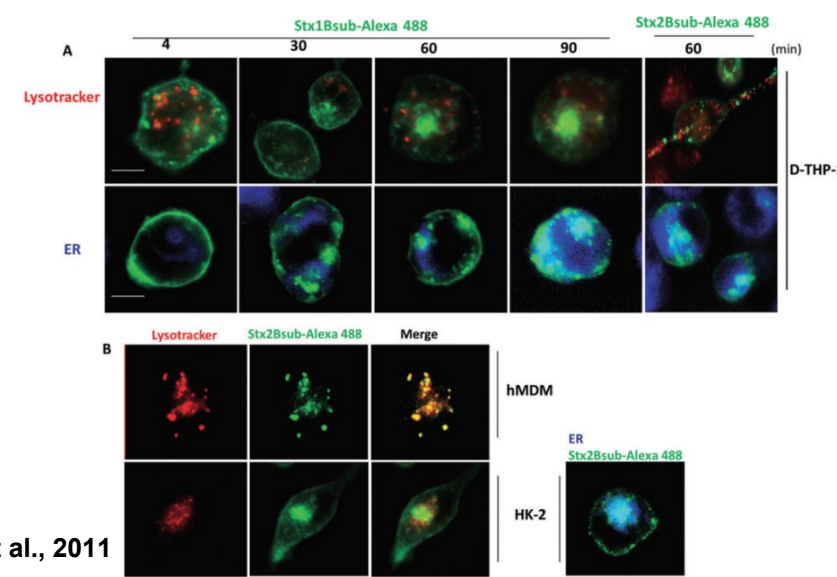
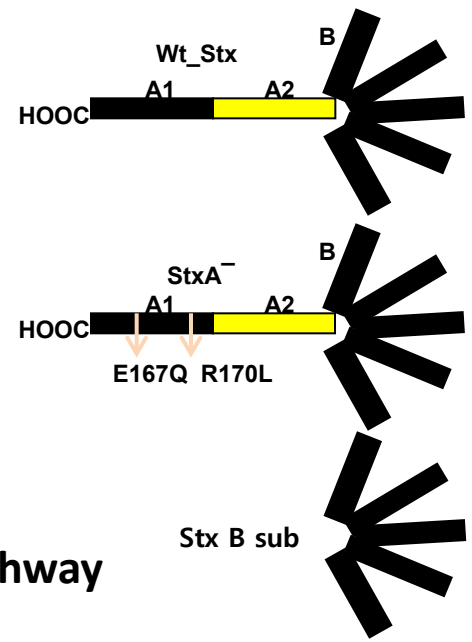
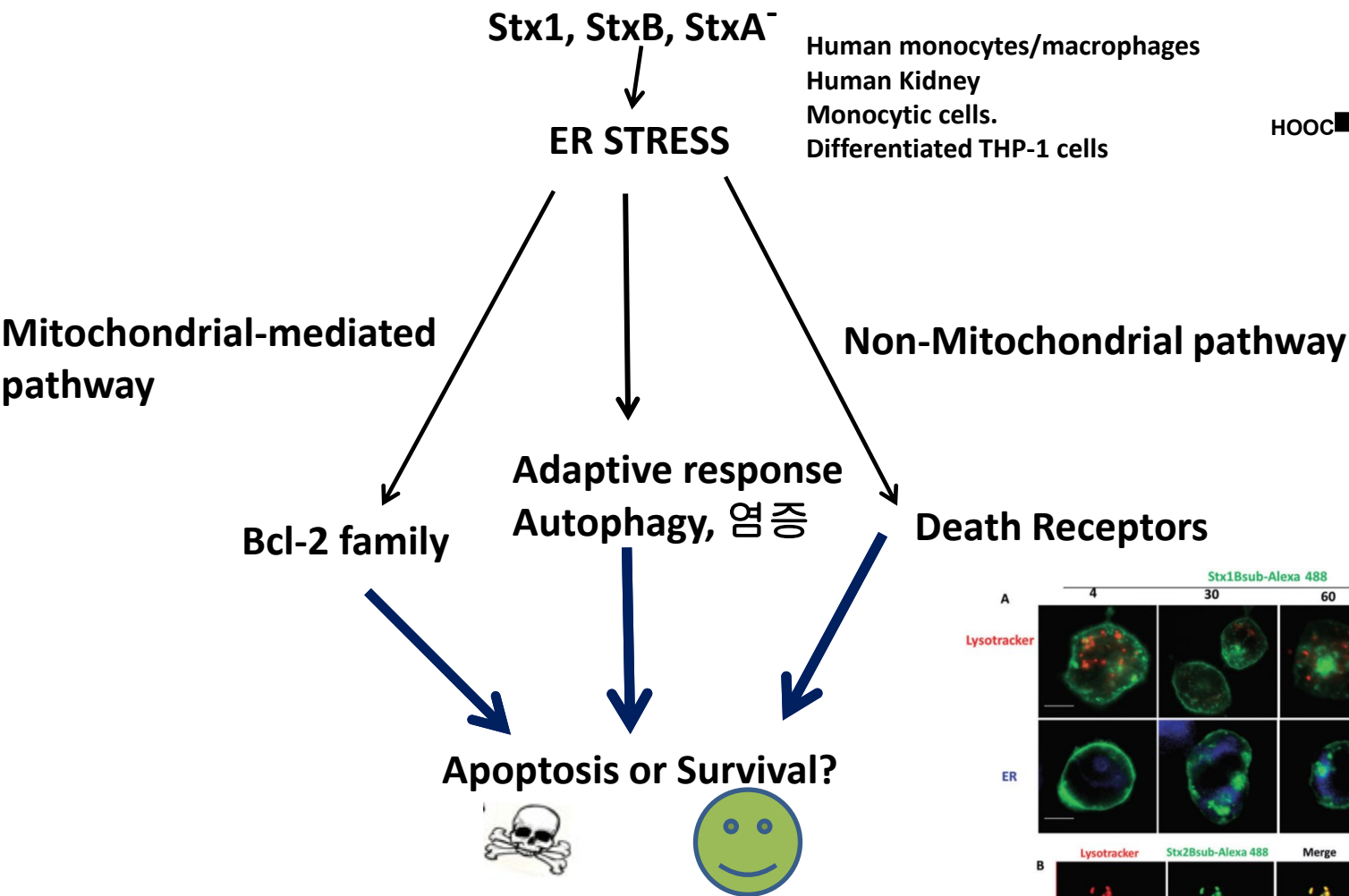


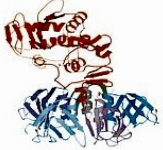
SUPER TWIG	M_r	Trisaccharide (mmol/g)
(1)2	1490	1.34
(1)3	2145	1.40
(1)5	3382	1.48
(1) ₂ 6	3973	1.51
(1) ₄ 6	4029	1.49
(1) ₅ 6	4057	1.48
(1.5)6	4101	1.46
(2)6'	4574	1.31

Kiyotaka Nishikawa et al., 2005 JID

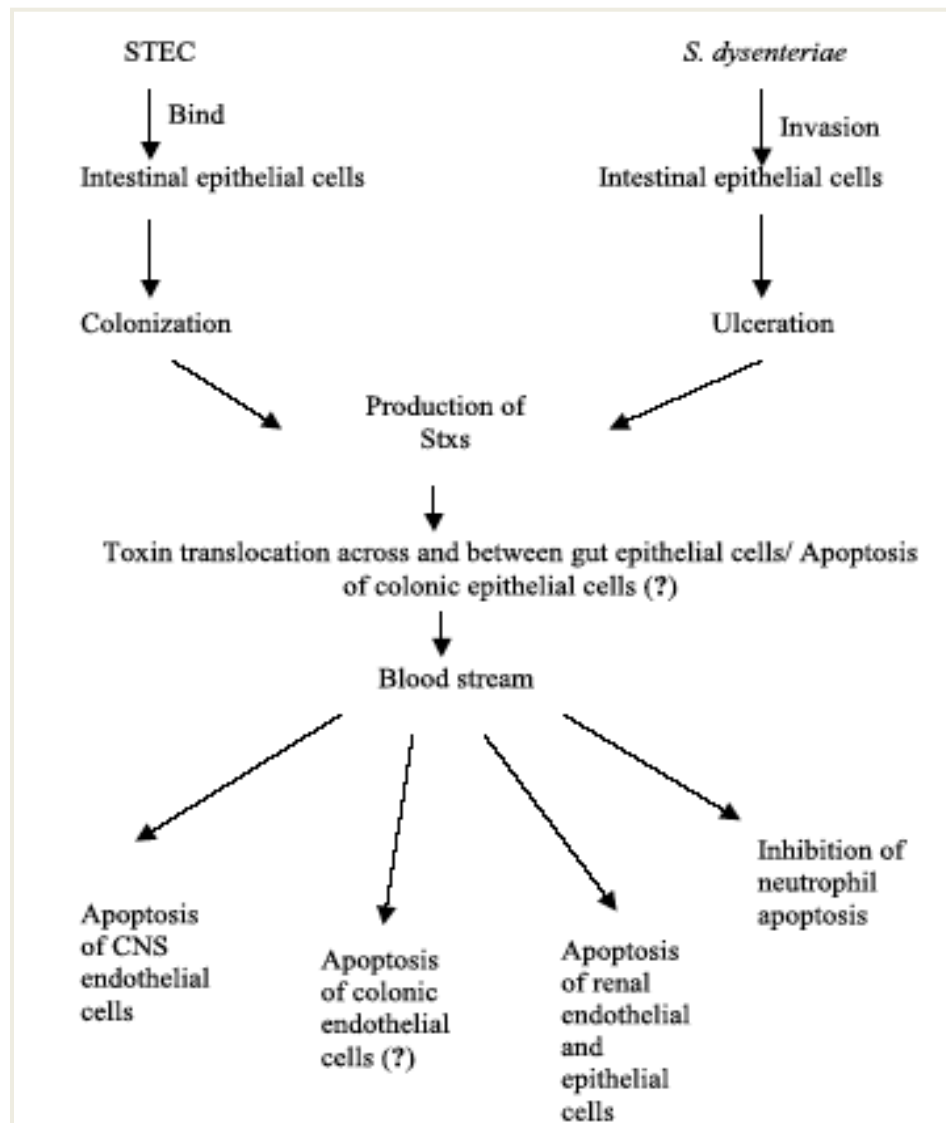


Host responses and defensive signals to Stxs





INTRODUCTION



Inward et al(1995) Vero cells

Kojio et al(2000) THP-1 cells

Fujii et al(2003) Hela cells

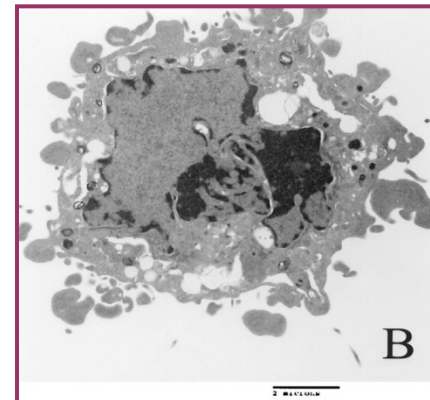
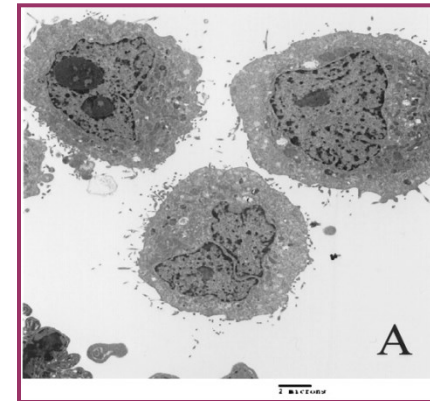


INTRODUCTION

Apoptosis

Apoptosis

- ✓ Programmed cell death
- ✓ Cytoplasmic condensation
 - ✓ Membrane blebbing
 - ✓ Loss of cell adhesion
- ✓ Fragmentation of chromosomes
- ✓ Driven by activity of caspases





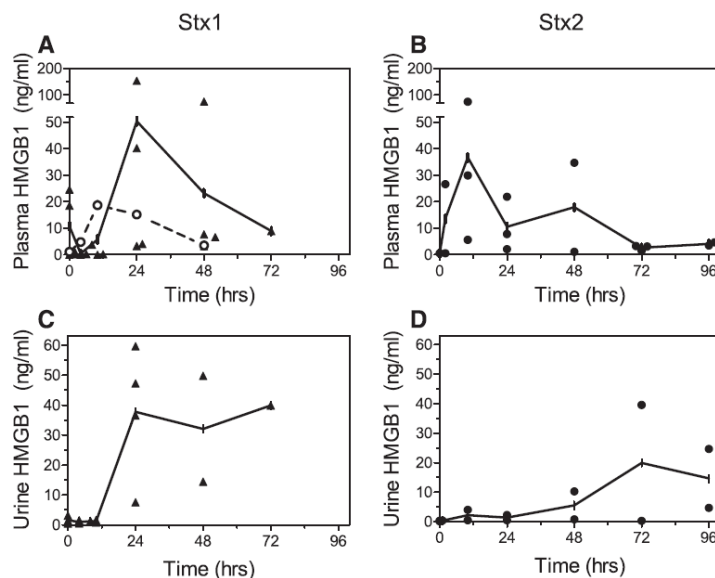
Quiescent complement in nonhuman primates during *E coli* Shiga toxin-induced hemolytic uremic syndrome and thrombotic microangiopathy

Benjamin C. Lee, Chad L. Mayer, Caitlin S. Leibowitz, D. J. Stearns-Kurosawa and Shinichiro Kurosawa

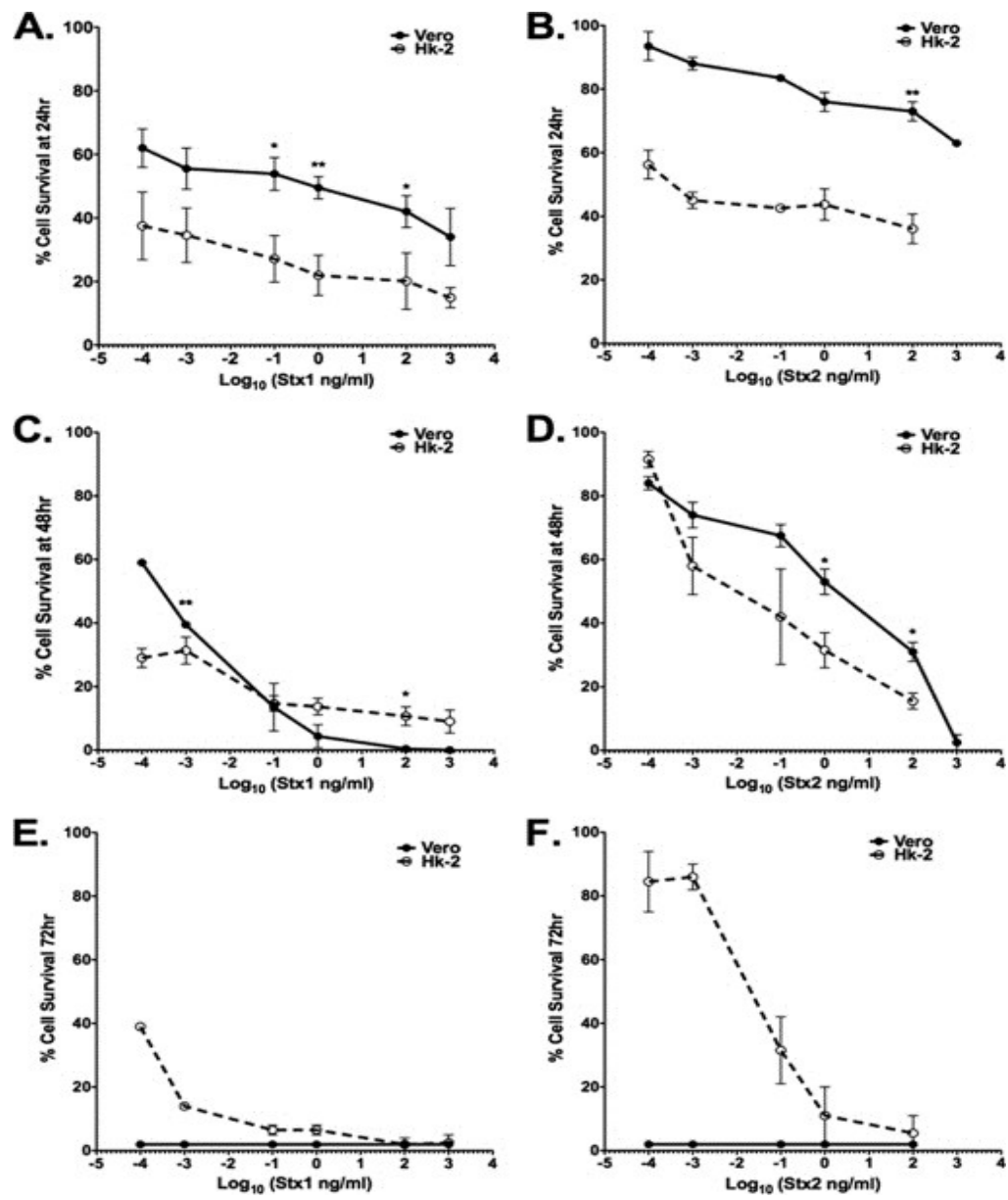
Cell injury markers.

Key Points

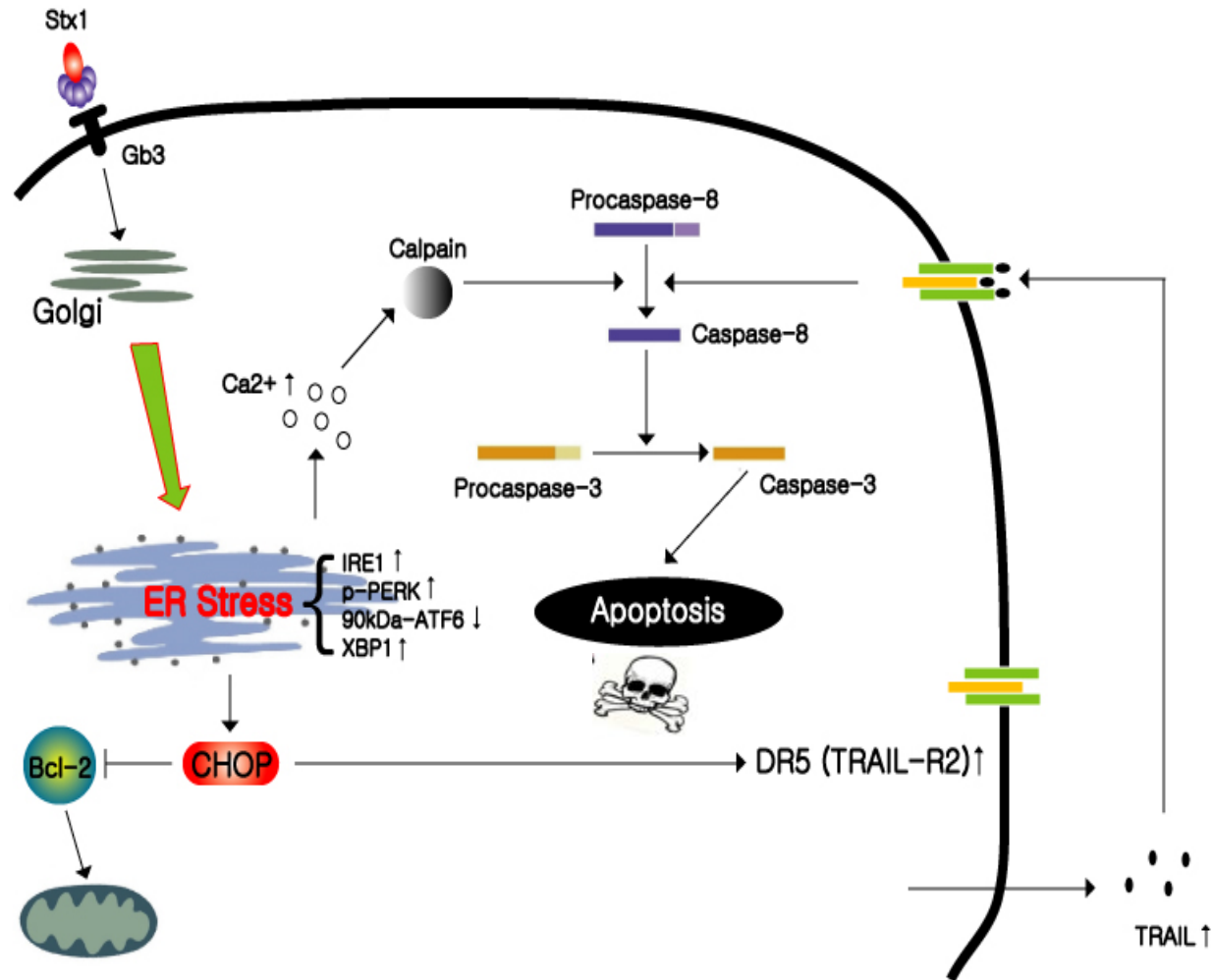
- Complement activation is not required for development of thrombotic microangiopathy and HUS induced by EHEC Shiga toxins in nonhuman primates.
- Complement is an important defense mechanism, and benefits or risks of therapeutic inhibition should be studied further for this infection.



대장균시가독소에 의한 인간신장세포의 괴사



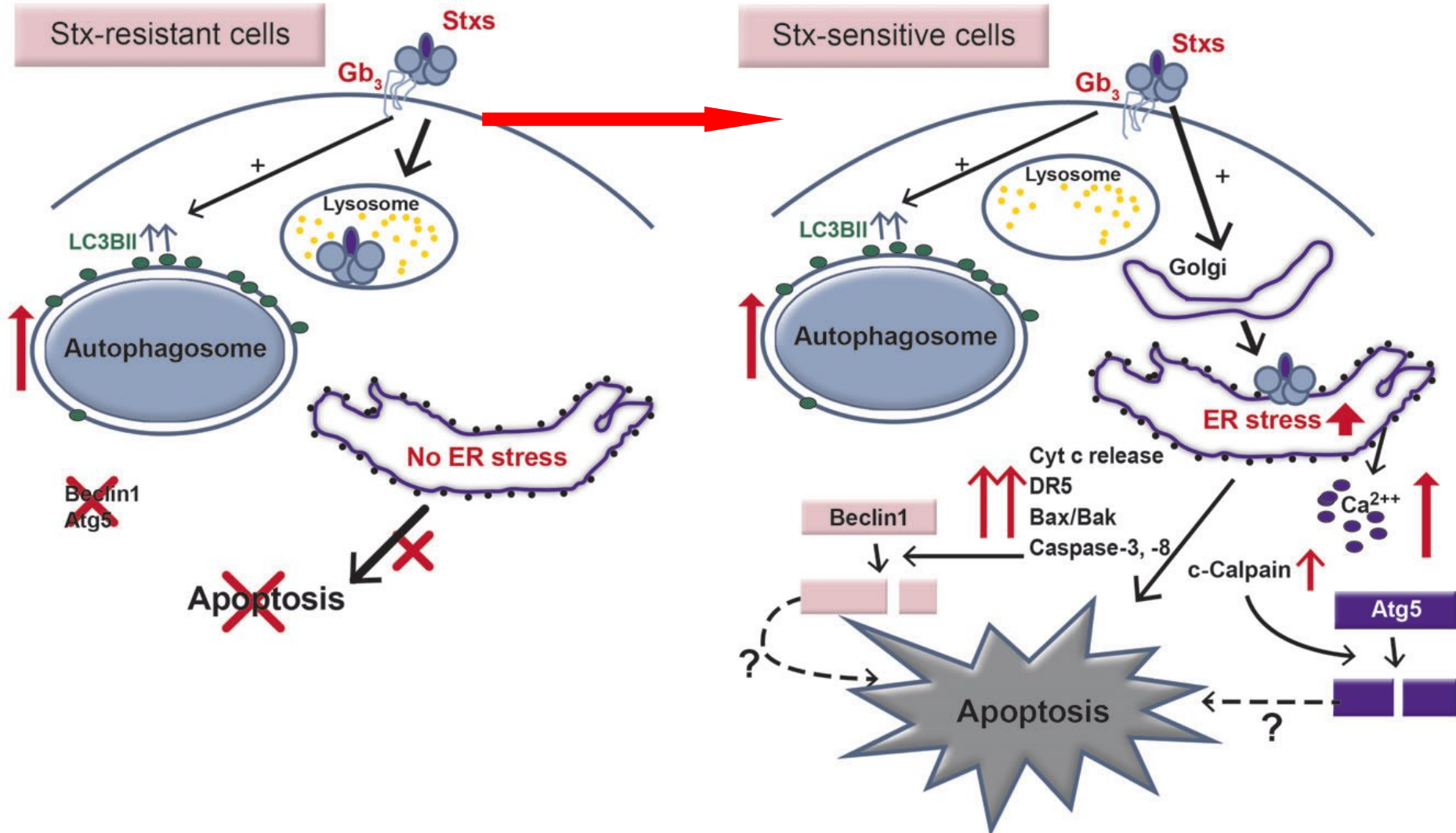
Monocytic cells : Stx1 induces apoptosis via ER stress



Sang-Yun Lee, Moo-Seung Lee, Rama P. Cherla and Vernon L. Tesh (2008) *Cellular Microbiology*

Proposed model of stress-activated cytotoxic signals by Stxs in toxin-resistant versus toxin-sensitive cells

Converting **pro-survival signal** to autophagic **death signal** by pathogenic bacterial Shiga toxin



SY Lee, M.S. Lee *et al.*, 2008, *Cell. Micro.*

M.S. Lee *et al.*, 2010, *IAI*.

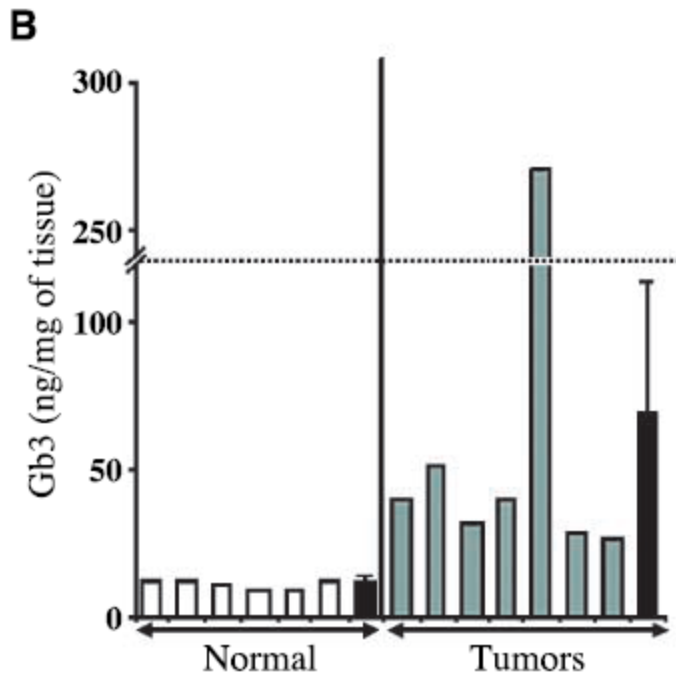
MS Lee *et al.*, 2011, *Cell.Micro.*

Gb₃ overexpressed in cancer cells: found it's very sensitive to the purified Shiga toxin. (Johansson *et al.*, 2009)

Shiga toxins — from cell biology to biomedical applications

MDR (P-gp)에 의한 항암치료 효능 감소, 약물에 대한 저항성 증가

1. 기존의 화학약물에 대해 efflux
2. 저항성이 강해진 악성종양에 대한 특이적인 biomarker를 통한 치료필요
3. Advanced tumor 치료 가능.
4. Targeting. Shiga toxin 자체가 Multi-drug resistance인 종양에 효과입증 ([Proulx et al., 2001](#); [Tarr et al., 2005](#); [Palermo et al., 2009](#))



Cancer Research, Johanness 2006

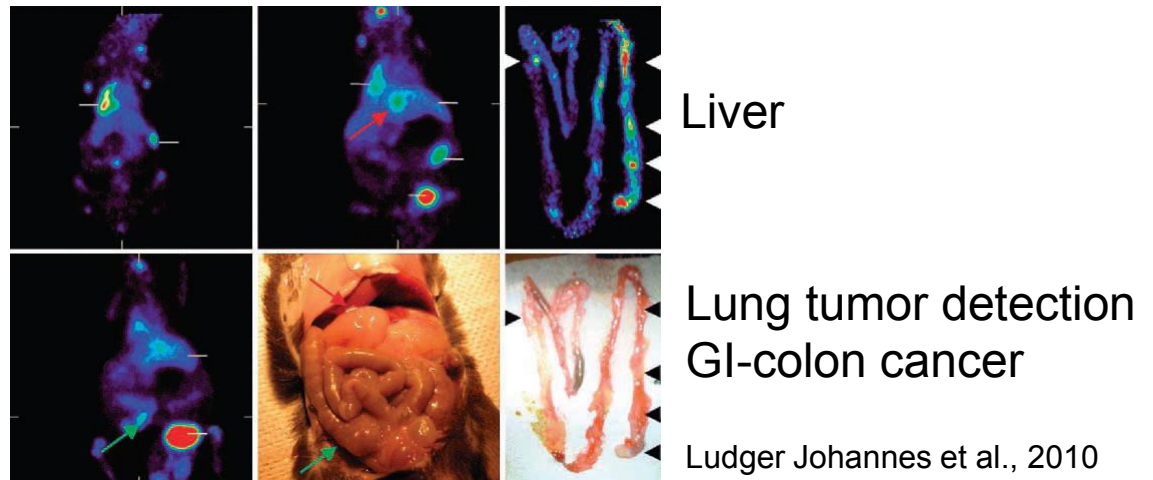
Perspectives of disease early detection

Shiga toxin as medical tool:

- Using non-toxic B subunit, it can be engineered for solid tumor targeting(TF)
- TRAIL would be clinically effective for combination cancer therapy - *in vivo* delivery of contrast agents to tumors(tumor detection), molecular imaging applications such as noninvasive PET

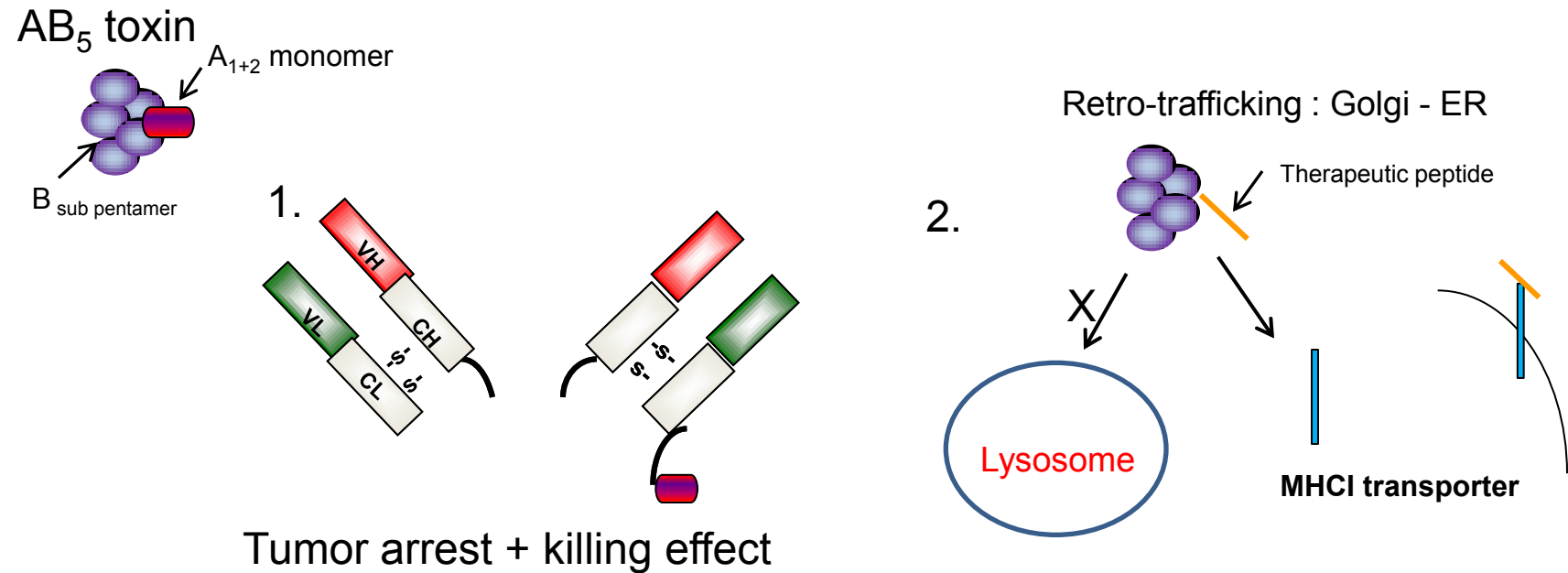
Shiga toxin-receptor as cancer biomaker:

- Toxin receptor, glycosphingolipid Gb3, which is highly expressed on human cancers and Inflammatory „Biomarker“ during early phase of the infections in nonhuman primates



Applications of utilizing the AB₅ toxin as powerful cytotoxic protein.

1. Generation of format to kill tumor: Site-specific conjugation, Fusion for chimeric protein
2. Powerful tools of therapeutic peptides delivery by utilizing StxB (T세포에 의한 살상효과 극대화)



Shiga toxin A₁ : N-glycosidase activity (Lingwood 1999 Nature)

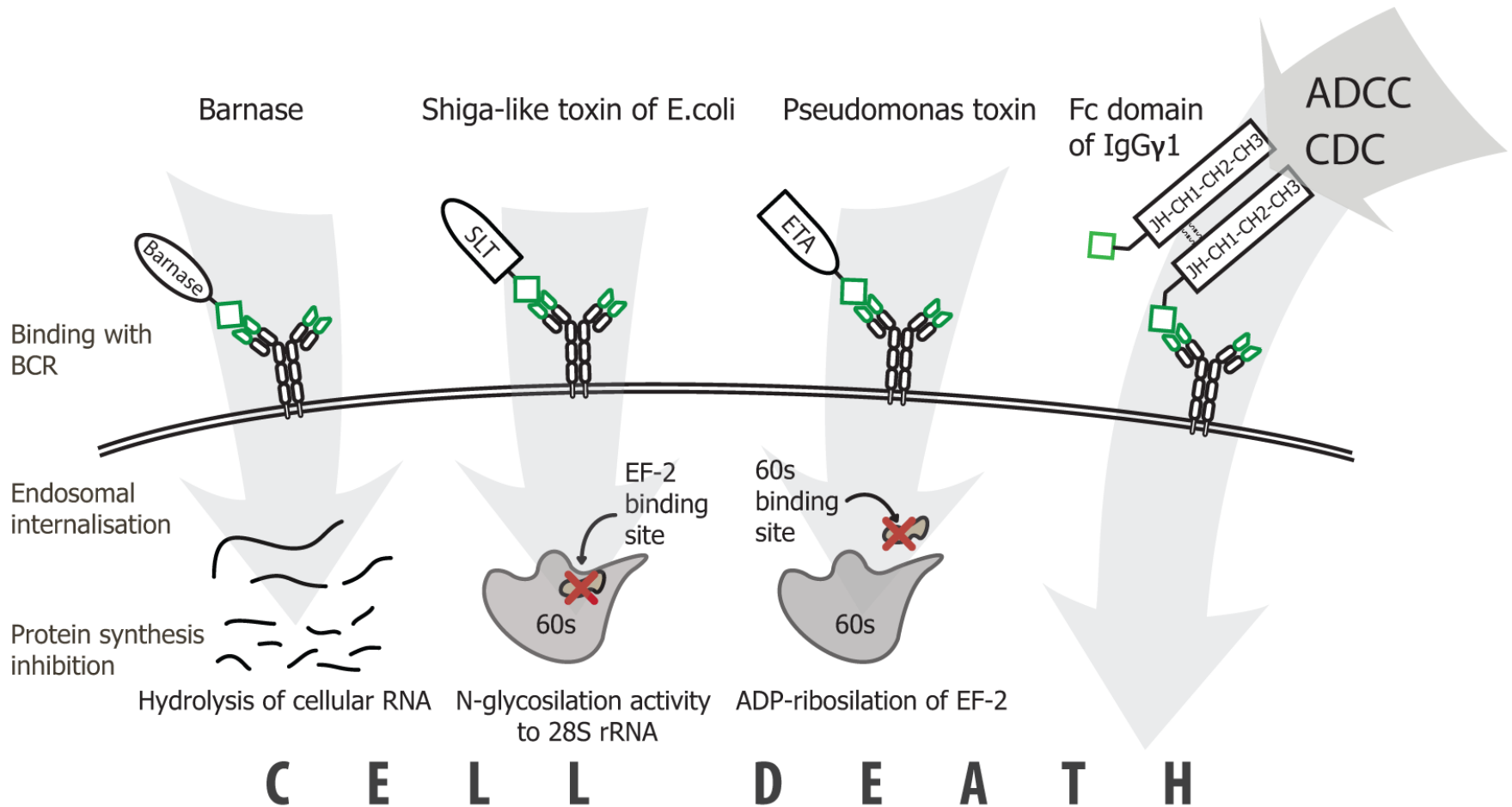
Subtilase : Charperone BiP cleavage-mediated target cell killing (Paton et al., 2006 Nature)

B-subunits bind Gb3 on target cells with high specificity followed by retro-trafficking.

Irreverisible cytotoxic effect with AB₅ toxin-toxic genetic fusion chimeric proteins

왜 장점인가? A subunit은 강력한 표적세포 살상작용 (new immunotoxin)

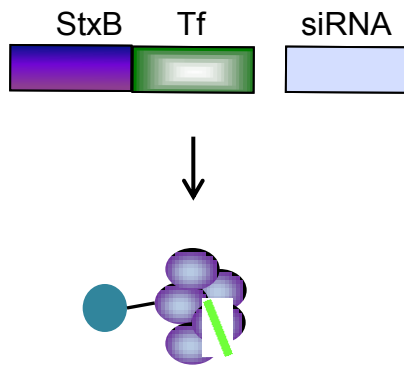
B subunit은 lysosome을 피해가는 유용한 natural 전달체.(gene therapy)



Stx B subunit 혹은 AB₅ type의 비독소부분이 가지고 있는 약물전달 잠재성 장점.

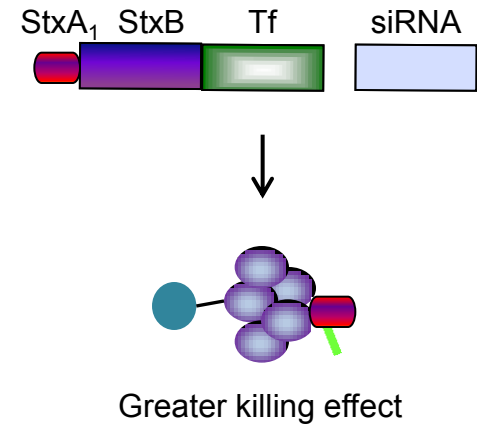
장점 : 극한의 pH와 세포내 **protease** 공격에서 안정성이 큼
약물 전달 시 발생하는 세포 내.외의 약물비활성신호체계에 저항성 강함
Immunogenecity를 발생시키지 않음.
독성이 전혀 없는 **Toxin**의 한 부분체 이므로 안정성 큼.
Endosomal low pH한 환경에서 구조와 성질에 영향 없이 전달됨.

1차 테스트 : StxB의 전달체로서의 적합성



전신투여 잠재성
Gb3에 binding 하는 StxB의 능력 억제
: 좀더 높은 specificity, delivery

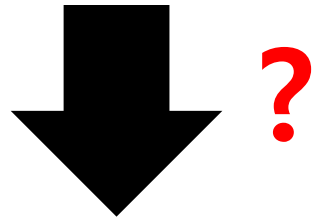

Aptamer?



성공시 유전자 치료제의 약물전달능력 향상기대

Challenge!

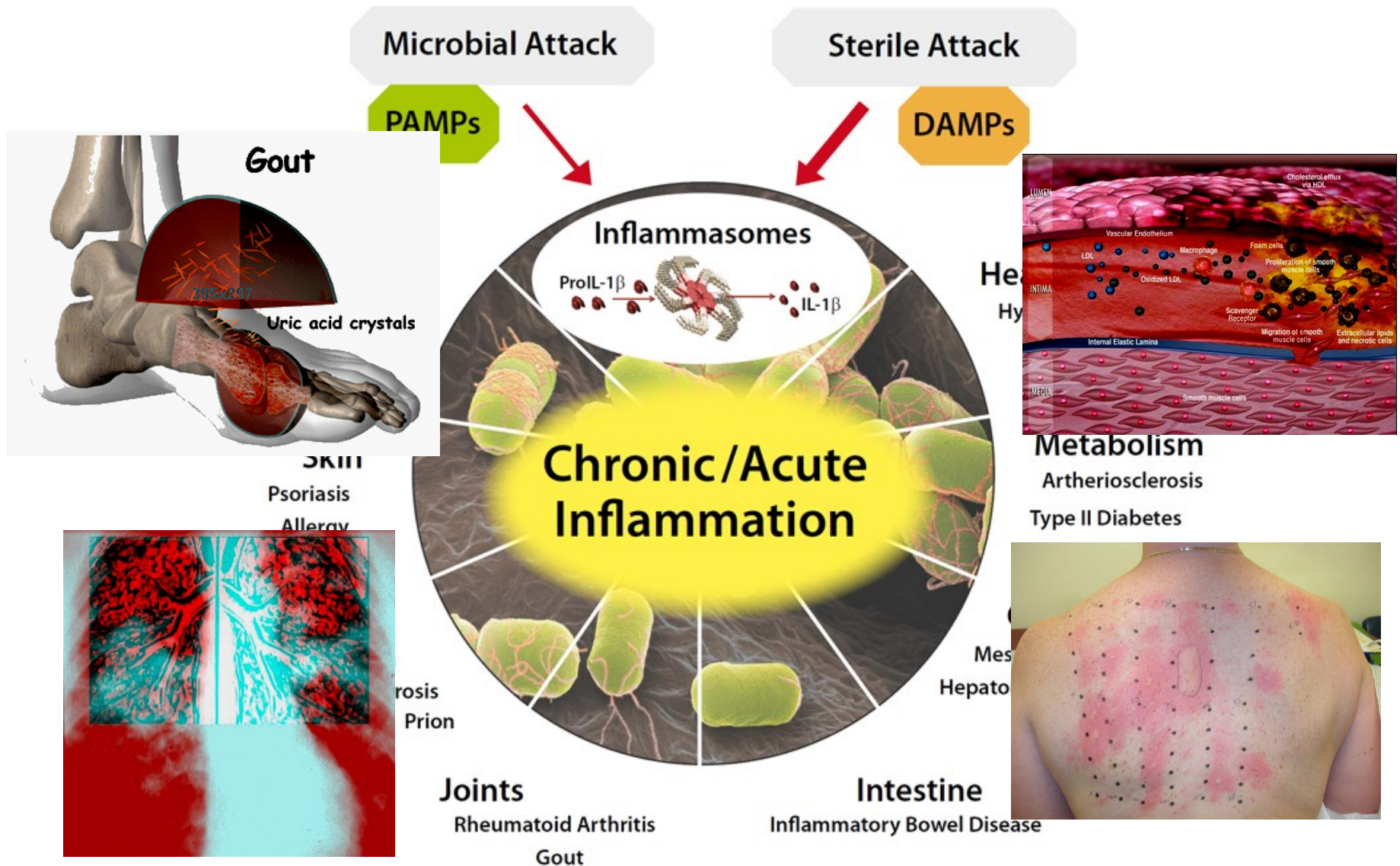
Shiga toxin type 1 or Shiga toxin type 2



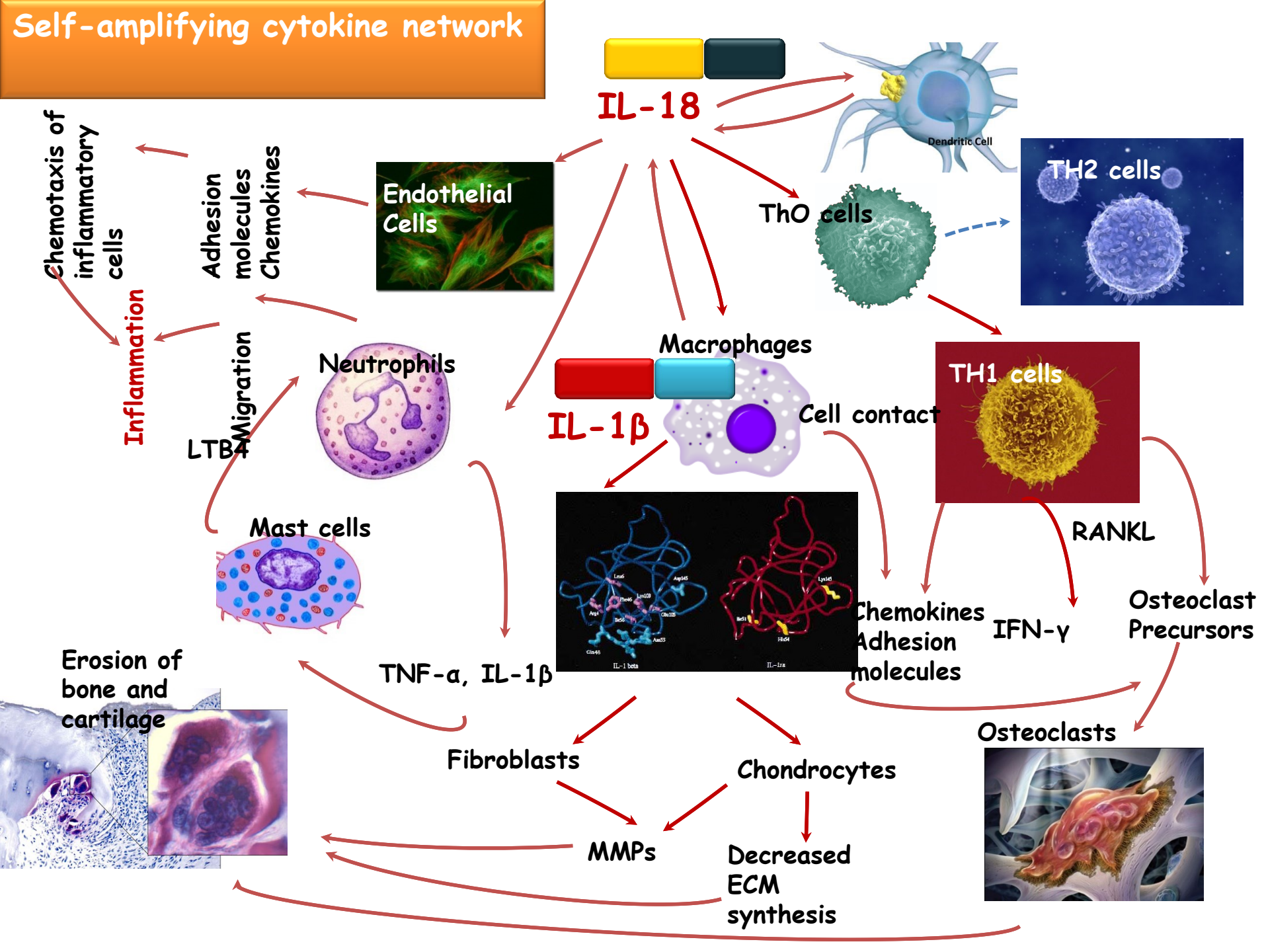
**Pro-inflammatory response
vs.**

Anti-inflammatory response

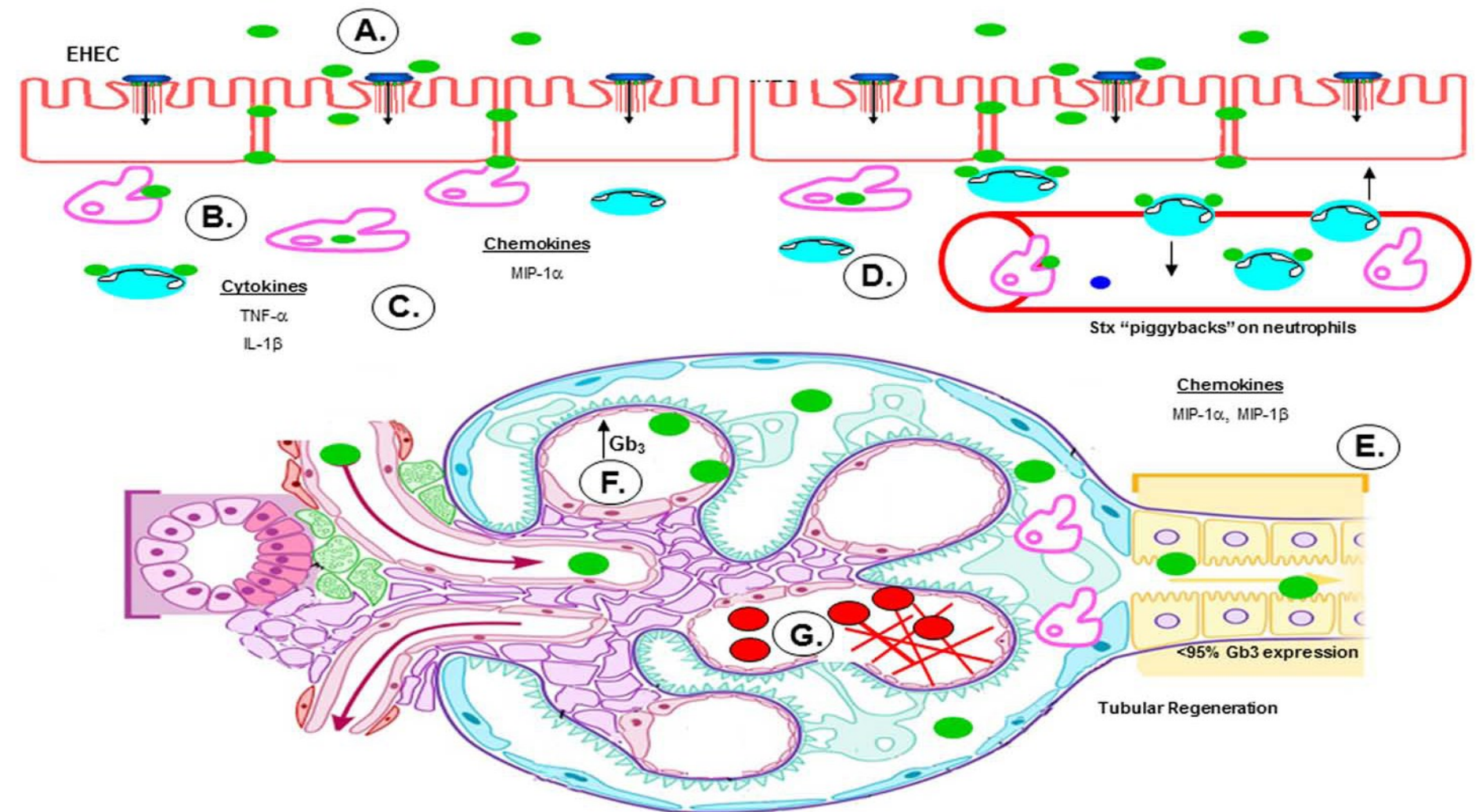
Inflammasome and Diseases



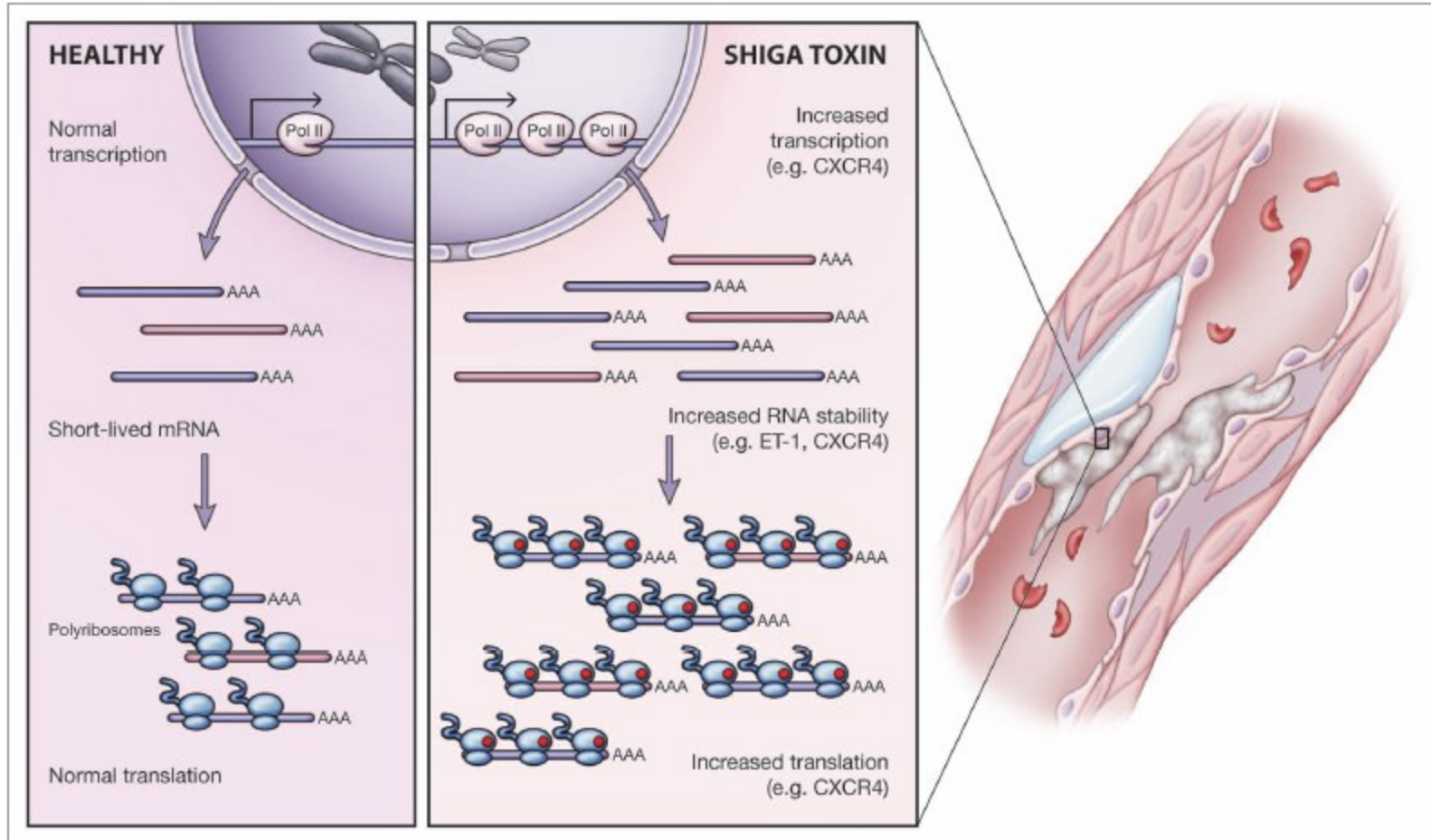
Self-amplifying cytokine network



Potential role of human proximal tubule epithelial cells in the pathogenesis of the Hemolytic Uremic Syndrome.

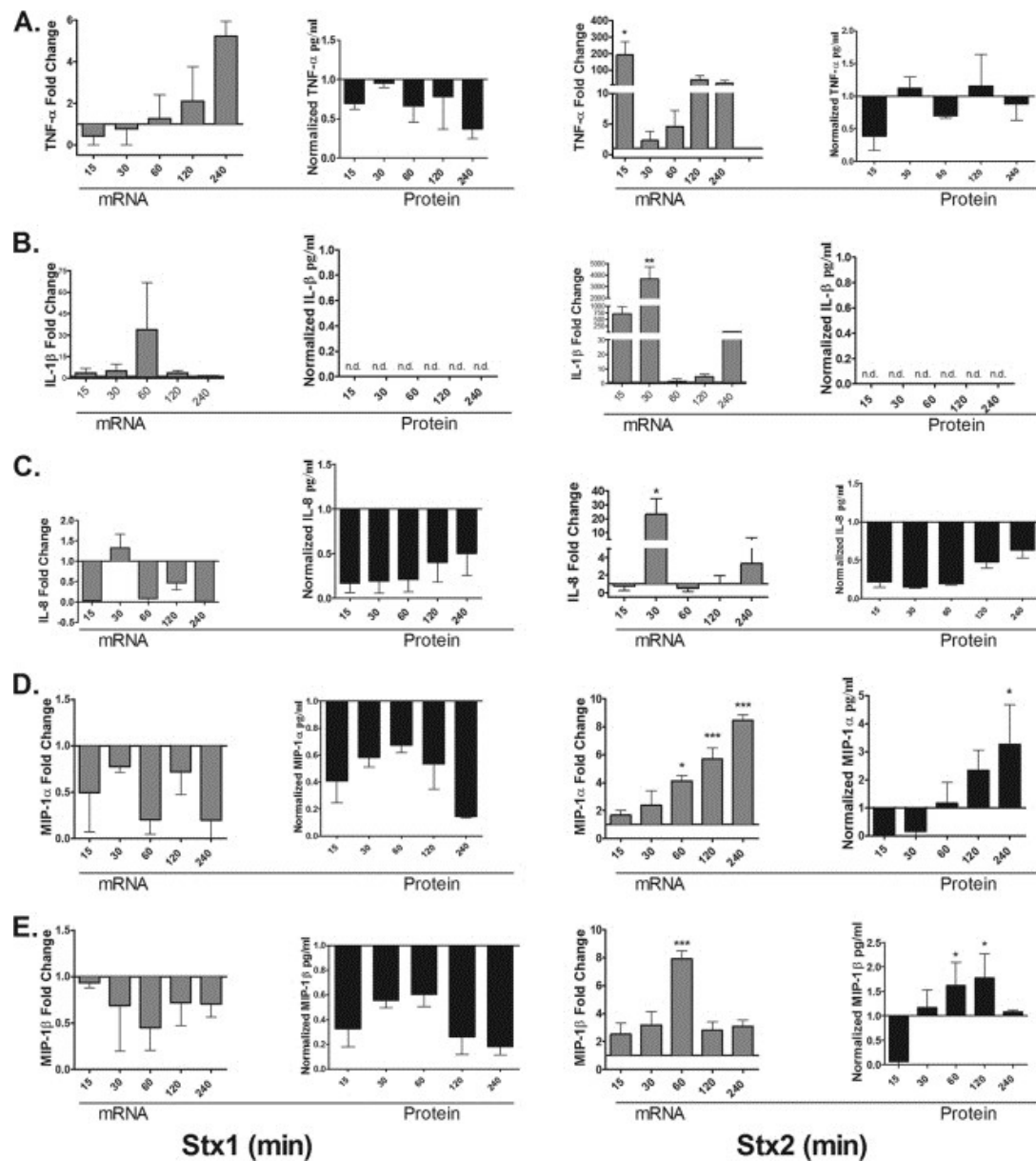


독소 체내이동시 혈관의 염증 증가 및 염증성사이토카인으로 인한 손상.

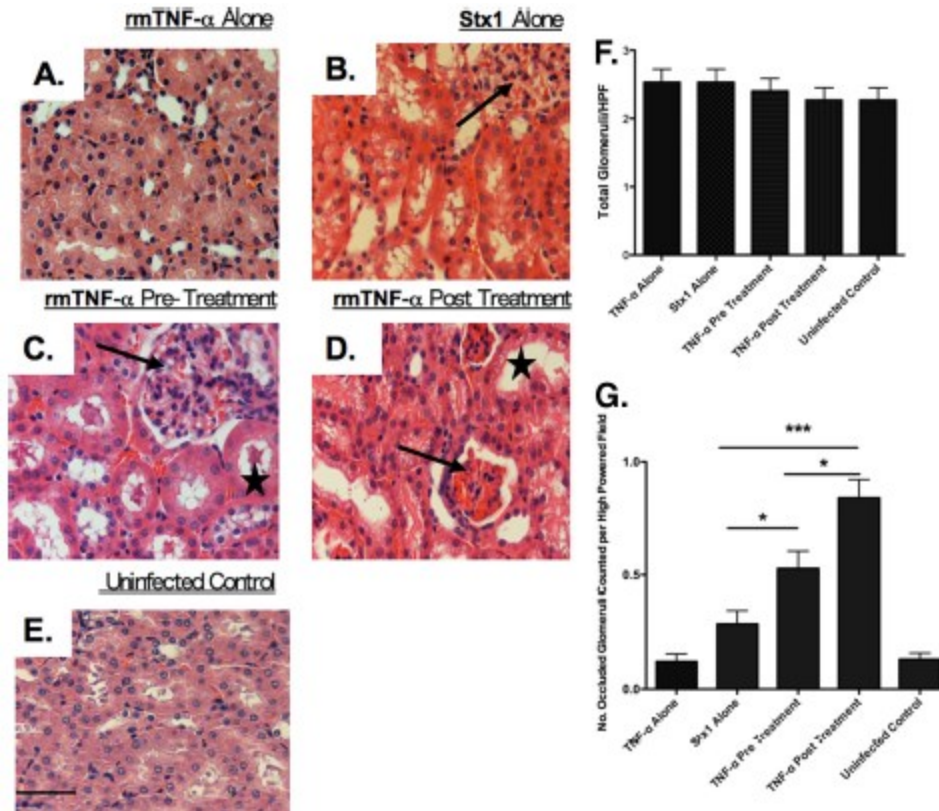


Tania N Petruzzello-Pellegrini

Virulence 4:6, 556–563; August 15, 2013; © 2013
Landes Bioscience



TNF- α administration to mice after intoxication alters renal histopathology



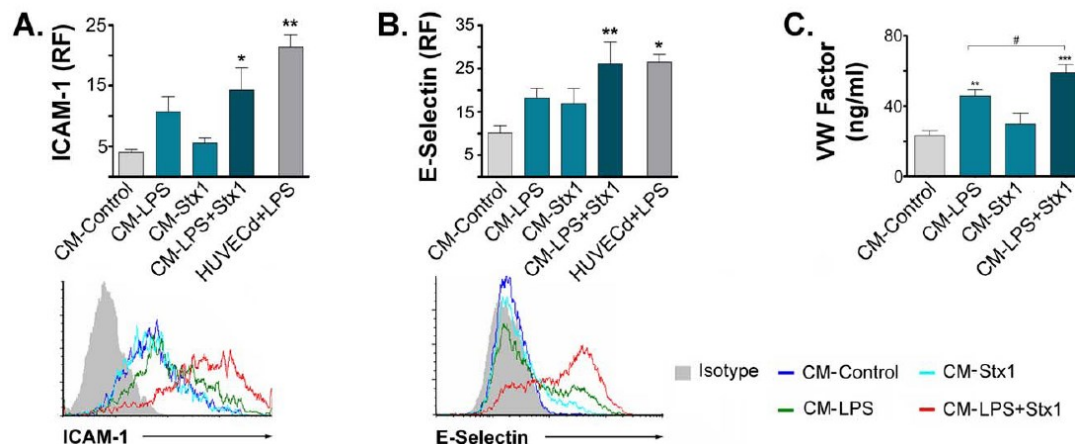
Confirmed by TUNEL

Lentz et al., *Infect Immun* 78:3689-3699 [2010]

Shiga Toxin 1 Induces on Lipopolysaccharide-Treated Astrocytes the Release of Tumor Necrosis Factor- α that Alter Brain-Like Endothelium Integrity

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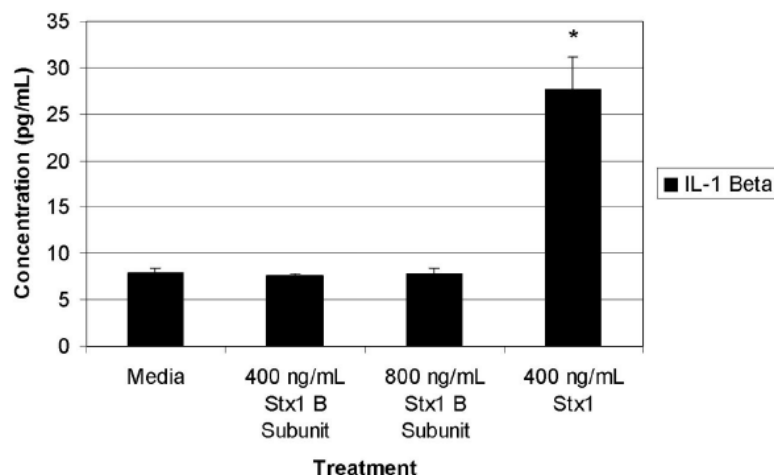


Regulation of Proinflammatory Cytokine Expression by Shiga Toxin 1 and/or Lipopolysaccharides in the Human Monocytic Cell Line THP-1

Lisa M. Harrison, Wilhelmina C. E. van Haaften, and Vernon L. Tesh*

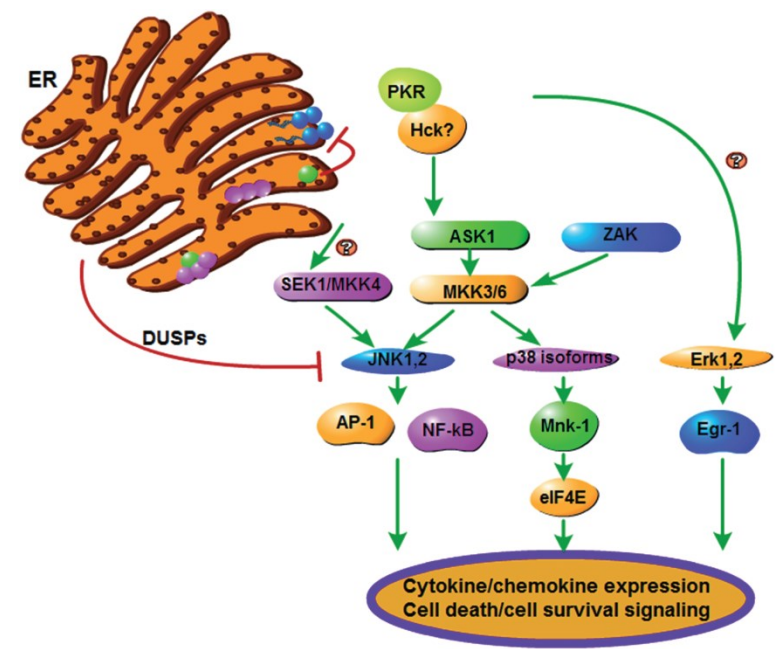
Department of Medical Microbiology and Immunology, Texas A&M University System Health Science Center, College Station, Texas 77843-1114

Received 20 October 2003/Returned for modification 6 January 2004/Accepted 2 February 2004

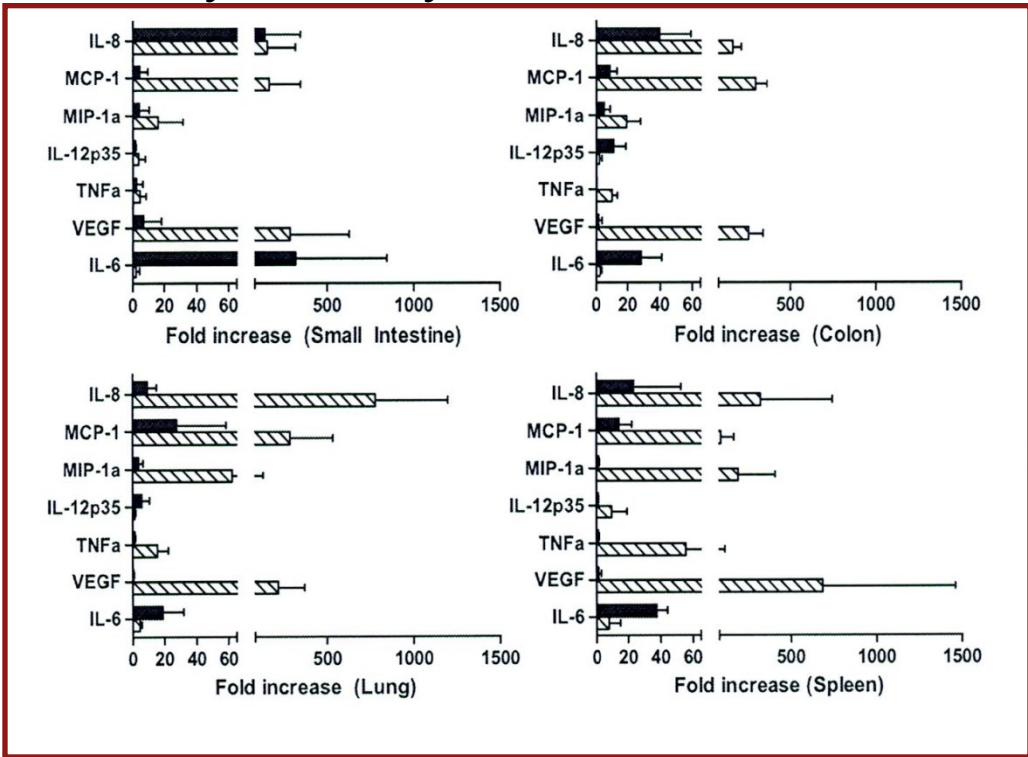


“Whether Stxs similarly affect inflammasome function and posttranslational processing mechanisms necessary for IL-1 secretion remains to be clarified..”

Shiga toxin is capable of activating MAPKs in host



Cytokine analysis from baboon tissues



Vernon Tesh 2012, *Cell.Micro.*
 Leyva-Illades and Lee *et al.*, 2012 IAI

Stearns-Kurosawa et al., 2013

The American Journal of Pathology, Vol. 182, No. 4, April 2013



CARDIOVASCULAR, PULMONARY, AND RENAL PATHOLOGY

Distinct Renal Pathology and a Chemotactic Phenotype after Enterohemorrhagic *Escherichia coli* Shiga Toxins in Non-Human Primate Models of Hemolytic Uremic Syndrome

Deborah J. Stearns-Kurosawa,* Sun-Young Oh,* Rama P. Cherla,† Moo-Seung Lee,† Vernon L. Tesh,† James Papin,* Joel Henderson,* and Shinichiro Kurosawa*

The American Journal of
PATHOLOGY
 ajp.amjpathol.org

After Shiga toxin challenge, inflammatory cytokines upregulated in baboon HUS model.

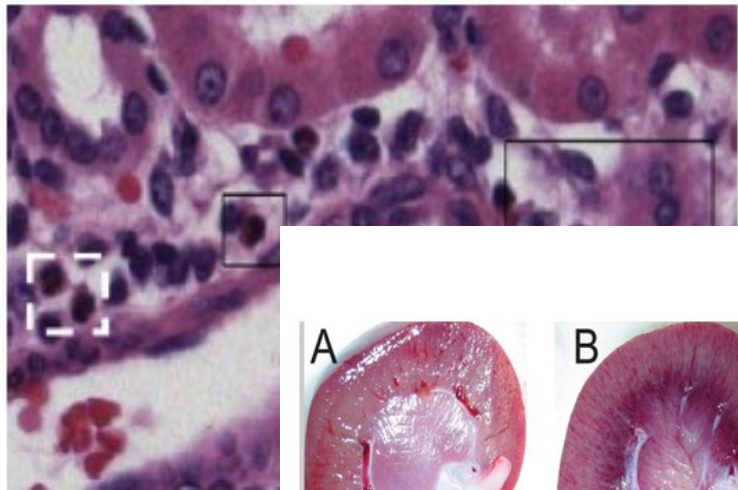
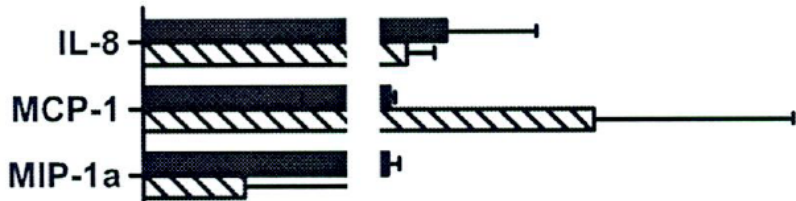
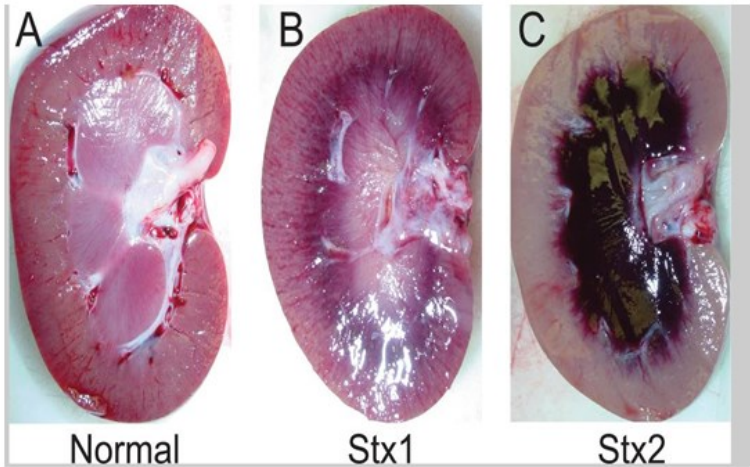


Figure 5 Eosinophilic Kidney tissue from an anim 168 hours after challenge), staining. **Black boxes:** pe eosinophils. **White box:** PI



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ase (Kidney)

etta study !

sing mechanisms

Lisa et al., 2004 IAI, "W necessary for IL-1 β secr
Thorpe et al., 2012 Curr. Top. Microbiol. " Inflammasome and host defensive signaling"

Urine biomarker in Stxs-challenged baboon : Chemokines

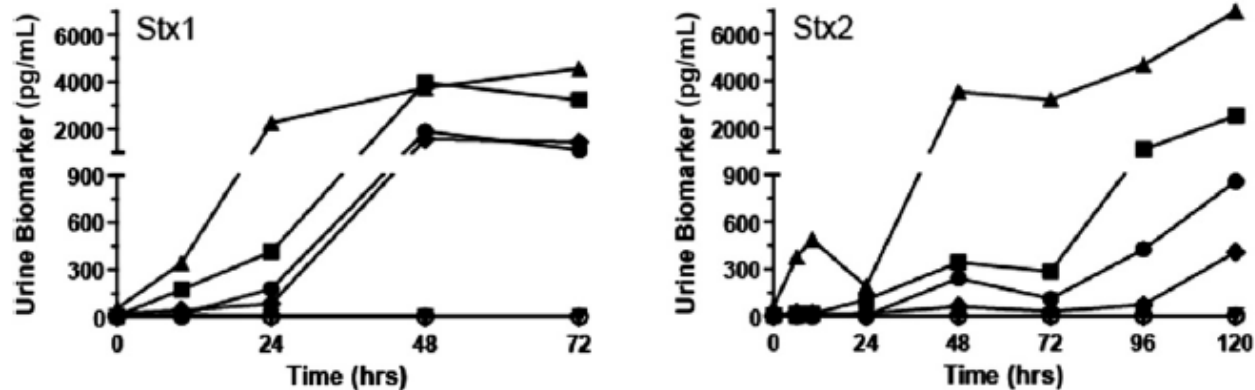
Table 3 Renal Interstitial Inflammatory Cell Infiltrates

Toxin	Dose ng/kg (No.)	Survival, average hours (range)	Mono (Range, 0–4)	Poly (Range, 0–4)
Stx1	10 (3)	168*	0 (0)	0 (0)
	50 (5)	69 (58–168)	0.20 (0–1F)	0.6 (0–2FE)
	100 (5)	57.4 (48–72)	0 (0)	0 (0)
Stx2	10 (4)	153.8 (111–168)	1.5 (0–2F)	1.0 (0–2FE)
	50 (7)	108.4 (84–128)	0.42 (0–2F)	1.3 (0–2FE)

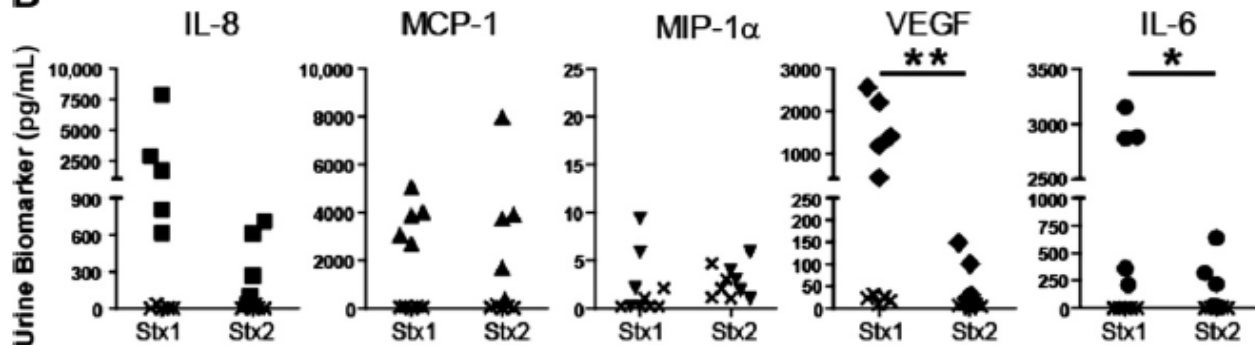
*Animal survived to day 7 (necropsy at approximately 168 hours).

E, infiltrate includes eosinophils; F, focal (otherwise diffuse); Mono, mononuclear; Poly, polymorphonuclear.

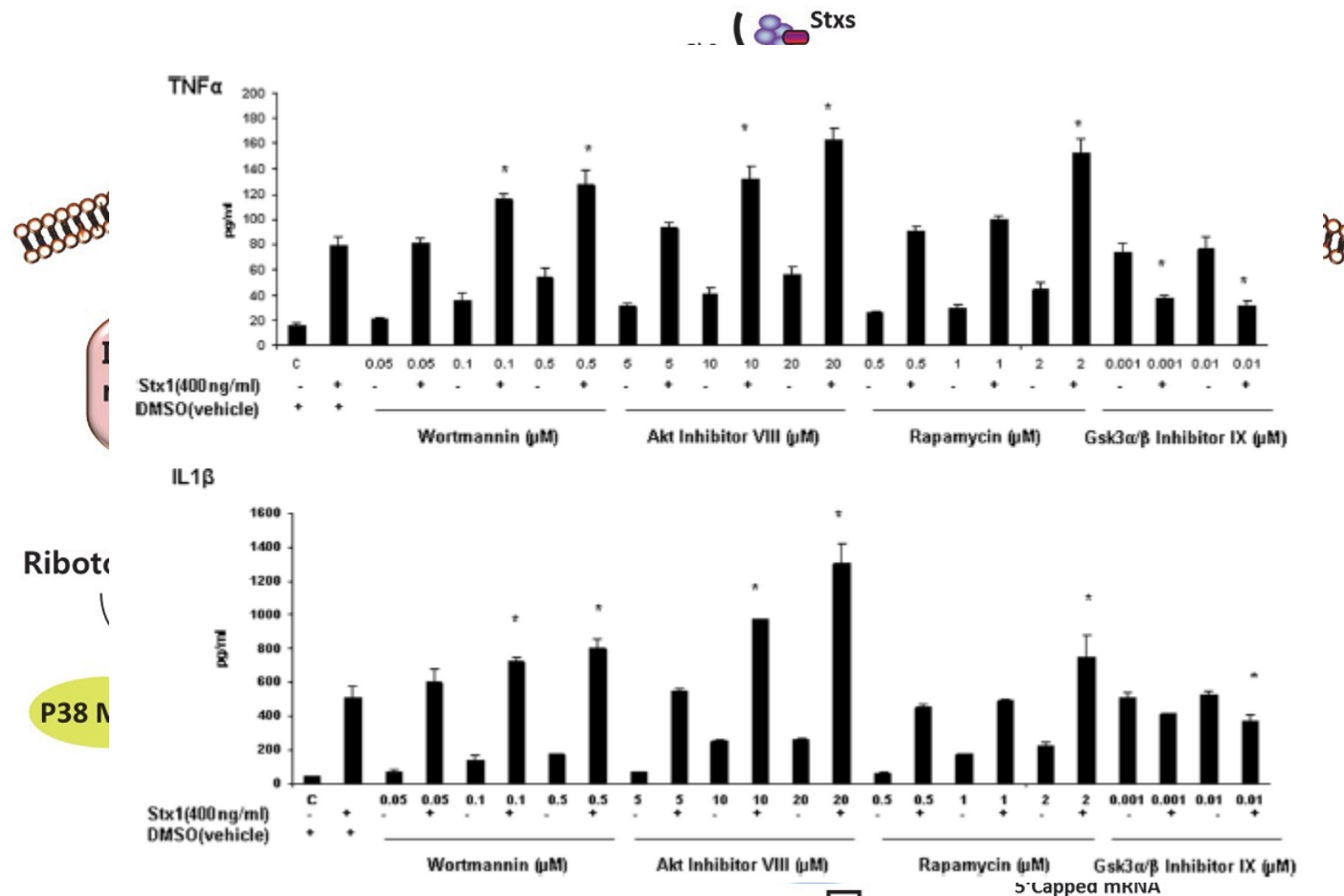
A



B



Shiga toxin 1-induced pro-inflammatory response regulated by mTOR signaling.



Pro-inflammatory responses



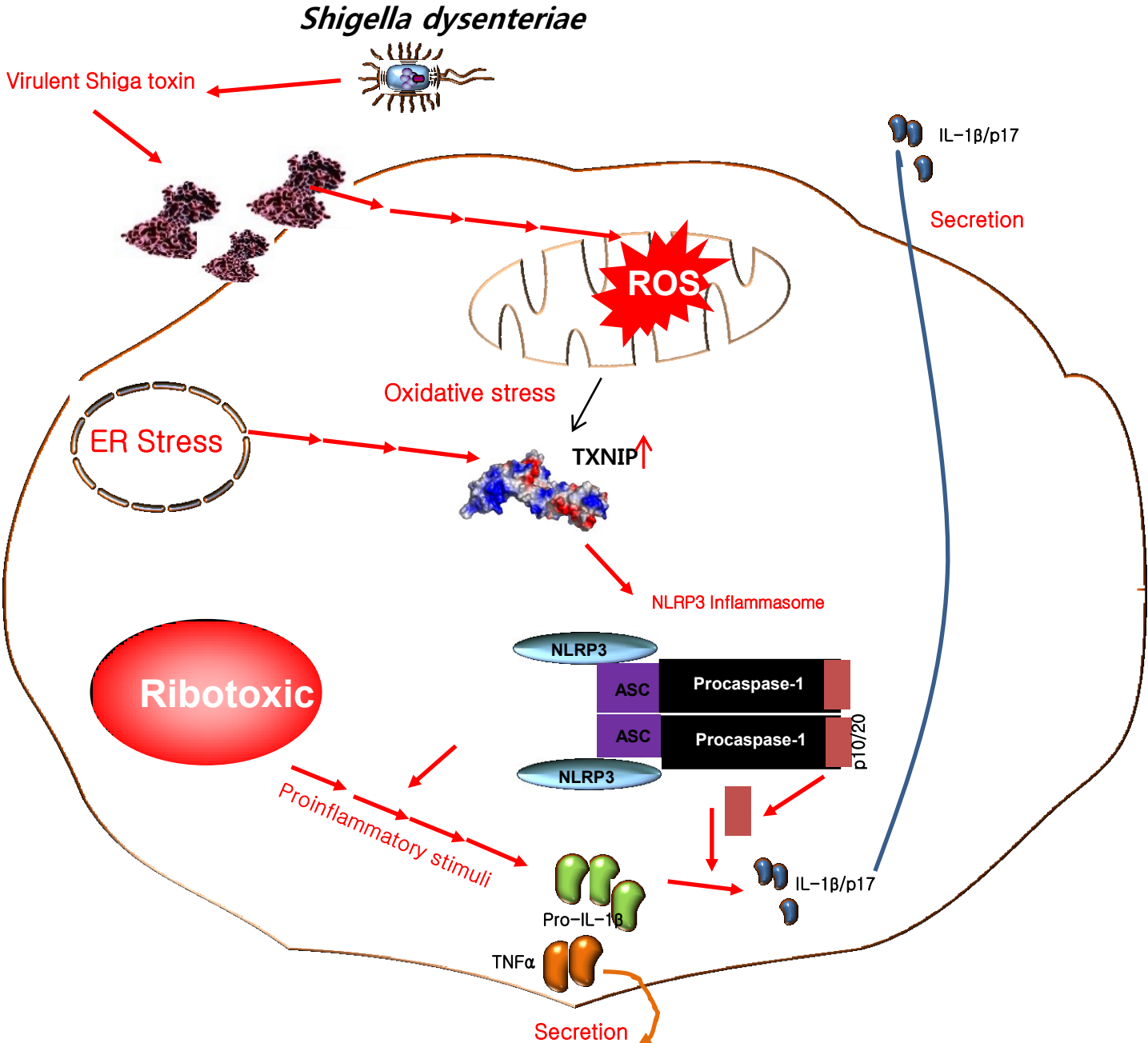
Differential cytokine/chemokine regulation

R.P Cherla *et al.*, 2006, *J. Leuko. Biol.*

R.P Cherla *et al.*, 2009, *IAI* ; Dinorah and MS Lee *et al.*, 2012, *IAI*

Fig. adapted from M.S Lee *et al.*, 2013 *J. Micro.*

Proposed model



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Prof. Vernon Tesh



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Prof. Shinichiro Kurosawa



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