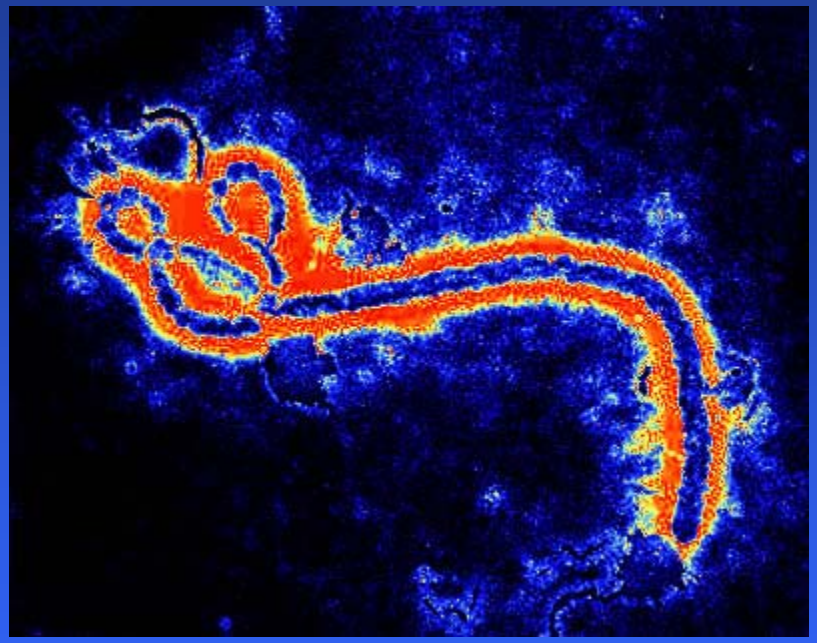
The role of antibodies and cell-mediated immunity in conferring protection against filoviruses



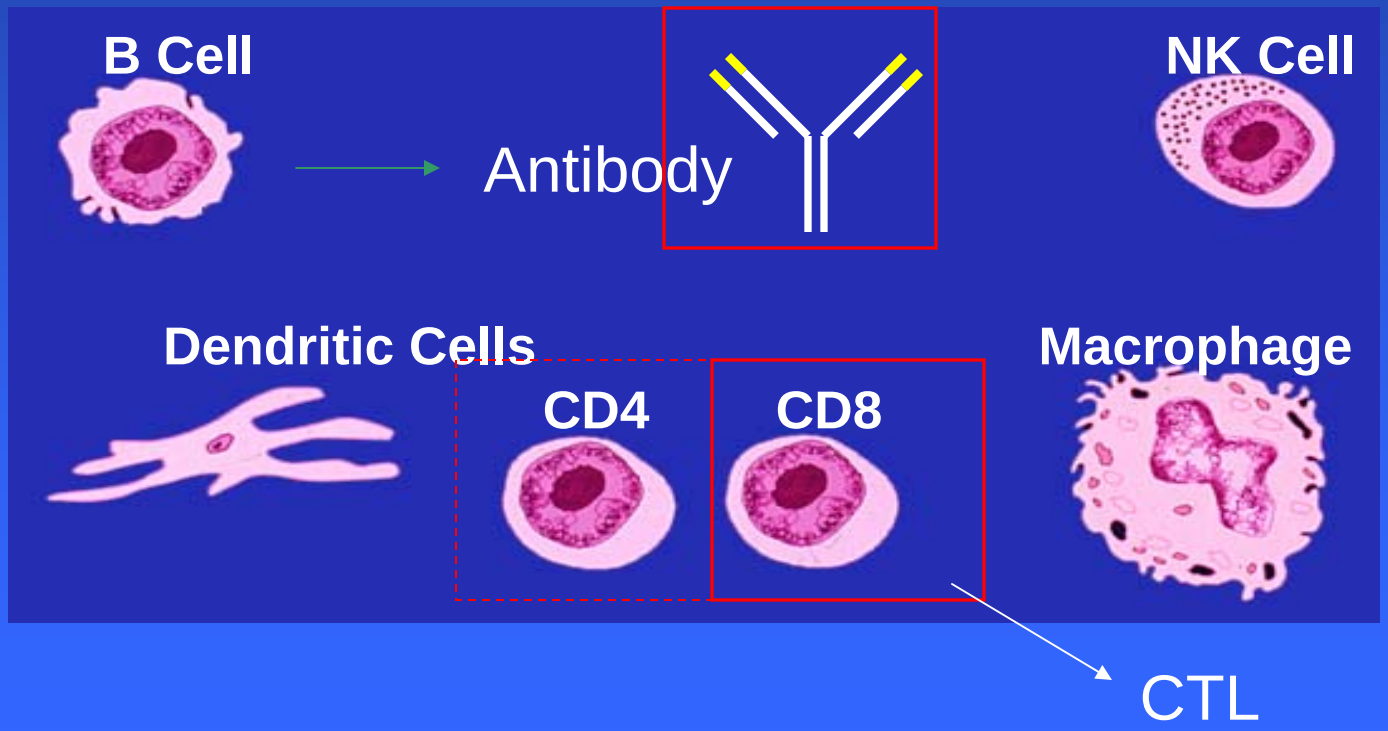
Mary Kate Hart, Ph.D.

Ebola Vaccine Development

- Different Platforms
  - DNA
  - Vector-based (adenovirus, VEE replicon, attenuated Vesicular stomatitis virus)
  - Virus-like particles (VLPs)
  - Liposomes
  - Inactivated virus
- Correlates of immunity poorly understood

What are the Protective Adaptive Immune Responses?

- Role of antibodies in protection
- Role of cytotoxic T lymphocytes (CTL) in protection



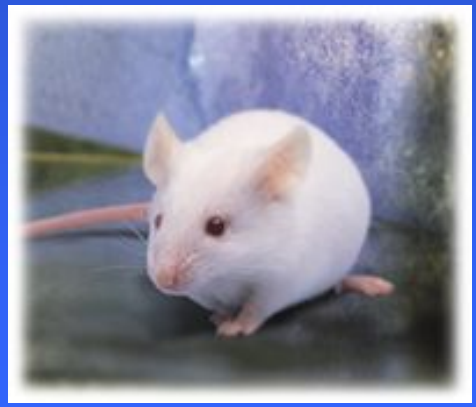
Ebola Virus Murine Model

- **Well-characterized immune system**
- **Availability of immunological reagents**
- **Access to inbred mouse strains**

C57BL/6

- Ebola Zaire 76 adapted by sequential passage in mice, plaque pick with 100% lethality
- Challenge Dose: 10 to 1000 PFU (~30,000 LD_{50})
- Ruffled fur observed from day 5 onward (Balb/c)
- Viremias on day 7 as high as 6 $\log_{10}$ PFU/ml
- LD_{50} < 1 PFU in adult mice (BALB/c) by i.p. route
- Death on day 6-11

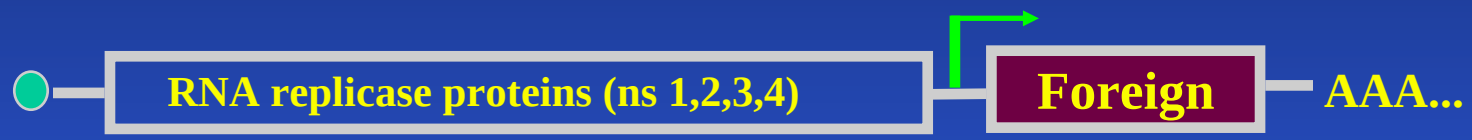
Bray et al, J. Infectious Diseases, 1998, Sep. 178: 651-61



BALB/c

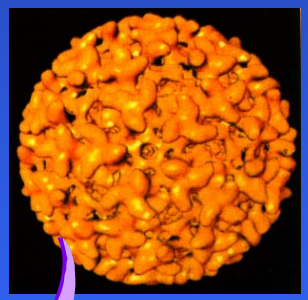
Generation of Venezuelan Equine Encephalitis (VEE) VRP Containing Ebola Genes

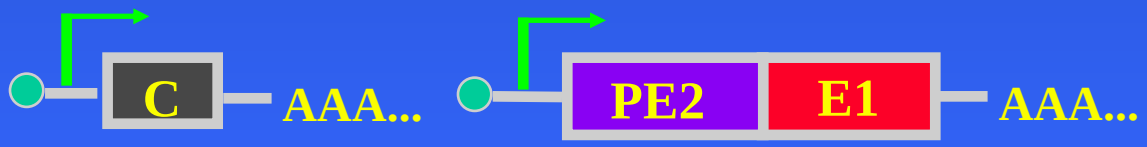
Alphavirus replicon RNA containing foreign gene



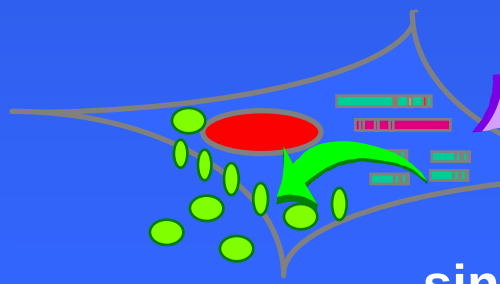
Transfect BHK cells

Helper RNAs express structural proteins which package replicon RNA into VEE-like particles





Helper RNAs: alphavirus structural genes



PROTEIN (ANTIGEN)

single-cycle event: no spread to new cells

Survival of Mice Vaccinated to Ebola and Identification of Protective Immune Responses

Antigen	Survivors/Total (%) BALB/c	C57Bl/6	Protection (Serum)
GP	9/10 (90%)	10/10 (100%)	32/40
NP	10/10 (100%)	15/16 (93%)	1/40
VP24	15/15 (100%)	0/40 (0%)*	0/20
VP30	25/25 (100%)	25/25 (100%)	0/20
VP35	38/40 (95%)	40/40 (100%)	0/20
VP40	15/15 (100%)	16/20 (80%)	0/20
Lassa N	0/30 (0%)	0/30 (0%)	0/40

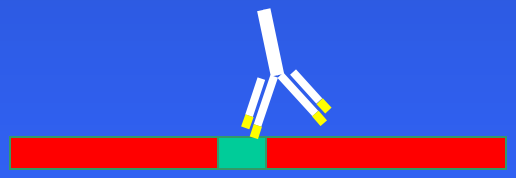
- No protection observed in C57Bl/6 mice vaccinated with VP24 expressing VRP.

Wilson J, Bray M, Bakken R, Hart MK. 2001. Virology 286: 384-390.

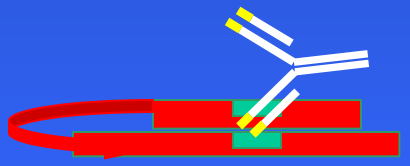
Olinger G, Bailey M, Dye J, Bakken R, Kuehne A, Kondig J, Wilson J, Hogan RJ, Hart MK. 2005. J. Virol. 79: 14189-14196.

Properties of Ebola GP Monoclonal Antibodies

MAb		Specificity			% Survival	Days given	Isotype
1	13F6	Zaire	GP_1 (401-417) ATQVEQHHRRTDNDSTA		90-100%	-1,+1	G2a
2	6D8	Zaire	GP_1 (389-405) HNTPVYKLDISEATQVE		90-100% 50-60%	-1,+1 +2	G2a
3	12B5	Zaire	GP_1 (477-493) GKLGLITNTIAGVAGLI		60-80%	-1,+1	G1
4	13C6	Zaire, IC, Sudan	GP_1, sGP	conformational	90-100%	-1,+1,+2	G2a
5	6D3	Zaire, IC	GP_1, sGP	conformational	80-100%	-1,+1,+2	G2a



Linear Epitope



Conformational Epitope

Wilson J, Hevey M, Bakken, R, Guest S, Bray M, Schmaljohn A, and Hart MK. 2000. Science 287: 1664-1666.

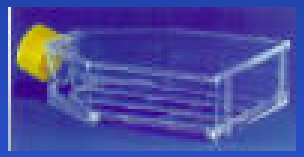
Positive Peptide = Potential CTL Epitope

Lytic CTL

Responsive but not lytic

Binds epitope but unresponsive

Positive Peptide



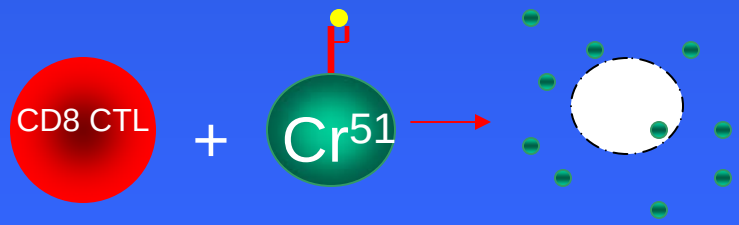
Bulk Re-stimulation for 7 days with peptide

Adoptive Transfer to Unvaccinated Mice

Challenge with Ebola

ELISpot

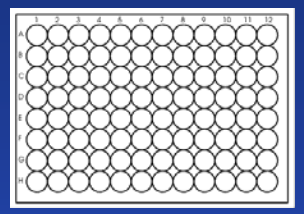
Intracellular cytokine (INF- γ) Assay (ICC)



Functional Assay Chromium Release

Method for Identifying Cellular Responses
Intracellular Cytokine Staining (ICC)

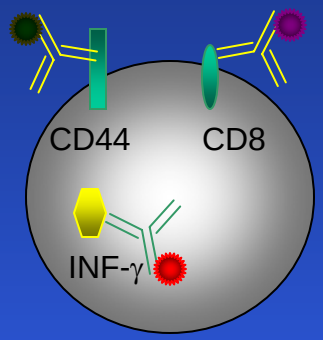
Vaccinate Mice



Spleen cells
+ Peptides

5 hr

Flow Cytometry
FACS



INF-γ + CD44 -	INF-γ + CD44 +
INF-γ - CD44 -	INF-γ - CD44 +

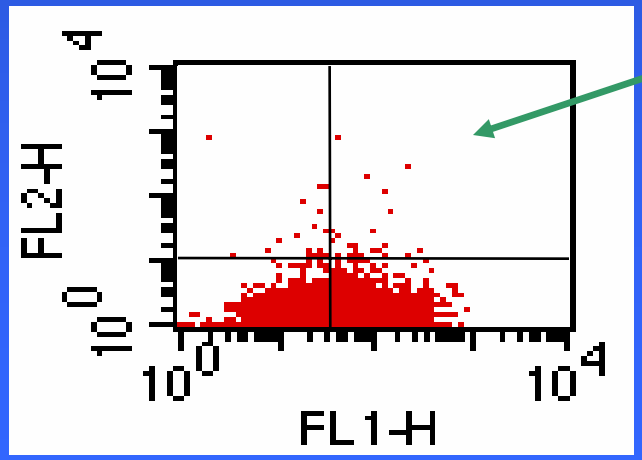
INF-γ

CD 44

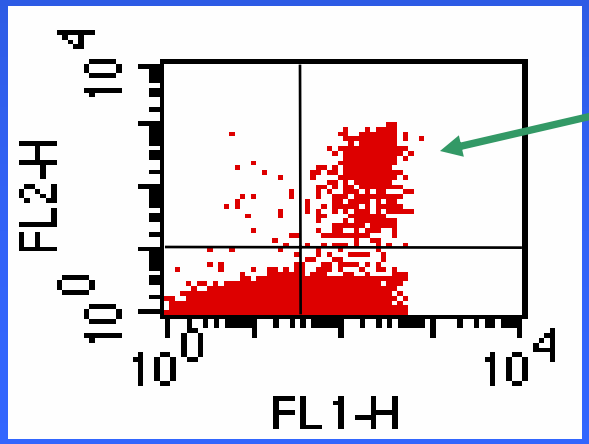
*Gate on CD8 cells

Intracellular Cytokine Staining (ICC)

CD8+, CD44+, INF-γ+



Negative



Positive

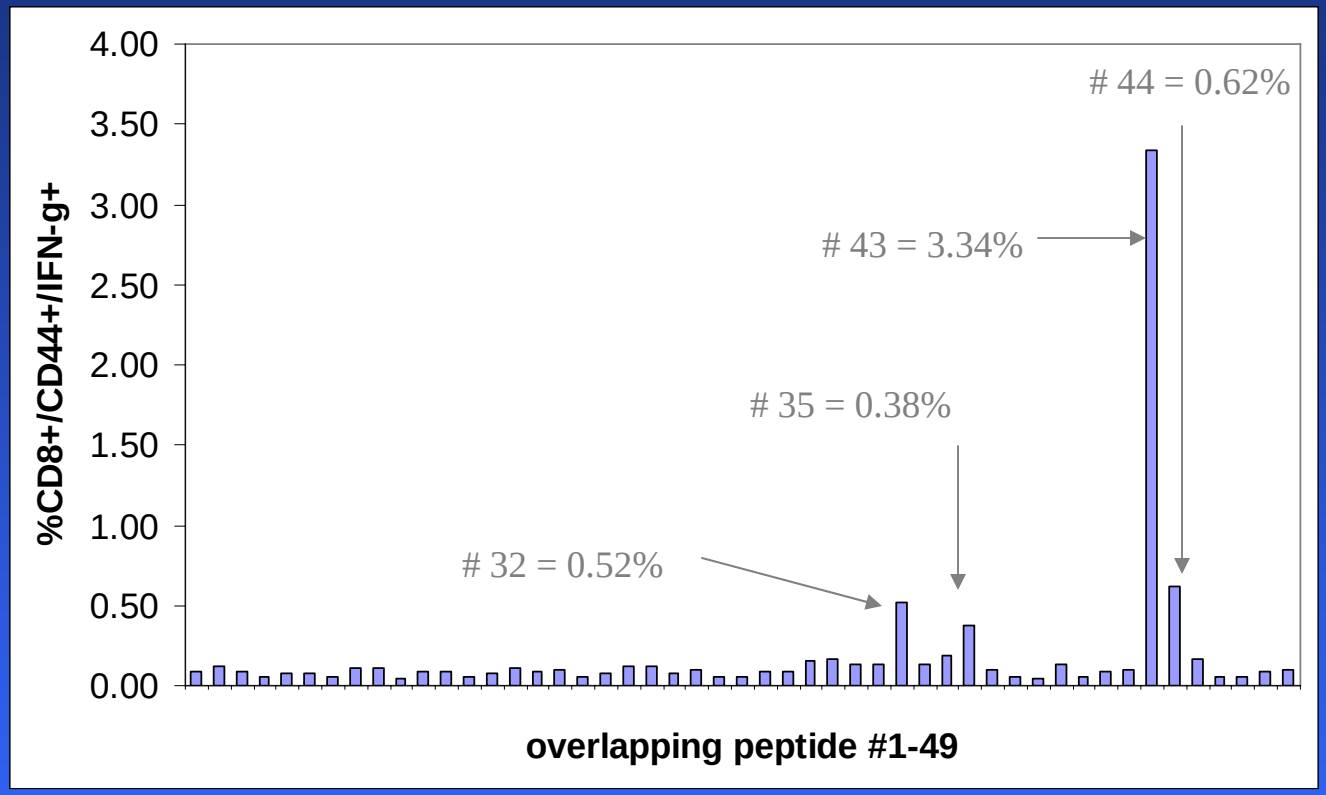
Matrix Approach to Identifying CTL Epitopes in Ebola Virus Proteins (VP24)

Pools

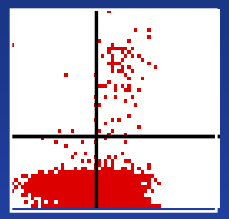
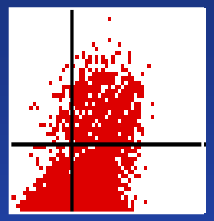
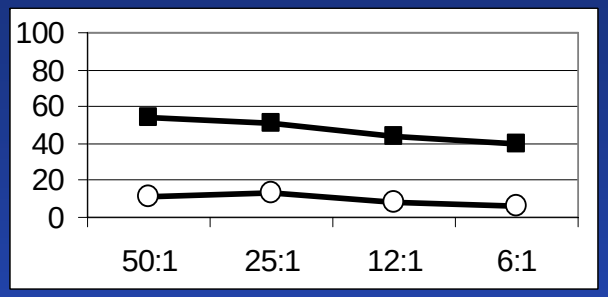
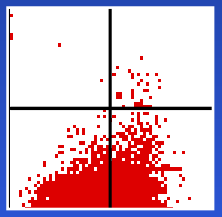
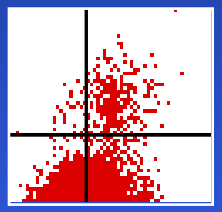
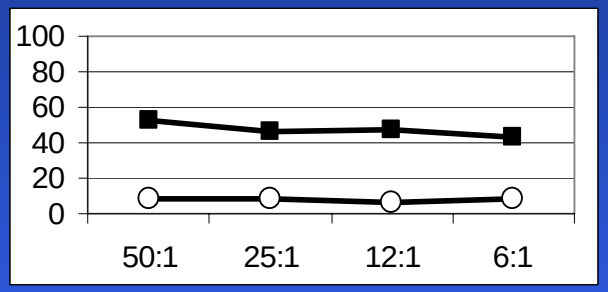
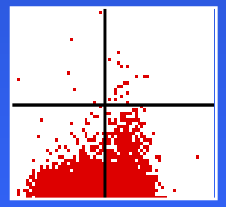
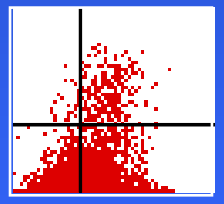
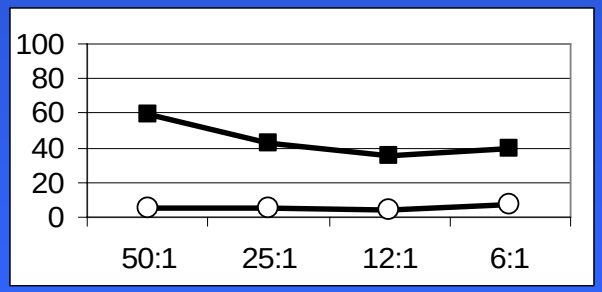
Pools

	0.07	0.14	0.08	0.14	**0.82**	0.13	**4.67**		
	1	**2**	**3**	**4**	**5**	**6**	**7**		
	1	*8*	*15*	*22*	***29***	*36*	***43***	**8**	**4.00**
	2	*9*	*16*	*23*	*30*	*37*	*45*	**9**	0.18
	3	*10*	*17*	*24*	*31*	*38*	*46*	**10**	0.13
	4	*11*	*18*	*25*	***32***	*39*	***47***	**11**	**0.21**
	5	*12*	*19*	*26*	*33*	*40*	*48*	**12**	0.08
	6	*13*	*20*	*27*	*34*	*41*	*49*	**13**	0.13
	7	*14*	*21*	*28*	***35***	*42*	**-**	**14**	**0.67**

VP 24 Individual Peptide Evaluation by ICC



- Peptides 32, 35, 43 and 44 positive
- Peptide 47 not positive but could be deduced by matrix.

	Ex-vivo	7d restim	^{51}Cr	Adoptive Transfer
$vP24_{171}$				20/20
$vP24_{159}$				19/20
$vP24_{214}$				17/20

Anti-INF-γ PE

Anti-CD44 FITC

Assay detection of murine CD8+ T cell responses to EBOV proteins

EBOV Protein	Amino acids	Restriction	IFN-γ ICC	IFN-γ ELISpot	51Cr-release	Survival
GP	141-155	H-2^d	0.24/0.02	Not detected	47%	10/10
	531-545	H-2^b	0.4/0.08	715	3%	0/10
NP	44-52	H-2^b	1.06/0.11	410	73%	10/10
	148-156	H-2^b	0.88/0.11	295	45%	19/20
	279-287	H-2^d	0.63/0.04	Not detected	43%	17/18
	663-671	H-2^b	0.99/0.11	910	57%	17/20

Summary of Protective Immune Mechanisms Induced in Mice by VRPs

Immunogen	BALB/c	C57Bl/6
GP	Ab/CTL(2*)	Ab/ CTL (1)
NP	CTL (1)	CTL (3)
VP24	CTL (3)	**None (0)**
VP30	CTL (3)	CTL (2)
VP35	CTL (2)	CTL (3)
VP40	CTL (3)	CTL (1)

Olinger G, Bailey M, Dye J, Bakken R, Kuehne A, Kondig J, Wilson J, Hogan RJ, Hart MK. 2005. J. Virol. 79:14189-14196

*Note: Rao et al. 2002. Vaccine 76: 9176 detected CTL to GP using different platform, including a third sequence not shown here.

Antibody and T cell responses to filovirus vaccines: Marburg GP

Platform	Animal	ELISA log_{10}	T cell	Reference
VRP	G pig	4.0	nd	Hevey et al. 1998. J. Virol 251: 28; and 2002. Vaccine 20: 586.
VRP	Cyno	3.3	nd	Hevey et al. 1998. J. Virol 251: 28.
cAdVax	Mouse	3.0-5.0	IFNγ ELISpot	Wang et al. 2006. Vaccine 24: 2975.
cAdVax	G pig	2.0-4.0	nd	Wang et al. 2006. Virology 353: 324.
VLP with VP40	G pig	4.0-5.0	Proliferation Index 2-4	Swenson et al. 2005. Vaccine 23: 3033. Warfield et al. 2004. Vaccine 22: 3495
Baculovirus Modified GP	G pig	3.5-4.8	nd	Hevey et al. 1997. Virology 239: 206; and 2002. Vaccine 20: 586.

Antibody and T cell responses to filovirus vaccines: Marburg GP, continued

Platform	Animal	ELISA log_{10}	T cell	Reference
DNA	G pig	2.5	nd	Hevey et al. 2002. Vaccine 20: 586.
DNA	Cyno	2.4-2.9 Note: some lack of protection observed below 2.4	nd	Riemenschneider et al. 2003. Vaccine 21: 4071-4080.

Antibody and T cell responses to filovirus vaccines: Marburg

Platform/Ag	Animal	ELISA log_{10}	T cell	Reference
VRP NP	G pig	3.4	nd	Hevey et al. 1998. J. Virol 251: 28.
VRP NP	Cyno	2.2	nd	Hevey et al. 1998. J. Virol 251: 28.
VRP VP35	G pig	1.9	nd	Hevey et al. 1998. J. Virol 251: 28.

Antibody and T cell responses to filovirus vaccines: Ebola GP

Platform	Animal	ELISA log_{10}	T cell	Reference
VRP	G pig Mouse	4.0 5.0	nd nd	Pushko et al. 2000. Vaccine 19: 142.
VLP with VP40	Mouse	4 logs	nd	Warfield et al. 2005. Expert Rev. Vaccines 4: 429.
cAdVax	Mice	5.0-6.0	nd	Wang et al. 2006. J. Virol. 80: 2738.
DNA	Mouse	1.5-3.0	CTL	Vanderzanden et al. 1998. Virology 246: 134.
DNA or DNA/ Ad; GP or sGP	G pig	2.8-4.4	Proliferation in g pig; CTL demonstrated in mice	Xu et al. 1998. Nat. Med. 6: 886. Sullivan et al. 2000. Nature 408: 605.
Vaccinia	G pig	1.6	nd	Gilligan et al. 1997.Vaccines 97: 87.

Antibody and T cell responses to filovirus vaccines: Ebola GP,NP

Platform	Animal	ELISA log_{10}	T cell	Reference
DNA (GP,NP)	G pig	3.5-3.8	Data not shown, discussed	Sullivan et al. 2000. Nature 408: 605.
DNA/ Adenovirus (GP,NP)	Cyno	4.5-5.0	Proliferation index 3-18; CTL not shown	Sullivan et al. 2000. Nature 408: 605.
DNA NP	Mouse	3.0	CTL	Vanderzanden et al. 1998. Virology 246: 134.
DNA NP	G pig	4.0-4.4	Proliferation negative	Xu et al. 1998. Nat. Med. 6: 886.
Liposomes	Mouse	3.0	IFN-g ELISpot, CTL	Rao et al. 2002. Vaccine 76: 9176.

Conclusions

- Different vaccine platforms induce Ab titers (ELISA) of 3-5 $\log_{10}$ to EBOV and MBGV in multiple animal models.
- T cell responses (CTL directly or cytokine-associated responses indicating a Th1 response) have been measured in animals vaccinated with different platforms for EBOV and MBGV.
- Antibodies and CTLs have been demonstrated to provide protection from EBOV challenge in a murine model. Additional studies evaluating NHP responses are warranted.
- Additional studies are required to identify the correlates that will bridge animal and human data for licensure under the Animal Rule.

Animal Use

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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