A Dominant EV71-specific CD4 T cell epitope is conserved among enteroviruses

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Hand, foot, and mouth disease (HFMD) and EV71

Mong How Ooi et al. Clinical features, diagnosis and ... Lancet Neurol 2010; 9: 1097–1105

HFMD : Severe complications

> ***Cardiorespiratory manifestations :***

- Pneumonia
- Pulmonary hemorrage
- Pulmonary oedema
- Myocarditis

> ***Neurological manifestations :***

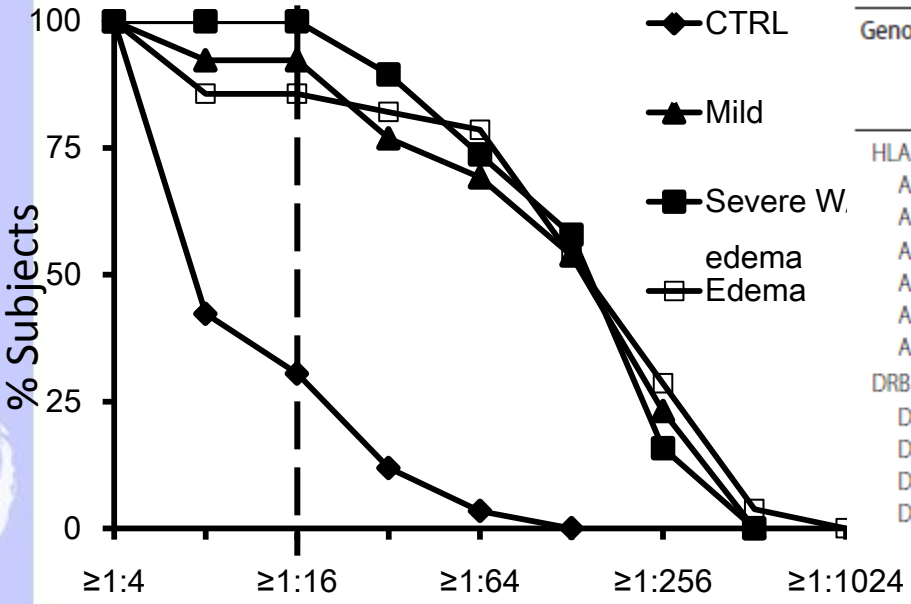
- Brainstem encephalitis (58%)
- Aseptic meningitis (36%)
- Encephalitis
- ANS dysregulation (4%)

> ***Systemic manifestations :***

Liver injury, myositis, thrombocytopenia

Since 2008,nearly 6 millions HFMD cases have been reported, and over 2000 children have been dead in China.

Cellular rather than humoral immunity is correlated with clinical outcome of HFMD



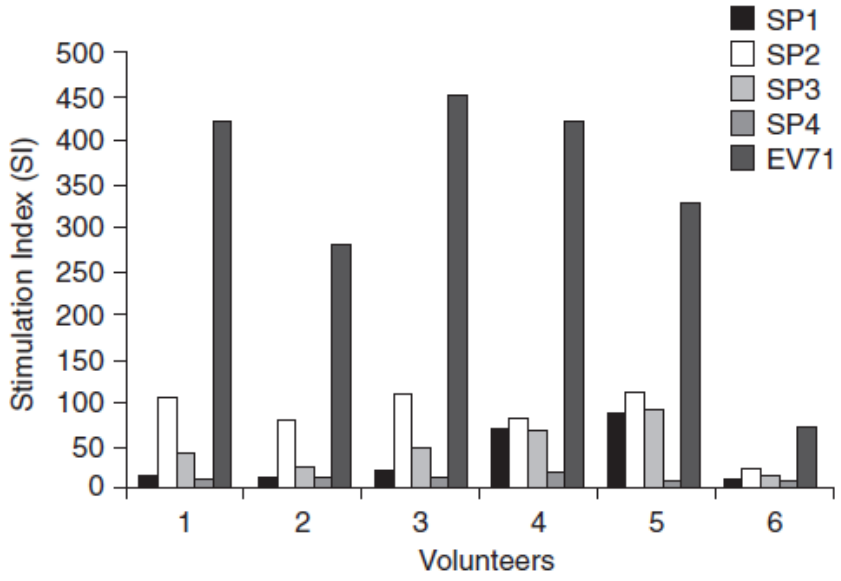
Genotype	*n* (%)		*P*	OR (95% CI)
	Case (*N* = 219)	Control (*N* = 97)		
HLA-A				
A33	65 (30)	14 (14)	.004	2.50 (1.33–4.73)
A11	119 (54)	60 (62)	.21	0.73 (0.45–1.20)
A24	64 (29)	32 (33)	.50	0.84 (0.50–1.40)
A26	12 (5)	2 (2)	.17	2.75 (0.60–12.55)
A31	9 (4)	2 (2)	.36	2.04 (0.43–9.60)
A2	104 (47)	52 (54)	.32	0.78 (0.49–1.26)
DRB1				
DR17	53 (24)	11 (11)	.008	2.51 (1.25–5.06)
DR15	35 (16)	21 (22)	.23	0.69 (0.38–1.27)
DR8	38 (17)	20 (21)	.50	0.81 (0.44–1.49)
DR12	63 (29)	23 (24)	.34	1.31 (0.75–2.27)

Yang C, et al. Virology J 2011

Chang LY, et al. Pediatr 2008

Three Human EV71 CD4+ T cell Epitopes Had Been discovered

Only EV71
VP1antigen alone



Foo DG et al. Viral Immunol.
2008 Jun;21(2):215-24.

SP2	E VV PQ L LQ YM FV P P G
EV71/GDFS/3/2008	---------- ----------
FY23	---------- ----------
EV71/Fuyang Anhui P.R.C/17.08/3	---------- ----------
EV71/Lanzhou01	---------- ----------
EV71/HENAN/DC/2010	---------- ----------
SZ/HK08-6	---------- ----------
SZ/HK08-5	---------- ----------
EV71/Jiangsu P.R.C/07.08/10	---------- ----------
BJ06-SJS06	---------- ----------
SHZH03	---------- ----------
EV71-Hubei-09-China	---------- ----------
BrCr	P--P ---- ----------
FY23-K14	L--P ---- ----------
FY23-K12	L--P ---- ----------
28/SHENZHEN/08	---R ---- ----------
EV71/Zhejiang08	---R ---- ----------
121/SHENZHEN/08/	Q--P ---- ----------

SP3	L V V R I Y M R M K H V R A W
EV71/GDFS/3/2008	----------
FY23	----------
EV71/Fuyang Anhui P.R.C/17.08/3	----------
EV71/Lanzhou01	----------
EV71/HENAN/DC/2010	----------
SZ/HK08-6	----------
SZ/HK08-5	----------
EV71/Jiangsu P.R.C/07.08/10	----------
BJ06-SJS06	----------
SHZH03	----------
FY23-K14	----------
FY23-K12	----------
28/SHENZHEN/08	----------
EV71/Zhejiang08	----------
121/SHENZHEN/08/	----------
EV71-Hubei-09-China	--I ----------
BrCr	--I ----------

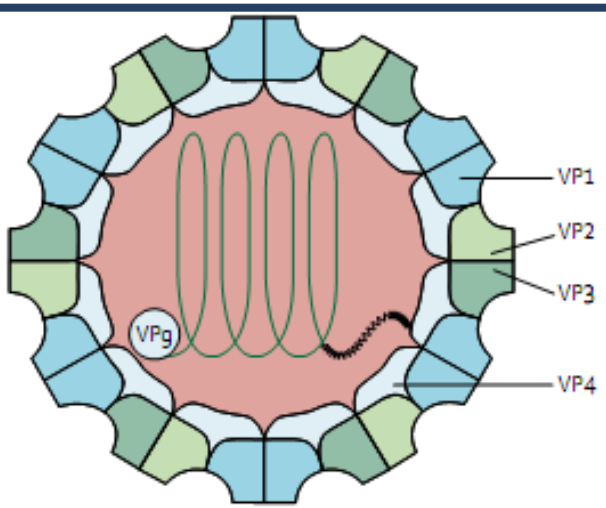
EpiMatirx Algorithm is applied to predict new EV71 CD4+ T cells epitopes

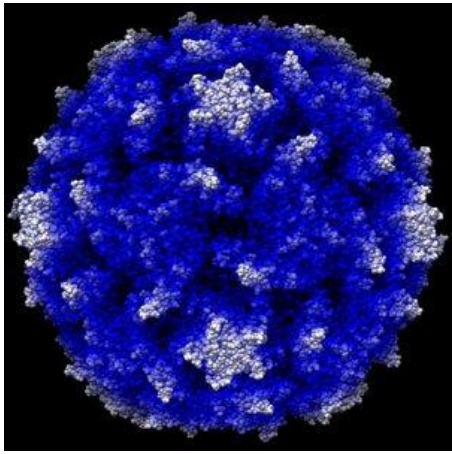
Anne De Groot

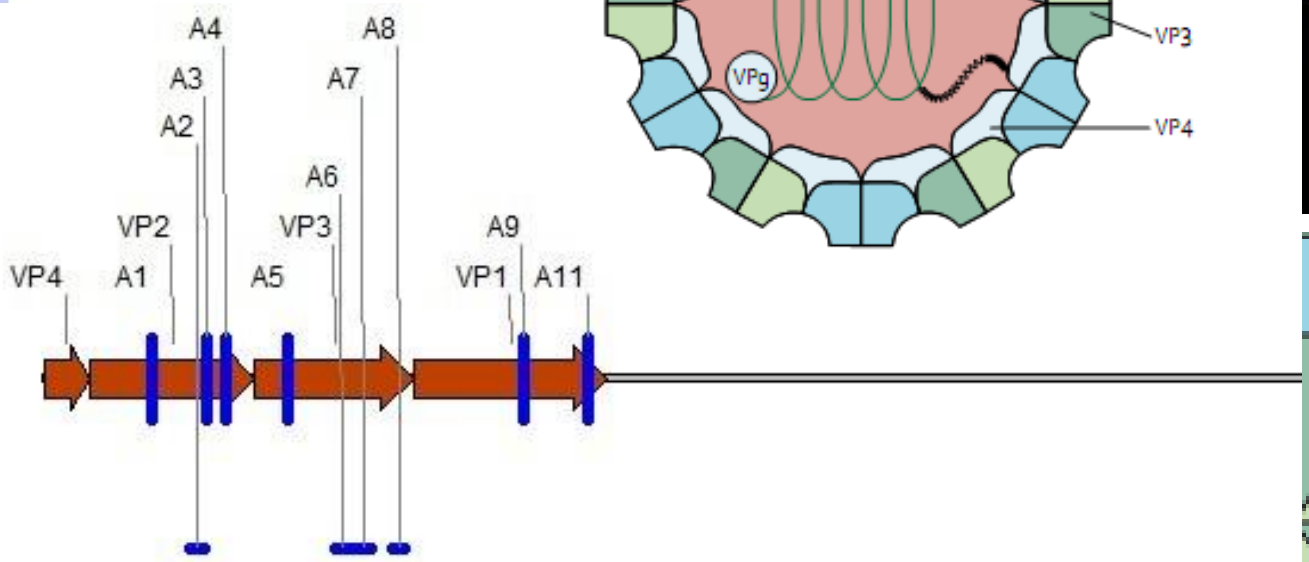
University of Rhode Island.

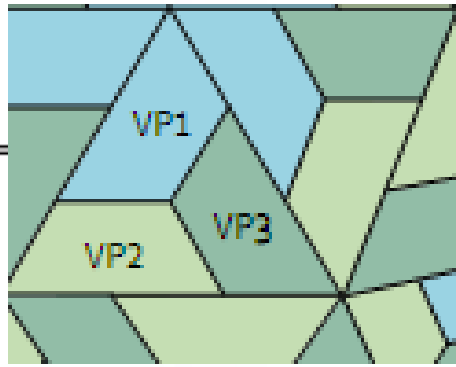
- **EpiMatrix has been successfully applied to the analysis of previously published epitopes, and in the prospective selection of epitopes from HIV, Mycobacterium tuberculosis, Tularemia to vaccinia virus.**
- **FY573 polyprotein sequence was subjected to analysis by EpiMatirx Algorithm.**

37 EV71 CD4+ T cells epitopes were predicted







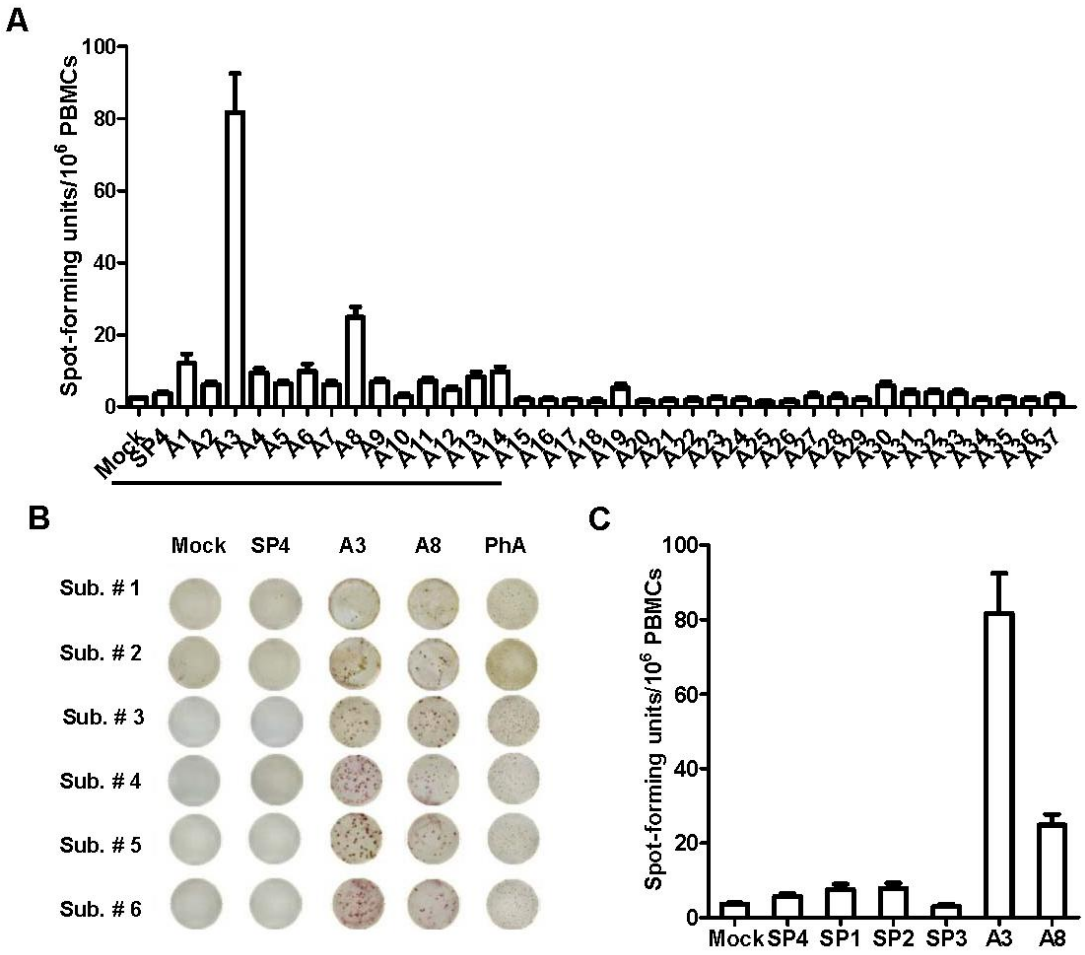


11 predicted epitopes are located in structural viral protein regions, and the rest are located in none-structural protein regions.

All tested 6 adults respond to A3 eiptope, and to various degree to other epitopes

Peptide	Sequence	Cluster Address	Proportion of Responding Donor (%)
A1	NAQFHYLYRSGFCIHVQ	164 - 180	16.7
A2	ADGFELQHPYVLDAGISISQL	224 - 244	33.3
A3	PHQWINLRTNNCATII	248 - 263	**100.0**
A4	HCNFGLLVVPISPLD	278 - 292	66.7
A6	TGSFMATGKMLIAYTPPGGPLP	445 - 466	50.0
A7	IWDFGLQSSVTLVIPWISNTH	479 - 499	16.7
A8	NTAYIIALAAAQKNFTMKL	533 - 551	**83.3**
A10	PLVVRIYMRMKHVRAWIP	811 - 828	16.7
A11	NQNYLFKANPNYAGNSI	833 - 849	33.3
A13	HYPVSFSKPSLIYVE	933 - 947	33.3
A14	SREVEALKNYFIGSE	1034 - 1048	50.0
A19	GLEWVSNKISKFIDWL	1128 - 1143	33.3
A23	GVSFTSKFVIASTNASNIIVP	1321 - 1341	16.7
A24	VSELIREYNNRSAIGNTIE	1418 - 1436	16.7
A27	RLEVDFEQALFSKYVGNTLYEP	1780 - 1801	16.7
A28	EAALHYANQLKQLEINTSQMSMEE	1807 - 1830	16.7
A30	DSVYLRMAFGHLYETF	1914 - 1929	66.7
A31	GHLYETFHANPGTITGSA	1923 - 1940	16.7
A33	SPVWFRALELVLREIGYSE	1973 - 1991	16.7
A34	THHVYRNKTYCVLGGMPSGCS	2003 - 2023	16.7
SP1	IETRCVLNSHSTAET	66-67	16.7
SP2	EVVPQLLQYMFVPPG	145-159	33.3

A3 and A8 are two dominant EV71 CD4+ T cell epitopes



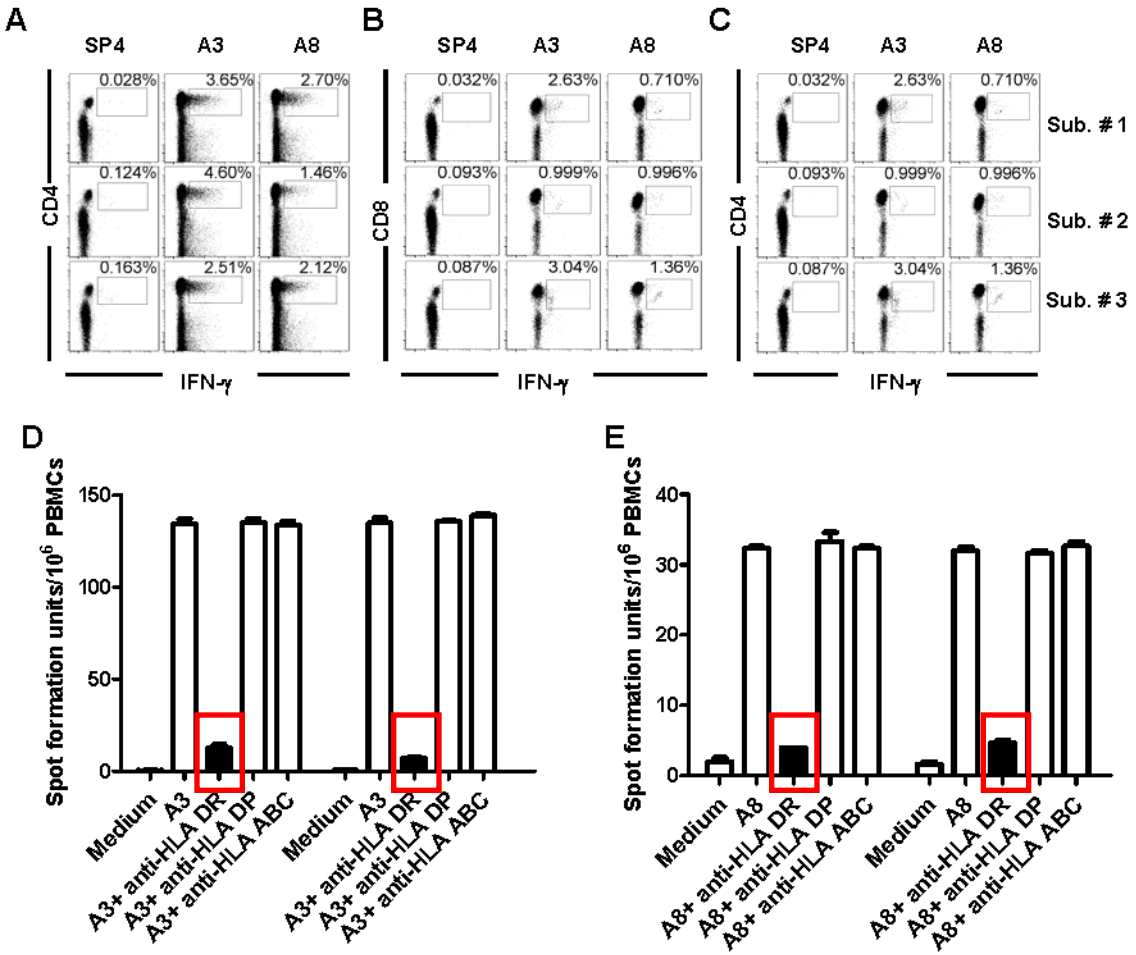
A3 and A8 epitopes are conservative among EV71 isolates, and A3 is a common CD4+ T cell epitope in EV71, Coxsackie viruses A4, A6 and A16.

A

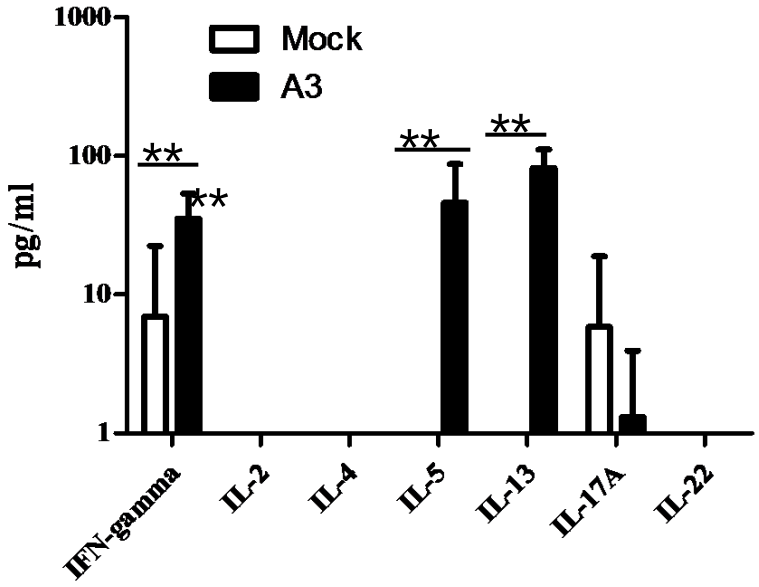
	A3	A8	A14	SP2
	PHQWINLRTNNCATII	NTAYIIALAAAQKNFTMKL	SREVEALKNYFIGSE	EVVPQLLQYMFVPPG
FY23	----------	----------	-----S--I----	----------
FY23-K12	----------	----------	-----H-I----	----------
FY23-K14	----------	----------	-----H-I----	----------
EV71/GDFS/3/2008	----------	----------	---------I----	----------
EV71-Hubei-09-China	----------	----------	---------I----	----------
EV71/HENAN/DC/2010	----------	----------	---------I----	----------
EV71/Lanzhou01	----------	----------	---------I----	----------
SHZH03	----------	----------	---------I----	----------
SZ/HK08-6	----------	----------	---------I----	----------
SZ/HK08-5	----------	----------	---------I----	P--P-------
121/SHENZHEN/08	----------	----------	---------I----	L--P-------
28/SHENZHEN/08	----------	----------	-----I----	L--P-------
EV71/Fuyang.Anhui.P.R.C/17.08/3	----------	----------	-----I----	---R-------
EV71/Jiangsu.P.R.C/07.08/10	----------	----------	-----I----	---R-------
BJ06-SJS06	----------	----------	-----I----	Q--P-------

B

	A3	A8
A3	PHQWINLRTNNCATII	NTAYIIALAAAQKNFTMKL
CAV A4	----------	T----V--A---DN-----
CAV A6	----------	-E-N---MG-G--N--L--
CAV A16	----------	SE-N----G---EN--L--
CAV A9	----------S---V	SSC--MCFVS-CND-SVR-
CAV B3	----------S---V	SDCK-LCFVS-CND-SVRM
CAV B5	----------S---V	SNCS-LCFVS-CND-SVRM
CAV B6	----------S---V	T-ST-LCFVS-CND-SVR-
Echovirus 30	----------S---V	-SSS-MCFAS-CND-SVRM
Echovirus 15	----------S---V	TDCIVLCFVS-CND-SVRM
Echovirus 5	----------V	TQCD-LGFVS-CND-SVRM
Poliovirus 1	---I---------LV	REMD-LGFVS-CND-SVR-
Poliovirus 2	---I---------LV	RKMD-LGFVS-CND-SVR-
Poliovirus 3	---I-------S---V	KSMSMLGFVS-CND-SVR-


A
SP4
A3
A8
0.028%
3.65%
2.70%
0.124%
4.60%
1.46%
0.163%
2.51%
2.12%
CD4
IFN-γ
B
SP4
A3
A8
0.032%
2.63%
0.710%
0.093%
0.999%
0.996%
0.087%
3.04%
1.36%
CD8
IFN-γ
C
SP4
A3
A8
0.032%
2.63%
0.710%
0.093%
0.999%
0.996%
0.087%
3.04%
1.36%
CD4
IFN-γ
Sub. #1
Sub. #2
Sub. #3
D
Spot formation units/10^6 PBMCs
150
100
50
0
Medium
A3
A3+ anti-HLA DR
A3+ anti-HLA DP
A3+ anti-HLA ABC
Medium
A3
A3+ anti-HLA DR
A3+ anti-HLA DP
A3+ anti-HLA ABC
E
Spot formation units/10^6 PBMCs
40
30
20
10
0
Medium
A8
A8+ anti-HLA DR
A8+ anti-HLA DP
A8+ anti-HLA ABC
Medium
A8
A8+ anti-HLA DR
A8+ anti-HLA DP
A8+ anti-HLA ABC

A3 can stimulate PBMCs derived from previous EV71 infected healthy adults PBMCs to produce multiple cytokines



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Acknowledgements

Mei Zeng
Fudan University Children Hospital

Zhaomin Zhu
Chongqi Medical University Children Hospital

Frances Terry et al/Anne De Groot
EpiVax Inc.& University of Rhode Island. USA

Ralf Altmeyer
Institute Pasteur of Shanghai