

What epitopes does the human immune system recognize in swine flu?

Introduction

The ongoing influenza H1N1 human swine flu outbreak has the potential to turn into a pandemic. Even if its impact on human health ends up being relatively benign, the outbreak underlines the need to rapidly understand emerging viral pathogens in order to develop timely countermeasures, which requires the rapid exchange and analysis of scientific information. Several researchers at institutes such as the CDC have made sequences from viral isolates publicly available, practically in real time through online resources such as Biohealthbase [1] [<http://www.biohealthbase.org/>], the NCBI influenza virus resource [2] [<http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html>] and GISAID [3] [<http://gisaid.org>]. The tools and analyses made available on these websites provide sequence comparisons which allow identifying the origin of the virus, and to compare it to past influenza strains.

The immune system recognizes a virus by other means than sequence comparisons: Adaptive immune responses against influenza (and other pathogens) are triggered upon T cell or B cell receptors recognizing a foreign entity. The molecular structures in the virus that are bound by these receptors are called immune epitopes. The recognition of such epitopes can provide immune memory protection if antibodies and T cells were primed to recognize them during past influenza infections or vaccinations. Therefore, a virus can change substantially in some sequence regions, but still be recognized by the immune system if it retains the epitopes of interest.

The Immune Epitope Database[4,5] [<http://www.iedb.org>] was developed for the purpose of cataloging epitopes and making them available in a single repository to the scientific community. All manuscripts published to date that characterize epitopes in influenza are contained in the IEDB (please notify us of any oversight!). The present report analyzes how these known epitopes, and therefore the immunological memory, is conserved in the emerging swine flu isolates.

The results reported here are preliminary, and subject to further updates. Specifically, new swine flu sequences are frequently made available, which will further enhance this analysis. Also, further reports describing influenza A epitopes are continuously added to the IEDB. Finally, the analysis itself is continuously evolving and should be considered preliminary. In this spirit, please communicate any inconsistencies and oversights to us to help improve this analysis.

Results

Assembly of epitope datasets

The entirety of epitope information in the IEDB related to influenza A is available at <http://iedb.org/sourceOrgId/197911> . At the time of writing, this encompassed information from 594 references (journal articles and direct submissions) describing 3724 distinct molecular structures (linear, and discontinuous peptides) derived from influenza A that were tested experimentally for interaction with immune receptors. This roughly doubles the amount of information on influenza epitopes that was

available in 2006 [6], which emphasizes the important contribution and greatly enhanced throughput of recent influenza epitope mapping efforts, which were stepped up since the emergence of H5N1 avian flu.

We focused our analysis on influenza A epitope mapping experiments which have most relevance for human immunity. We considered an experiment relevant if it showed epitope recognition by antibodies or recognition by T cells in the context of human MHC, and if the epitopes were mapped in the context of whole influenza organisms or proteins. B cell epitopes mapped in the context of any host organism were included, as exact mapping of such epitopes is still done infrequently in humans, as noted previously [6]. This is justified as studies have shown substantial overlap for B cell epitopes defined in different species presumably because the structural constraints associated with their recognition is similar across species.

We distinguished between two primary categories of epitopes: those recognized by B-cells or antibodies and those recognized by T cells. If possible, the later were further categorized into those recognized by CD8+ T cells in the context of MHC class I molecules and those recognized by CD4+ T cells in the context of MHC class II. If multiple epitopes in the same category had sequences that were nested in each other, the optimal epitope was chosen that gave the highest specific frequency of recognition at minimal length. Full details of the specific IEDB queries are given in the Method section.

The first column in Table 1 gives an overview of the number of epitopes in the different categories. For a majority of $130 + 190 = 320$ out of 454 T cell epitopes it could be determined if the T cells are CD4+ or CD8+, and both types of responses are well represented. Notably, there are nearly three times more known T cell epitopes (454) than B cell epitopes (162). This is in agreement with our previous analysis[6], but it is nevertheless striking giving the importance of antibody mediated immunity for vaccine induced protection.

Epitope category	All influenza epitopes	Present in recent H1N1 seasonal flu	Conserved in H1N1 swine flu
B cell	162	31	11
T cell	454	216	111
T cell - CD8+	130	78	52
T cell - CD4+	190	95	39

Table 1 - Number of influenza A derived epitopes in the IEDB

Next, we refined our analysis to consider only epitopes found in sequences from seasonal flu viruses of subtype H1N1 in the period of 1988-2008 which were isolated from human hosts. It is expected that there are immune reactivities against these epitopes in the present population of adults of age 20 years and above. To exclude rare sequence isolates that cannot be considered as representative of seasonal flu strains, we only consider epitopes conserved in 30% or more of the total strains reported in any given year (see Methods for details). The second column in Table 1 summarizes the distribution of these epitopes. Notably, only about 20% of the B cell epitopes in influenza are present in seasonal H1N1 flu strains, while up to half of the T cell epitopes are shared. This indicates a higher variability of the

sequence regions containing B cell epitopes compared to those containing T cell epitopes, and is in line with the greater variability of surface proteins between influenza strains of different subtypes.

For the final epitope set, we asked which of these epitopes defined on the basis of pre-existing immunity against H1N1 are found to be totally conserved in the emerging swine flu strains. Human H1N1 swine flu sequences were retrieved from isolates originating in Mexico, the US and elsewhere as described in the Methods section. The list of individual epitopes is given as a **Supplementary Table** at the end of this manuscript. As shown in the third column of Table 1, a substantial number of epitopes are present in swine flu sequences for which immune memory exists based on past exposure to seasonal H1N1 strains. Remarkably, less than a third of B cell epitopes, but more than half of the T cell epitopes for which immune memory exists from seasonal H1N1 flu strains are conserved in swine flu sequences.

Next, we assessed if exposure to seasonal influenza viruses of the H3N2 subtype contributes additional epitopes with pre-existing immunity in swine flu strains. A repeat of the analysis above shows that this is not the case, as all epitopes with

Distribution of epitopes in different influenza proteins

Based on the observation that B cell epitopes are less conserved than T cell epitopes, we postulate that the majority of non-conserved epitopes is found on highly variable surface protein such as HA. As shown in Table 2, this is indeed the case: For both T cells and B cells, around 80% of epitopes from the HA protein present in seasonal flu strains are not conserved in swine flu sequences. In contrast, the majority of T cell epitopes from the NP, PB1 and M1 proteins are conserved. The NP protein also contains nine H1N1 B cell epitopes, five of which are conserved in swine flu sequences. This supports that the lower degree of conservation for B cell epitopes is primarily a result of higher variability in the targeted antigens.

Protein	Present in recent H1N1 seasonal flu		Conserved in H1N1 swine flu	
	T cell	B cell	T cell	B cell
HA	38	9	4	2
NA	5	2	1	1
M1	44	4	27	1
M2	4	4	0	1
NS1	4	1	2	0
NS2	2	0	1	0
NP	62	9	36	5
PB1	44	2	33	1
PB1-F2	0	0	0	0
PA	8	0	5	0
PB2	5	0	2	0

Table 2 – Distribution of epitopes in different influenza proteins

Discussion

We here provide an analysis of immune epitopes found in the emergent H1N1 swine flu strains. We find that a significant number of T cell epitopes and some B cell epitopes are found in swine flu that have

also been present in circulating seasonal H1N1 influenza strains. Based on this, we formulate the hypothesis that a significant fraction of the adult population has some immune memory responses against swine flu. Furthermore, our analysis suggests that such memory response will predominantly consist of T cell responses, in particular CD8+ T cell responses.

There are a number of caveats to the present study. For one, to our knowledge there are no good data available on the frequency of circulating influenza strains in seasonal flu for different years. We are therefore taking the number of sequenced influenza strains in each year as a proxy for the sequence distribution of circulating strains. Along the same lines, it is not straightforward to identify from public databases what sequences can be considered seasonal influenza, and which are rare isolates from e.g. a rare case of animal to human transmission. Both issues would be addressed by making quantitative epidemiological monitoring data on the circulation of different isolates available.

In the present analysis, we consider an epitope to be conserved between two strains if 100% of its sequence is present in both strains. Clearly, this is a very conservative assumption, as cross reactivities between epitopes with individual residue substitutions have been observed frequently. The qualitative findings of our analysis should not be altered significantly when allowing for such cross reactivities.

More to come ...

Methods

Querying for epitopes with human relevance in the IEDB

The following **query parameters (bolded)** were used to extract epitopes with human relevance from the IEDB:

Epitope source organism = influenza A. We consider epitopes that were derived from any influenza A strain.

B cell assay or (T cell assay AND (host = human OR restricting MHC = human)). We limit ourselves to epitopes characterized in experiments that demonstrate the presence of adaptive immune receptors (antibody / BCR or TCR) that recognize the epitope. For TCR recognition, the epitopes are presented by MHC molecules. As these molecules are highly divergent in different species, we limit T cell epitopes to those identified in the presence of human MHC molecules.

Positive measurement. The outcome of the experiment has to be reported as a positive response against the epitope. We make no attempt to enforce a common set of criteria for defining immunogenicity and protective efficacy, because widely divergent methodologies were used by different laboratories to measure immune responses. Rather, we record, for each epitope the specific assay category and conditions used, and conform to the criteria for defining positive and negative measurements as reported by the authors themselves in each published article.

Exclude Antigen = Epitope AND Immunogen = Epitope. We excluded epitopes that were defined solely by their use as both immunogen (to induce the responses) and as antigen (to measure the response). In

such experiments, it is not possible to evaluate the relevance of the induced response for antiviral immunity, as the structural context of the epitope is missing. We considered only epitopes shown to be recognized by Abs or TCR in the context of the whole influenza virus or proteins.

Exclude small sequences: Linear sequences of <7 residues and discontinuous sequences <3 residues. These constitute partial epitope sequences, and will be discarded, as their conservation cannot be accurately determined.

The resulting IEDB epitope IDs were retrieved and stored in a local database. For T cell epitopes with nested sequences, the optimal epitope(s) were chosen manually by comparing the assay descriptions associated with each epitope in the IEDB to identify the peptide with the most frequent responses, or to choose multiple peptides if the epitopes were restricted by different MHC molecules. For linear B cell epitopes with nested sequences, the shortest sequence ≥ 7 residues was chosen as the representative. For discontinuous epitopes no removal of nested sequences was performed.

Querying for Influenza sequences

Sequence set of circulating H1N1 and H3N2 sequences Flu sequences were are obtained from the NCBI Influenza Viral resource [<http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html>]. The following query parameters were set:

- Virus Species = Influenza A AND (Subtype = H1N1 OR Subtype = H3N2).
- Host = Human
- Year FROM 1988 TO 2008
- Whole genome only

Swine flu sequences were obtained from two sources. The NCBI the NCBI Influenza Viral resource was queried for:

- Virus Species = Influenza A AND Subtype = H1N1
- Host = Human
- Year 2009

This query retrieved the same set of sequences listed on the main website as being swine flu outbreak related. NCBI does not contain sequences from Mexican isolates. These were retrieved from the GISARD database. The table below lists the isolates for which sequences were obtained, all of which were submitted by the Centers for Disease Control and Prevention, Atlanta(Rebecca Garten)

EPI_ISOLATE_ID	NAME	TYPE	PASSAGE	DATE	HOST	LOCATION
EPI_ISL_29610	A/Mexico/4482/2009	A / H1N1	ORIGINAL	14-APR-09	Human	Mexico
EPI_ISL_29611	A/Mexico/4486/2009	A / H1N1	ORIGINAL	14-APR-09	Human	Mexico
EPI_ISL_29612	A/Mexico/4108/2009	A / H1N1	ORIGINAL	02-APR-09	Human	Mexico
EPI_ISL_29613	A/Mexico/4115/2009	A / H1N1	ORIGINAL	07-APR-09	Human	Mexico
EPI_ISL_29614	A/Mexico/4108/2009	A / H1N1	C1-IR	02-APR-09	Human	Mexico
EPI_ISL_29615	A/Mexico/4482/2009	A / H1N1	C1-IR	14-APR-09	Human	Mexico

EPI_ISL_29616	A/Mexico/4603/2009	A / H1N1	C1	19-APR-09	Human	Mexico
EPI_ISL_29617	A/Mexico/4604/2009	A / H1N1	C1	19-APR-09	Human	Mexico
EPI_ISL_29710	A/Mexico/4482/2009	A / H1N1	C1	14-APR-09	Human	Mexico
EPI_ISL_29711	A/Mexico/4486/2009	A / H1N1	C1	14-APR-09	Human	Mexico
EPI_ISL_29712	A/Mexico/4108/2009	A / H1N1	C1	02-APR-09	Human	Mexico
EPI_ISL_29717	A/Mexico/4115/2009	A / H1N1	C1	07-APR-09	Human	Mexico

All Sequences were stored in a local database for subsequent analysis

References

1. Squires B, Macken C, Garcia-Sastre A, Godbole S, Noronha J, et al. (2008) BioHealthBase: informatics support in the elucidation of influenza virus host pathogen interactions and virulence. *Nucleic Acids Res* 36: D497-503.
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4. Peters B, Sette A (2007) Integrating epitope data into the emerging web of biomedical knowledge resources. *Nat Rev Immunol* 7: 485-490.
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Supplemental Table - Epitope sequences conserved in swine flu

IEDB ID	Protein	Sequence	Epitope Category
97820	HA	S109, K113, N165	B cell discontinuous
77474	NA	S364, D395, N396	B cell discontinuous
76949	NP	E53, R98, E107	B cell discontinuous
20836	HA	GLFGAIAGF	B cell linear
37043	M1	LKTRPILSPLTKGILGFVFTLTVPSERGLQRRRFVQNALNGND	B cell linear
97650	M2	SLLTEVET	B cell linear
15381	NP	FDERRNKYLEEHPSAGKDPKKTGGPI	B cell linear
17539	NP	FQTAAQR	B cell linear
45359	NP	NPGNAEIEDLIFLAR	B cell linear
67436	NP	TYQRTRALV	B cell linear
97236	PB1	DAVATTHSWIPKRNRSIL	B cell linear
20837	HA	GLFGAIAGFI	T cell class I
97183	M1	AGKNTDLEALMEWLKTR	T cell class I
27350	M1	ILSPLTKGIL	T cell class I
20356	M1	GILGFVFTLV	T cell class I
54953	M1	RMVLASTTAK	T cell class I
4349	M1	ASCMGLIY	T cell class I
33844	M1	KTRPILSPLTK	T cell class I
97506	M1	MSLLTEVETYVLSII	T cell class I
28309	M1	IRHENRMVL	T cell class I
53918	M1	RGLQRRRFVQNALNGNG	T cell class I
20354	M1	GILGFVFTL	T cell class I
58567	M1	SIIPSGPLK	T cell class I
62486	NA	SWPDGAELPF	T cell class I
97772	NP	YERMCNILKG	T cell class I
97173	NP	AEIEDLIFLA	T cell class I
60867	NP	SRYWAIRTR	T cell class I
97614	NP	RMVLSAFDER	T cell class I
53890	NP	RGINDRNFV	T cell class I
55738	NP	RRSGAAGAAVK	T cell class I
35590	NP	LELRSRYWAI	T cell class I
35589	NP	LELRSRYWA	T cell class I
97298	NP	FEDLRVSSF	T cell class I
27283	NP	ILRGVAHK	T cell class I
27126	NP	ILKGKFQTA	T cell class I
97178	NP	AFDERRNKYLEEHPSAGK	T cell class I
7136	NP	CTELKLSDY	T cell class I
13263	NP	ELRSRYWAI	T cell class I
97583	NP	QLVWMACHSAA	T cell class I
19312	NS1	GEISPLPSL	T cell class I

97405	NS2	ITFMQALQLL	T cell class I
97503	PA	MRRNYFTAEVSHCRATEY	T cell class I
17119	PA	FMYSDFHFI	T cell class I
1166	PA	AESRLLLLI	T cell class I
62180	PA	SVKEKDMTK	T cell class I
97781	PB1	YRRPVGISSMVEAMVSRA	T cell class I
42143	PB1	MMMGMFNML	T cell class I
21574	PB1	GPATAQMAL	T cell class I
4177	PB1	ARLGKGYMF	T cell class I
32289	PB1	KMARLGKGY	T cell class I
10514	PB1	DTVNRTHQY	T cell class I
70898	PB1	VSDGGPNLY	T cell class I
97299	PB1	FEFTSFFY	T cell class I
97693	PB1	TLARSICEK	T cell class I
97314	PB1	FVANFSMEL	T cell class I
65880	PB1	TQIQTRRSF	T cell class I
16681	PB1	FLKDVMEISM	T cell class I
6174	PB1	CEKLEQSGL	T cell class I
17780	PB1	FSMELPSFGV	T cell class I
97309	PB1	FNMLSTVLGV	T cell class I
97682	PB1	TFPYTGDPYPYSHGTGTGY	T cell class I
63635	PB1	TFEFTSFFY	T cell class I
97779	PB2	YMLERELVRKTRFLPVA	T cell class I
95905	HA	TGMVDGWYGYHHQNEQGS	T cell class II
96007	HA	WTYNAELLVLENERLTD	T cell class II
95623	HA	NKVNSVIEKMNTQFTAVG	T cell class II
97482	M1	LTKGILGFVFTLTVPSER	T cell class II
51249	M1	QKRMGVQMQRFK	T cell class II
97613	M1	RMVLASTTAKAMEQM	T cell class II
97403	M1	IRHENRMVLASTTAKAM	T cell class II
97740	M1	VLASTTAKAMEQMAGSSEQA	T cell class II
67496	M1	TYVLSIIPSGPLKAEIAQRL	T cell class II
21087	M1	GLQRRRFVQNALNGNDPNN	T cell class II
65112	M1	TLTVPSERGLQRRRFVQNAL	T cell class II
37217	M1	LLENLQAYQKRMGVQMQRFK	T cell class II
97418	M1	KGILGFVFTLTVPSE	T cell class II
65389	M1	TNPLIRHENRMVLASTTAKA	T cell class II
97730	M1	VFTLTVPSERGLQRRRFV	T cell class II
2754	M1	ALMEWLKTRPILSPLTKGIL	T cell class II
1579	M1	AGKNTDLEALMEWLKTRPIL	T cell class II
97280	M1	ERGLQRRRFVQNALNGNG	T cell class II
97306	NP	FLARSALILRGSVAHK	T cell class II

97448	NP	LILRGsvAHKsCLPACVY	T cell class II
45297	NP	NPAHKSQLVWMACHSAAFED	T cell class II
97487	NP	LVWMACHSAAFEDLR	T cell class II
14070	NP	ERRNKYLEEHPSAGKDPKKT	T cell class II
41793	NP	MIWHSNLNDATYQRTRALVR	T cell class II
97637	NP	SFDMSNEGSYFFGDNA	T cell class II
49220	NP	PRMCSLMQGSTLPRRSGAAG	T cell class II
97416	NP	KFQTAAQRAMMDQVRESR	T cell class II
97609	NP	RMCNILKGFQTAAQRAM	T cell class II
36692	NP	LIRMIKRGINDRNFWRGENG	T cell class II
7655	NP	DATYQRTRALVRTGMDPRMC	T cell class II
97361	NP	GQISVQPTFSVQRNLPF	T cell class II
97269	NP	ELIRMIKRGINDRNFWR	T cell class II
10014	NS1	DRLRRDQKS	T cell class II
97623	PA	RSKFLMDALKLSIE	T cell class II
97655	PB1	SPGMMMGMFNMLSTV	T cell class II
97610	PB1	RMFLAMITYITRNQP	T cell class II
97489	PB1	MAFLEESHPIGIFENS	T cell class II
97713	PB1	TVLGVsilNLGqKkYtK	T cell class II
97501	PB1	MMGMFNMLSTVLGVs	T cell class II
59323	M1	SLLTEVETYVL	T cell unknown
32182	NP	KLSTRGVQIASNEN	T cell unknown
97411	NP	KATNPiVPSFDMSNEGSY	T cell unknown
70712	NP	VRESRNPGNAEIEDLIFLARS	T cell unknown
9745	NP	DPRMCSLMQGSTLP	T cell unknown
67439	NP	TYQRTRALVRTGMDP	T cell unknown
38689	NP	LPRRSGAAGAAVKG	T cell unknown
36863	NP	LKGKFQTAAQRAMMDQVRES	T cell unknown
97701	PB1	TNTETGAPQLNPIDGPL	T cell unknown
97354	PB1	GMFNMLSTVLGVsILNL	T cell unknown
97392	PB1	IFENSCLetMEVVQqTRV	T cell unknown
97389	PB1	HRGDTQIQTRRSfELKkL	T cell unknown
97559	PB1	PQLNPIDGPLPEDNEPSGY	T cell unknown
97228	PB1	CKLVGINMSKkKsYINK	T cell unknown
97611	PB1	RMFLAMITYITRNQPEWF	T cell unknown
97237	PB1	DCVLEAMAFLEESHPIGIF	T cell unknown
97747	PB1	VNRTHQYSEKkKwTTNTE	T cell unknown
97353	PB1	GLPVGgNEKkAKLANVVR	T cell unknown
97704	PB1	TQGRQTYDWTlnRNQPAA	T cell unknown
97519	PB2	NFVNranQRLNPMHQllR	T cell unknown
