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Peptide Sharing Between Influenza A H1N1 Hemagglutinin and Human Axon Guidance Proteins

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Epidemiologic data suggest that maternal microbial infections may cause fetal neurodevelopmental disorders, potentially increasing susceptibility to heavy psychopathologies such as schizophrenia, schizophreniform disorder, autism, pervasive developmental disorders, bipolar disorders, psychosis, epilepsy, language and speech disorders, and cognitive impairment in adult offspring. However, the molecular pathomechanisms underlying such a relationship are not clear. Here we analyze the potential role of the maternal immune response to viral infection in determining fetal brain injuries that increase the risk of neurological disorders in the adult. We use influenza infection as a disease model and human axon guidance pathway, a key process in the formation of neural network during midgestation, as a potential fetal target of immune insults. Specifically, we examined influenza A H1N1 hemagglutinin (HA), an antigenic viral protein, for amino acid sequence similarity to a random library of 188 axon guidance proteins. We obtain the results that (1) contrary to any theoretical expectations, 45 viral pentapeptide matches are distributed throughout a subset of 36 guidance molecules; (2) in 24 guidance proteins, the peptide sharing with HA antigen involves already experimentally validated influenza HA epitopes; and (3) most of the axon guidance vs HA peptide overlap is conserved among influenza A viral strains and subsets. Taken together, our data indicate that immune cross-reactivity between influenza HA and axon guidance molecules is possible and may well represent a pathologic mechanism capable of determining neurodevelopmental disruption in the fetus.

Key words: influenza A H1N1 virus/hemagglutinin/immune cross-reactivity/schizophrenia/autism/bipolar disorder

Introduction

Little information exists about the molecular basis underlying schizophrenia. Abnormalities in neurotransmitters (eg, dopamine, 5-hydroxytryptamine, glutamate, and gamma amino butyric acid systems),[1–4] intracellular cytoskeletal assembly,[5] and synapse-associated proteins[6,7] have been thoroughly studied, but attempts to unequivocally relate schizophrenia to alterations in specific molecules are unsatisfying. The old dopamine hypothesis of schizophrenia[1] turned toward a more complex context, involving neurotransmitter interactions and neurocircuits.[8] Also, in many cases, genetic mutations and polymorphisms of genes putatively associated to schizophrenia do not appear related to an increased risk for schizophrenia.[9–16]

Likewise, autism and an ample category of autism-like disorders are of unknown etiology.[17–19] Several hypotheses have been advanced to explain risk for autism, pervasive developmental disorders, speech or language impairment, Asperger's syndrome, and Rett syndrome. Genetic factors[20–22] and prenatal exposure to ultrasounds,[23] environmental stress,[24,25] and microbial infections,[26–29] have been invoked as risk factors that can disrupt fetal brain development. However, notwithstanding worldwide extensive and intensive research efforts to solve the mystery of autism and autism-like pathologies, science and medicine are still facing a lack of knowledge of the molecular basis of such neurodisorders.[17–19]

Actually, it seems that the *primum movens* in autism and schizophrenia is not related to a single molecule or lesion but might deal with a defective neural connectivity originating during neurodevelopment[30–32] as a result of prenatal insult(s).[33] In particular, an association between prenatal viral influenza infection and an increased risk for autism and schizophrenia in the adult offspring has been repeatedly studied,[33–38] and maternal immune activation during pregnancy, rather than direct infection of the fetus, has been advanced as a possible causative insult.[39–42] The time of exposure to influenza virus has been invoked to be a critical factor, and a risk of schizophrenia in humans

appears to be higher for influenza exposure from early to midgestation.[33,39,43] Accordingly, in an experimental model of prenatal exposure to human influenza virus in mice, Fatemi and colleagues[37,44] demonstrated that abnormal changes occur in the offspring following influenza infection at E9 (which corresponds to the middle of the first trimester in humans),[37,45] E16 (which corresponds to the middle of the second trimester in humans),[46] and E18 (which corresponds to late second trimester in humans).[38] The extent of aberrant changes was higher in offspring of E16-infected mice, thus suggesting that infection during middle second trimester leads to heavier effects in the exposed offspring than during middle first or late second trimester.[46] On the other hand, in spite of the epidemiologic and experimental evidence that prenatal exposure to influenza infection may be linked to autism, schizophrenia, and other neurological alterations in the childhood,[29–46] the mechanicistic role(s) of influenza infection in the etiology of such disorders remain to be elucidated.[33]

In the last decade, the possibility of accessing complete human and microbial proteomes provided a new platform for dissecting the host-virus relationships at the phenetic peptide level.[47–50] Indeed, sharing of peptide modules between microbes and the human host may prelude to a subversion of host cellular processes and immune responses.[49–53] Hence, analysis of the shared motifs and of the proteins involved in the overlap may help identify altered cellular functions and associated (immune)pathologies and, possibly, open the way to new preventive/therapeutic approaches[54–58] and more specific diagnostic tools.[58] In exploring the hexapeptide identity platform between the influenza A H5N1 and *Homo sapiens* proteomes, it was found that peptide sharing involves human proteins such as reelin, neurexin I-α, myosin-IXa, Bardet-Biedl syndrome 10 protein, Williams syndrome transcription factor, disrupted in schizophrenia 1 protein, amyotrophic lateral sclerosis 2 chromosomal region candidate gene 17 protein, fragile X mental retardation 2 protein, and jouberin.[48] That is, the influenza A H5N1 polyprotein-vs-human proteome peptide overlap involves human antigens that, when altered, have been reported to be potentially associated with multiple neurological disorders that can include autism, schizophrenia, epilepsy, amyotrophic lateral sclerosis, and sensorineural deafness.

According to this line of research, to understand how maternal immune activation during pregnancy might cause neurodevelopmental alterations and result in psychopathologies in the offspring, in this study we analyzed the pentapeptide overlap between influenza A H1N1 hemagglutinin (HA), a highly antigenic protein,[55,59] and human proteins associated to the axon guidance pathway. In fact, guidance molecules have important roles in neurodegenerative diseases during neural development.[60,61] Moreover, the relationship between prenatal infection and risk of neurological disorders seems to be temporally circumscribed to midgestation,[33,39,46,62] a time frame coincident with guidance protein expression (~16 week of gestation).[63]

We searched HA and axon guidance molecules for common pentapeptide motifs because the optimal amino acid (aa) length of a B-cell epitope is 5 aa.[64–69] Likewise, scientific literature indicates that 5 residues may represent minimal antigenic determinants in T-cell epitopes,[70–75] and numerous pentapeptide epitopes able to bind major histocompatibility complex molecules and induce T-cell proliferation have been described.[76–86] Therefore, pentapeptide sharing between the viral protein and axon guidance proteins might be indicative of potential immune cross-reactions.

Here, we report that HA and axon guidance molecules have a vast pentapeptide overlap that quantitatively exceeds the expected values and qualitatively is endowed with an immunological potential. Our findings support the hypothesis of an immune basis for increased risk of schizophrenia, autism, and other psychopathologies in the offspring following influenza prenatal infection.

Methods

HA protein sequence, UniProtKB/Swiss-Prot accession number: Q67010, 565 aa long, from influenza A H1N1 virus (National Center for Biotechnology Information Taxonomic identifier: 382845, isolate A/swine/Cambridge/1939) was analyzed for pentapeptide sharing with human axon guidance proteins as follows. First, a viral pentapeptide library was constructed by dissecting the HA primary sequence into pentapeptides offset by 1 residue, ie, MKARL, KARLL, ARLLV, RLLVL, etc. Then, each of the final 561 pentamers was analyzed for occurrence(s) within a library consisting of primary sequences of human proteins involved in axon guidance pathway. To eliminate possible bias in the analysis, the axon guidance library was constructed at random using UniProtKB Database (http://www.uniprot.org/)[87] and utilizing the following 5 key words: axon, guidance, proteins, homo, sapiens. The key word-guided search produced 188 human protein entries (see table 1) that directly or indirectly relate to axon guidance pathway and are of different length from 93 aa (SDF1) to 4640 aa (MYCB2) for a total of 176 648 aa. Axon guidance proteins are reported as UniProtKB/Swiss-Prot entry names throughout the article, unless when discussed in detail. Any viral occurrence in the set of guidance proteins was termed a match. Proteins hosting viral match(es) were recorded by UniProtKB/Swiss-Prot entry name and briefly described.

The 2 libraries were searched for aa groupings that were common sequences using pentapeptides as probes. As discussed above, a pentapeptide can be a sufficient minimal determinant for epitope-paratope interaction and thus can act as an immune unit and play a crucial role in cellular immunoreactivity and antigen-antibody recognition.[64–86,88,89]

Multialignment sequence analysis was carried with T-Coffee program[90,91] using HA aa primary sequences corresponding to the following HA Swiss-Prot entries: Q9WFX3, 566 aa (strain A/Brevig Mission/1/1918 [H1N1]); P03455, 566 aa (strain A/Swine/New Jersey/11/1976 [H1N1]); Q9WCE3, 566 aa (strain A/Duck/Australia/749/1980 [H1N1]); P26142, 122 aa (strain A/Camel/Mongolia/1982 [H1N1]); Q07FI5, 565 aa (strain A/China:Nanchang/11/1996 [H1N1]); Q289M7, 565 aa (strain A/New Zealand:South Canterbury/35/2000 [H1N1]); B4URD6, 565 aa (strain A/Russia:St.Petersburg/8/2006 [H1N1]); and A8C8J4, 565 aa (strain A/USA:Texas/UR06-0195/2007 [H1N1]).

The Immune Epitope Database and Analysis Resources (IEDB; www.immuneepitope.org)[92] was used to search for influenza A HA-derived B- and/or T-cell epitopes raised in the human host.

Results

Pentapeptide Sharing Between Influenza A H1N1 HA and Human Axon Guidance Proteins

Table 1 lists the 188 human guidance molecules derived from UniProtKB databank and analyzed for exact pentapeptide matching to HA protein (UniProtKB/Swiss-Prot accession: Q67010) from influenza A H1N1 virus, isolate A/swine/Cambridge/1939. The viral protein was chosen based on the following criteria: (1) known to belong to an influenza strain (swine/Cambridge/1939) that had been studied in the context of the relationship between schizophrenia and the occurrence of influenza epidemics[93] and (2) of significant antigenic and immunogenic impact.[55,59]

Pentapeptide matching to influenza A H1N1 HA protein was carried out as follows. A pentapeptide library of 561 pentamers overlapped by 4 residues was created for influenza A H1N1 HA protein; then, a protein library was created by downloading aa sequences of the 188 guidance molecules listed in table 1 from UniProtKB database. Each viral pentapeptide from the first library was used to search for instances of the same pentapeptide in the axon guidance protein library, and the protein(s) sharing the match(es) were recorded.

The final data on the peptide sharing are reported in table 2. It can be seen that 36 out of the 188 guidance proteins listed in table 1, collected as described under Methods, have pentapeptide matches to HA. Exactly, 45 viral pentapeptide matches are distributed throughout 36 human proteins related to human axon guidance pathway. The 36 axon guidance proteins are briefly described under table 2[94–135] (further details and related references are available at http://www.uniprot.org/). The pentapeptide matching pattern varies, with guidance proteins sharing 2 matches (ANK2, AP2A2, CO6A3, FARP2, FES, GLI2, KS6A1, KS6A2, MYCB2, NFASC, ROBO1, and SEM6A) or even 3 matches (AP2M1 and FEZ2) with HA protein.

Number of Occurrences of HA Pentapeptide Matches in Human Axon Guidance Proteins Is Independent of the Protein Length

Mathematically, the number of times a perfect *n*-peptide match might occur at random in 2 proteins is directly proportional to the product of the protein aa lengths and inversely proportional to the number of possible aa (20) raised to *n*. In the present case, for a protein to have a

Table 1. Random Library of Human Proteins Associated to Axon Guidance Pathway Downloaded from UniProtKB Database

ABL1(1130); ABL2 (1182); ABLM2 (611); ABLM3 (683); ACTB (375); ACTG (375); AGAP2 (1192); ANK1 (1881); ANK2 (3957); AP2A1 (977); AP2A2 (939); AP2B1 (937); AP2M1 (435); AP2S1 (142); ARHGB (1522); ARHGC (1544); B3GN2 (397); BOC (1114); CADH4 (916); CAP1 (475); CAP2 (477); CD166 (583); CD5R1 (307); CDC42 (191); CDK1 (297); CDK5 (292); CHL1 (1208); CLAP1 (1538); CLAP2 (1294); CNTN1 (1018); CO1A1 (1464); CO4A2 (1712); CO4A3 (1670); CO6A3 (3177); COF1 (166); CREB1 (341); CSK21 (391); CSK22 (350); DCC (1447); DLG1 (904); DLG3 (817); DOCK1 (1865); DPYL1 (572); DPYL2 (572); DPYL3 (570); DPYL5 (564); DRAXI (349); DVL1 (695); EFNB1 (346); EGFR (1210); ENAH (591); EPHA7 (998); EPHA8 (1005); EPHB3 (998); ERBB4 (1308); EVL (416); EXT1 (746); EZRI (586); FAK1 (1052); FARP2 (1054); FES (822); FEZ1 (392); FEZ2 (353); FGFR1 (822); FOXD1 (465); FOXD4 (439); FX4L2 (416); FX4L4 (416); FX4L5 (416); FX4L6 (417); FYN (537); FZD3 (666); GFRA1 (465); GFRA3 (400); GLI2 (1586); GLI3 (1580); GRB2 (217); GSK3B (420); HS90A (732); HS90B (724); ITA2 (1181); ITAV (1048); ITB1 (798); ITB3 (788); JIP3 (1336); K319L (1049); KDIS (1771); KGP1 (671); KIF5C (957); KS6A1 (735); KS6A2 (733); KS6A3 (740); LGI1 (557); MET (1390); MK01 (360); MP2K1 (393); MYCB2 (4640); MYH11 (1972); MYH9 (1960); MYO10 (2058); NET1 (604); NFASC (1347); NFIB (420); NOGG (232); NRCAM (1304); NRX1A (1477); NRX3A (1643); PAK1 (545); PAK2 (524); PI51C (668); PLCG1 (1290); PLXA1 (1896); PLXA2 (1894); PLXA3 (1871); PLXA4 (1894); PLXB1 (2135); PLXB2 (1838); PLXB3 (1909); PLXD1 (1925); PO4F2 (409); PRIO (253); PSD95 (724); PTN11 (597); RAC1 (192); RAC2 (192); RAF1 (648); RASH (189); RASK (189); RASN (189); RGMA (450); RHG35 (1513); RHG39 (1083); RHOA (193); RHOB (196); RHOC (193); RHOG (191); RND1 (232); ROBO1 (1651); ROBO2 (1378); ROCK1 (1354); ROCK2 (1388); RRAS (218); RTN4R (473); RYK (604); SCC4 (613); SCN3B (215); SDCB1 (298); SDF1 (93); SEM3E (775); SEM4A (761); SEM4C (833); SEM4D (862); SEM4F (770); SEM4G (838); SEM6A (1030); SEM6D (1073); SH3G2 (352); SHH (462); SHOT1 (631); SIA8B (375); SIAH1 (282); SIAH2 (324); SLIT1 (1534); SLIT2 (1529); SLIT3 (1523); SMO (787); SPON2 (331); SPTA1 (2419); SPTB1 (2137); SPTB2 (2364); SPTN1 (2472); SPTN2 (2390); SPTN4 (2564); SRC (536); SRGP1 (1085); SRGP2 (1071); STIP1 (543); TBB3 (450); TLN1 (2541); TNR16 (427); TRPC4 (977); UBP33 (942); UNC5A (842); UNC5B (945); UNC5D (953); VASP (380); WASL (352); WNT3A (352).

Note: Proteins are given as UniProtKB/Swiss-Prot entry names and listed in alphabetical order with aa length in parentheses.

Table 2. Distribution of HA Pentapeptide Matches in Human Axon Guidance Proteins

Sequence[a]	Pos[b]	Human Axon Guidance Protein[c]
ARLLV	3	SEM4G. Semaphorin-4G. It is a ligand of Plexin-B2, required in cerebellar development[94]
LLVLL[d]	5	ARHGB. Rho guanine nucleotide exchange factor 11. Modulator of RhoGEF glutamate transport[95]
		CO4A3. Collagen α-3(IV) chain. Goodpasture antigen. Binds to a protein that is highly expressed in neurons of the cerebral cortex, hippocampal formation, the basal ganglia, the olfactory bulb and nuclei of the thalamus, the hypothalamus, and the septal area[96]
TLAAT	11	SPTA1. Spectrin α chain, erythrocytic 1. Defects in SPTA1 are the cause of epileptic encephalopathy early infantile type 5[97]
LLEKN	36	SPTN4. Spectrin beta chain, nonerythrocytic 4. Involved in nervous system membrane biogenesis and in ion channel clustering[98]
SVNLL	46	AP2M1.[e] AP-2 complex subunit mu. Involved in axonal growth cone motility[99]
VNLLE	47	CO6A3.[f] Collagen α-3(VI) chain. Protects against neuronal apoptosis[100]
LLEDS	49	GFRA1. GDNF family receptor α-1. Involved in establishing synaptic contacts and promoting the assembly of presynaptic terminals[101]
HNGKL	54	SEM6A.[f] Semaphorin-6A. It is as a major contributor to the guidance of corticospinal tract axons at multiple choice points[102]
GNPEC	80	KS6A1.[f] Ribosomal protein S6 kinase α-1. Involved in signaling pathways responsible for tuberous sclerosis complex 2 phosphorylation. Involved in hippocampal signaling cascades in consolidation of fear memory[103–106]
SLLPA	86	GLI2.[f] Zinc finger protein GLI2. Gli2 alterations affect Slit function in pioneer longitudinal guidance[107]
LLPAR	87	GLI2.[f] See previous entry
NSETG	101	AP2M1.[e] See previous entry
SETGA	102	AP2M1.[e] See previous entry
PGDFI	109	CLAP2. CLIP-associating protein 2. Regulates microtubule plus-end dynamics at the cell cortex[108]
EELRE	116	FEZ2.[e] Fasciculation and elongation protein zeta-2. Involved in axonal outgrowth and fasciculation[109,110]
ELREQ	117	FEZ2.[e] See previous entry
LREQL	118	FEZ2.[e] See previous entry
		PSD95. Postsynaptic density protein 95. Disks large homolog 4. Required for synaptic plasticity associated with NMDA receptor signaling[111,112]
EQLSS	120	FGFR1. Fibroblast growth factor receptor 1. Required for normal mesoderm patterning and correct axial organization during normal development of the gonadotropin-releasing hormone neuronal system[113]
SSVSS	123	ROBO1.[f] Roundabout homolog 1. Receptor for SLIT1 and SLIT2. Acts as molecular guidance cue in axonal navigation at the ventral midline of the neural tube and projection of axons to different regions during neuronal development[114,115]
SVSSL	124	DOCK1. Dedicator of cytokinesis protein 1. Required for projection of commissural axons in the neural tube[116]
SSLER	126	SEM6A.[f] See previous entry
PNHNT	141	MYCB2.[f] E3 ubiquitin-protein ligase MYCBP2. Key regulator for axon guidance, outgrowth, and synapse development[117]
LTKKG	167	FARP2.[f] FERM, RhoGEF and pleckstrin domain-containing protein 2. Involved in the response of neuronal growth cones to class-3 semaphorins[118]
TKKGN	168	ANK1. Ankyrin-1. Integral membrane protein. Isoform Br21 is expressed in brain[119]
		ANK2.[f] Ankyrin-2. Brain ankyrin. Structurally defines terminal microdomains of peripheral somatosensory axons[120]
VNNKG	182	MYCB2.[f] See previous entry
RFTPE	225	ANK2.[f] See previous entry
GDTII	253	NFASC.[f] Neurofascin. Involved in neurite extension, axonal guidance, synaptogenesis, and myelination[121]
DTIIF	254	NFASC.[f] See previous entry
EATGN	259	ROBO1.[f] See previous entry
GNLIA	262	AP2A1. AP-2 complex subunit α-1. Component of the adaptor protein complex 2. Expressed in forebrain, spinal cord, cerebellum[122]
		AP2A2.[f] AP-2 complex subunit α-2. Component of the adaptor protein complex 2. Huntingtin-interacting protein 9[122,123]
NLIAP	263	FARP2.[f] See previous entry
TSNAS	283	DLG1. Disks large homolog 1. Dlg1-PTEN interaction regulates myelin thickness to prevent peripheral nerve overmyelination[124]
SLPFQ	305	KS6A1.[f] See previous entry
		KS6A2.[f] Ribosomal protein S6 kinase α-2. Chronic activation of p90RSK has been shown in human epileptic hippocampus. Involved in hippocampal signaling cascades in consolidation of fear memory[104–106]
KYVRS	321	FES.[f] Tyrosine-protein kinase Fes/Fps. Cooperates to induce neurite extension[125]
TGLRN	332	SLIT1. Slit homolog 1 protein. Molecular guidance cue. Function seems to be mediated by interaction with ROBO receptors[114,115,126]
GLRNI	333	NRX3A. Neurexin-3-α. Neuronal synaptic adhesion molecule[127]

Table 2. Continued

Sequence[a]	Pos[b]	Human Axon Guidance Protein[c]
SIQSR	339	ROCK2. Rho-associated protein kinase 2. Plays a role in the regulation of spine and synaptic properties in the hippocampus[128,129]
SRGLF	342	ITA2. Integrin α-2. Integrin-ECM interactions regulate axonal outgrowth and pathfinding[130]
AELLV	439	FES.[f] See previous entry
LLVLL[d]	441	ARHGB. See previous entry
		CO4A3. See previous entry
AKEIG	473	RAC1. Ras-related C3 botulinum toxin substrate 1. Plays an essential role in neuronal development[131]
QILAI	528	SEM4F. Semaphorin-4F. Expressed during neurodevelopment and in the adult brain. Attached to PSD95 in glutamatergic synapses[132]
STVAS	534	AP2A2.[f] See previous entry
VASSL	536	ARHGC. Rho guanine nucleotide exchange factor 12. LARG protein. Mediates the action of repulsive guidance molecule[133]
		KS6A2.[f] See previous entry
LVLLV	540	CO6A3.[f] See previous entry
SNGSL	555	DCC. Netrin receptor DCC. Mediates axon attraction of neuronal growth cones during neurodevelopment upon ligand binding[134]
		EFNB1. Ephrin-B1. Regulates axon guidance by reverse signaling through a PDZ-dependent mechanism[135]

[a]Pentapeptide sequence, with aa given in 1-letter code.
[b]aa position along the HA sequence.
[c]Human axon guidance proteins given with UniProtKB/Swiss-Prot entry and recommended name.
[d]LLVLL occurs twice in HA, at aa pos 5 and 441.
[e]Guidance protein with three pentapeptide matches to HA.
[f]Guidance protein with two pentapeptide matches to HA.

HA pentapeptide match, it must have a minimal length equal to 5664 aa. For the 2 libraries analyzed here (eg, the HA pentapeptide set amounting to 565 aa, and 176 648 aa, respectively), the theoretical number of pentapeptide matches is 31.2.

Hence, it has to be observed that, as an average value, the comprehensive overlapping extent reported in table 2 (equal to 45 pentapeptide matches) is about 1.5-fold higher than the theoretical value.

In addition, it is of interest that HA pentapeptide matching varies among the 36 guidance molecules listed in table 2 independently of the protein length (figure 1). In fact, short polypeptides such as FEZ2 and AP2M1 (353 and 435 aa, respectively; see table 1) have up to 3 HA pentapeptide occurrences (table 2). That is, the actual values are respectively higher 48 and 39 times than the theoretical ones. The theoretical number of HA pentapeptide matches for each of the 36 human axon guidance proteins, which are listed in table 2 and range from 192 aa (RAC1) to 4640 aa (MYCB2), is reported in figure 1A. The ratio between theoretical and actual HA pentapeptide matching is shown in figure 1B. On the whole, figure 1 shows that, mostly, the actual number of HA pentapeptide matches in the 36 human axon guidance proteins is independent of the protein length.

HA-vs-Axon Guidance Peptide Overlap Is Highly Conserved Among Influenza A H1N1 Viral Strains

Next, we investigated whether the pentapeptide commonality with human guidance molecules was a property unique to the HA sequence under study (Q67010, from isolate A/H1N1/swine/Cambridge/1939). To this end, a multialignment sequence analysis was carried out on HA aa sequences from the following influenza A HIN1 strains: Brevig Mission/1/1918 (Q9WFX3); Swine/New Jersey/11/1976 (P03455); strain A/Duck/Australia/749/1980 (Q9WCE3); Camel/Mongolia/1982 (P26142); China:Nanchang/11/1996 (Q07FI5); New Zealand:South Canterbury/35/2000 (Q289M7); Russia:St.Petersburg/8/2006 (B4URD6); and USA:Texas/UR06-0195/2007 (A8C8J4). The multialignment analysis is reported in figure 2. It can be seen that the HA-vs-axon guidance peptide overlap described in table 2 is highly conserved among influenza A H1N1 strains, even at the heptapeptide level (see the EELREQL heptapeptide shared with FEZ2). In other words, the HA peptide sequences shared with guidance molecules and derived from an influenza strain that characterized a H1N1 swine infection in 1939 are highly conserved in influenza A H1N1 strains that temporally succeeded from 1918 to 2007, are present in different world geographical areas, and have been responsible for infections in humans and other hosts (birds, pigs, camels).

Similar data of conservativeness were found in examining other influenza A subtypes (data not shown).

The Pentapeptide Overlap Between HA and Guidance Proteins Has an Immunologic Potential

As discussed under Introduction, neuropathologic diseases such as autism, schizophrenia, bipolar disorder with

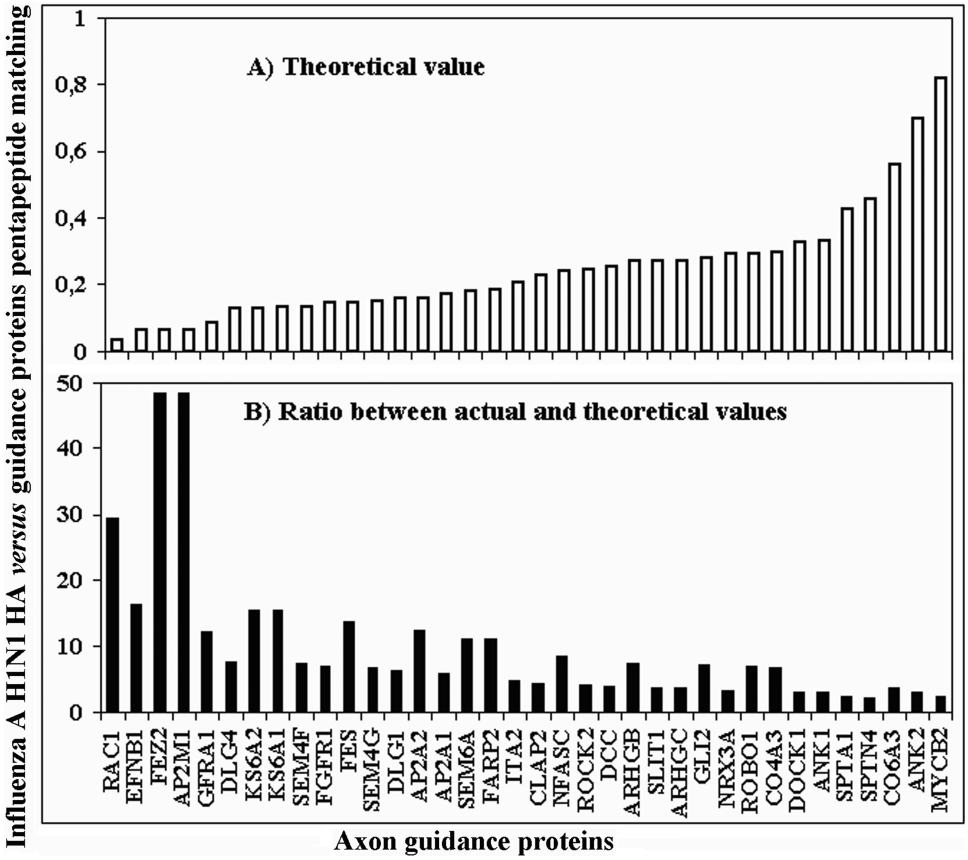

Fig. 1. Actual-vs-theoretical pentapeptide matching between HA and human axon guidance proteins. Axon guidance proteins are listed along the *x*-axis according to increasing aa length. Actual matching values derived from table 2.

psychotic features, brief psychosis, and psychosis could potentially ensue from maternal immune responses to influenza developed during the pregnancy.[29,33–46] Aiming at understanding the molecular mechanism(s) linking anti-influenza maternal immune response(s) and fetal brain insult(s), we proceeded trying to determine the immunologic potential of the HA-vs-axonal proteins pentapeptide overlap described in table 2, and figures 1 and 2.

For this purpose, we investigated the potential cross-reactivity among influenza A epitopes and human axonal guidance molecules using IEDB (www.immuneepitope.org), a public database developed to classify epitope data that had been experimentally validated and controlled in a number of research laboratories worldwide.[92] Specifically, the IEDB database was searched for B- and T-cell peptide epitopes derived from influenza A H1N1 HA, targeted by human humoral and/or cellular response(s), and sharing pentapeptide(s) with axon guidance molecules (see table 2). The results obtained following the IEDB search are reported in table 3. It can be seen that 24 out of the 45 pentapeptides shared between HA and axon guidance proteins are, sometimes repeatedly, present in 42 influenza A H1N1 HA-derived epitopes as consecutively overlapping and/or adjacent pentapeptide sequences as exemplified in epitope ID 798, ADY*EELREQLSSVSS*, and epitope ID 80050, *TGLRNIPSIQSR*G (with peptide fragments shared between HA and guidance proteins given in italics). Data similar to those reported in table 3 were also found in B- and T-cell epitopes derived from other influenza A subtypes (data not shown).

In short, table 3 suggests that 24 fundamental axon guidance molecules (ARHGB, ARHGC, CLAP2, CO4A3, CO6A3, DCC, EFNB1, FARP2, FES, FEZ2, FGFR, GFRA1, ITA2, KS6A1, KS6A2, NFASC, NRX3A, PSD95, RAC1, ROBO1, ROCK2, SEM4F, SEM6A, and SLIT1; see table 2 for a brief protein description) are potentially hittable by cross-reactions following human immune responses against influenza A H1N1. In this regard, it is pertinent to note that autoantibodies against neurofascin (UniProtKB/Swiss-Prot entry: NFASC, an axon guidance protein that shares a pentapeptide with the HA epitope IEDB ID 79792, see tables 2 and 3) result in axonal injury.[136] Moreover, it has to be noted that table 3 describes only linear epitopic sequences. Actually the issue of conformational epitopes might add to the cross-reactivity potential illustrated in tables 2 and 3. As a matter of fact, a search through IEDB epitopes shows, eg, that the discontinuous HA epitope, $S^{138}S^{139}P^{141}N^{142}K^{171}G^{172}S^{173}S^{174}Y^{175}P^{176}K^{177}$

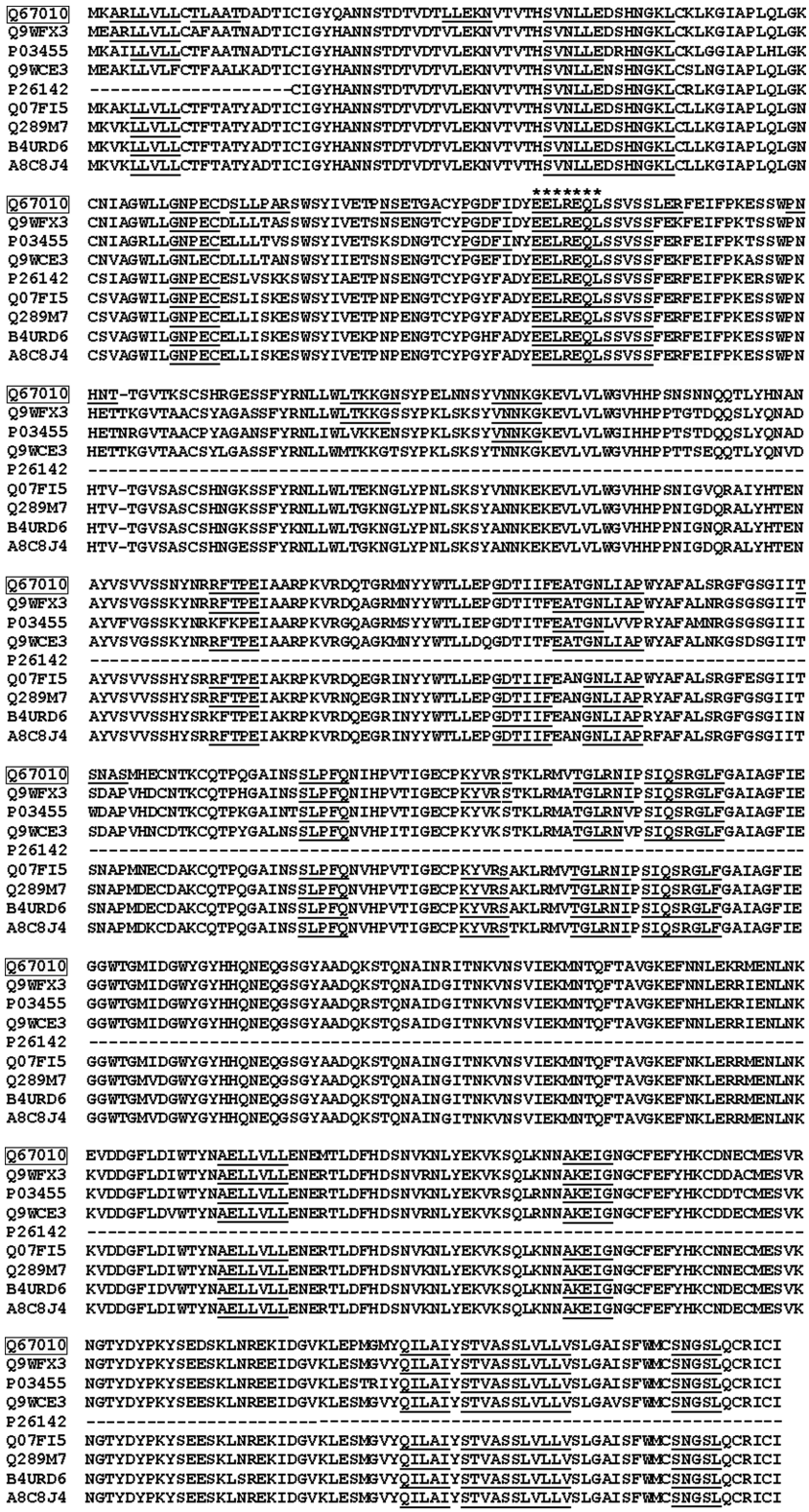

Fig. 2. HA peptide modules common to human axon guidance proteins are highly conserved among influenza A virus H1N1 strains. CLUSTAL W multiple sequence alignment of HA sequences from influenza A virus H1N1 strains was obtained using the T-Coffee program.[90,91] HA UniProtKB/Swiss-Prot accessions correspond to: Q67010 (swine/Cambridge/1939); Q9WFX (Brevig Mission/1/1918); P03455 (Swine/NewJersey/11/1976); Q9WCE3 (Duck/Australia/749/1980); P26142 (Camel/Mongolia/1982); Q07FI5 (China:Nanchang/11/1996); Q289M7 (New Zealand: South Canterbury/35/2000); B4URD6 (Russia: St.Petersburg/8/2006); A8C8J4 (USA:Texas/UR06-0195/2007); HA sequence under study (Q67010) is given boxed; aa given in 1-letter code; viral peptide modules common to human axon guidance molecules (see table 2) are given in italics; asterisks highlight the heptapeptide common to viral HAs and human FEZ2.

Table 3. Peptides Shared Between Influenza H1N1 HA and Guidance Proteins Are Present in B- and/or T-Cell Epitopes

IEDB ID[a]	Epitope Sequence[b,c]	Immune Recognition Context	Influenza A H1N1 Virus Strain
798	ADY*EELREQLSSVSS*	T	New Caledonia/20/1999
6801	CP*KYVRS*AKL	T	Puerto Rico/8/1934
26959	ILAIY*STVASSL*	T	California/04/2009
28063	IP*SIQSR*GL	T	Puerto Rico/8/1934
29691	IY*STVASSL*VL	T	Puerto Rico/8/1934
35415	LEDS*HNGKL*	T	Denver/1957
61460	*SSVSS*FERF	T	camel/Mongolia/1982
79792	*DTIIF*EAN*GNLIA*	T	New Caledonia/20/1999
79809	E*LLVLL*ENERTLD	T	New Caledonia/20/1999
79864	IIFEAN*GNLIAP*W	T	New Caledonia/20/1999
80034	S*SLPFQ*NVHPVTI	T	New Caledonia/20/1999
80050	*TGLRNI*P*SIQSR*G	T	New Caledonia/20/1999
113324	DGFLDIWTYN*AELLV*	T	New Caledonia/20/1999
113375	*EQLSSVSS*FERFE	T	New Caledonia/20/1999
113595	*KYVRS*AKLRMVT	T	New Caledonia/20/1999
125913	E*LLVLL*ENERTLDYHDS	T	California/04/2009
126340	ITF*EATGN*LVVPRYAFA	T	California/04/2009
128403	DDGFLDIWTYN*AELLV*L	T	New Caledonia/20/1999
129178	KVKSQLKNN*AKEIG*NG	T	New Caledonia/20/1999
129221	LEKNVTVTH*SVNLLEDS*	T	New Caledonia/20/1999
129319	LRMV*TGLRNI*P*SIQSR*G	T	New Caledonia/20/1999
129320	LRNIP*SIQSRGLF*GAIA	T	New Caledonia/20/1999
129890	SFWMC*SNGSL*QCRICI	T	New Caledonia/20/1999
129938	SLGAISFWMC*SNGSL*Q	T	New Caledonia/20/1999
130394	Y*QILAI*Y*STVASSL*VLL	T	New Caledonia/20/1999
144630	E*LLVLL*ENERTLDYHDSNVK	T	California/04/2009
144688	IDY*EELREQLSSVSS*FERFE	T	California/04/2009
144696	IY*QILAI*Y*STVASSL*VLVVSLGA	T	California/04/2009
144732	NLYEKVRSQLKNN*AKEIG*NG	T	California/04/2009
144810	VLEKNVTVTH*SVNLLE*DK	T	California/04/2009
144813	VTH*SVNLLE*DK*HNGKL*CK	T	California/04/2009
150978	EKNVTVTH*SVNLLE*D	B	California/04/2009
150998	IL*GNPEC*ESLSTASS	B	California/04/2009
150999	IP*SIQSRGLF*GAIAG	B	California/04/2009
151026	*LREQLSSVSS*FERFE	B	California/04/2009
151036	NLLEDK*HNGKL*CKLR	B	California/04/2009
151056	SQLKNN*AKEIG*NGCF	B	California/04/2009
151063	TF*EATGN*LVVPRYAF	B	California/04/2009
151064	TPKGAINT*SLPFQ*NI	B	California/04/2009
151075	YN*AELLVLL*ENERTL	B	California/04/2009
151076	Y*PGDFI*DY*EELREQL*	B	California/04/2009
152919	*QLSSVSS*FERFEIFPKTSSW	T	California/04/2009

Note: IEDB was searched for influenza A HA epitopes that had produced humoral or cellular immune responses in the human host. The search generated 537 epitopes; of which, 120 refer to H1N1 strains, and 42 correspond to H1N1 HA-derived sequences containing peptide fragments (in italics) shared between HA and guidance proteins (see table 2).
[a]For epitope further details and references, refer to www.immuneepitope.org.[92]
[b]aa sequences given in 1-letter code.
[c]Peptide fragments shared between HA and guidance proteins are given in italics.

$S^{179}K^{180}S^{181}V^{183}N^{211}E^{260}T^{262}$ (IEDB ID: 1779502) from the A/California/04/2009 H1N1 virus,[137] hosts the pentapeptide KGSSY that is present in the axon guidance molecule ROBO1 (aa pos 1525–1529).

Discussion

This study demonstrates that anti-HA humoral and/or cellular immune responses that follow influenza A H1N1 infection in humans have the potential to cross-react with human axon guidance molecules. In the context of our random guidance molecule selection, such a potential immunologic cross-reactivity involves 24 crucial guidance proteins (ARHGB, ARHGC, CLAP2, CO4A3, CO6A3, DCC, EFNB1, FARP2, FES, FEZ2, FGFR, GFRA1, ITA2, KS6A1, KS6A2, NFASC, NRX3A, PSD95, RAC1, ROBO1, ROCK2, SEM4F, SEM6A, and SLIT1; see table 2 for a brief protein description). Qualitatively,

the immunogenic potential of influenza A H1N1 HA epitopes is often multitargeting. The sequence EELREQLSSVSS (pos 116–127, table 2) is exemplar in this regard. It contains an heptapeptide in common with FEZ2 (EELREQL), and shares a pentapeptide with PSD95 (LREQL), FGFR1 (EQLSS), and ROBO1 (SSVSS). That is, an anti-influenza immune response targeting a viral epitope containing the EELREQLSSVSS sequence might cross-react with 4 fundamental axon guidance proteins that have been already implicated in the genesis of schizophrenia, autism, and bipolar disorders.

- FEZ2 (or Fasciculation and elongation protein zeta-2) is an ortholog of the *C. elegans* unc-76 gene,[109] and is involved in axonal outgrowth and fasciculation.[110] That is, it is necessary for normal axonal bundling and elongation within axon bundles.
- PSD95 (or Postsynaptic density protein 95; alternative names: SAP90, Synapse-associated protein 90; and DLG4, Disks large homolog 4) is highly expressed in postsynaptic density of neurons in the forebrain and in presynaptic region of inhibitory synapses formed by cerebellar basket cells on axon hillocks of Purkinje cells. PSD95 is required for synaptic plasticity associated with NMDA receptor signaling. Indeed, complex and crucial functions are supported by PSD95 as reified by an extremely high number of molecular interactions (see http://www.uniprot.org/uniprot/P78352 for details and references). Of course, these molecular interactions (and related functions) might be perturbed by an immune attack targeting PSD95. For example, PSD95 associates with TMEM16B, a protein with calcium-dependent chloride channel activity,[138–141] involved in deciphering and modulating the dynamic neuronal signaling in neurons important for learning and memory.[142] Moreover, overexpression or depletion of PSD95 changes the ratio of excitatory to inhibitory synapses in hippocampal neurons.[111,112,143–146] Hence, it is feasible to hypothesize (and address research to verify) that an immune attack against PSD95 might disrupt interaction(s) with TMEM16B and alter the mechanisms of learning and memory, thus generating learning disabilities as pathologic consequences.
- FGFR1 (or Fibroblast growth factor receptor 1) is required for normal mesoderm patterning and correct axial organization during normal development of the gonadotropin-releasing hormone neuronal system;[147–151]
- ROBO1 (or Roundabout homolog 1) is a receptor for SLIT1 and SLIT2 and acts as molecular guidance cue in cellular migration, including axonal navigation at the ventral midline of the neural tube and projection of axons to different regions during neuronal development.[114,115,152,153]

In essence, an immune response against influenza A infection may cross-react with FEZ2, PSD95, FGFR1, ROBO1 and other crucial guidance proteins, in this way disrupting fundamental molecular interactions in the developing brain. Later, infection(s) with (or vaccination protocols against) different influenza A H1N1 strains or A subtypes might evoke immune responses cross-reactive with the same epitopic targets already damaged during the fetal life. Indeed, as discussed above (see also figure 2), sequence analyses and epitope-coverage information reveal highly conserved protein regions in influenza strains that can be recognized by the human immune system as possible targets for inducing cross-reactions.[154] Moreover, cross-reactivity as a pathogenic mechanism linking maternal infections and neuropsychological disturbances in the future adult appears feasible considering the experimental evidence emerged from the many well-known data according to which, eg, (1) short synthetic peptides may define T-cell influenza epitopes[155]; (2) an exceptionally broad pattern of immunodominance characterizes the primary HLA-DR1-restricted CD4 T-cell response to influenza virus HA in HLA-DR1 transgenic mice[156]; and (3) a high level of cross-reactivity exists between an influenza virus HA-specific CD4+ T-cell clone derived from a patient with multiple sclerosis and 14 influenza HA variants, 11 viral, 15 human, and 3 myelin-derived peptides.[157] Thus, the present data might also represent a clue to investigate the origin and nature of brain reactive autoantibodies that have been repeatedly detected and hypothesized to play a role in neuropsychiatric disorders.[158–163] In this framework, our findings indicate that influenza infections and, obviously, anti-influenza vaccination have the potential to cause neuropathologies because of the extensive influenza-vs-human peptide commonality.[164] This study might help disentangle the intricated and mysterious context of the risk of autistic and schizophrenic disorders following maternal infections, thus also addressing the issue of influenza vaccination during pregnancy. Indeed, using sequence uniqueness as a criterion for constructing anti-influenza vaccines would eliminate potential autoimmune cross-reactions.[48,55,164] More in general, this study represents a methodological approach to dissect the links between microbial infections and neuropathologies at the molecular level.

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