

Molecular Mimicry Revisited: Gut Bacteria and Multiple Sclerosis

Fred C. Westall*

Institute for Disease Research, P.O. Box 890193, Temecula, California 92589

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Molecular mimicry is a possible explanation for autoimmune side effects of microorganism infections. Protein sequences from a particular microorganism are compared to known autoimmune immunogens. For diseases such as multiple sclerosis (MS), where the infectious agent is unknown, guesses to its identity are made. Mimics are assumed to be rare. This study takes a radically different approach. Reported sequences from all known human bacterial and viral agents were searched for autoimmune immunogen mimics. Three encephalitogenic peptides, whose autoimmune requirements have been studied extensively, were selected for comparison. Mimics were seen in a wide variety of organisms. For each immunogen, the mimics were found predominantly in nonpathogenic gut bacteria. Since the three immunogens used in this study are related to MS, it is suggested that a microorganism responsible for autoimmune activity in MS could be a normally occurring gut bacterium. This would explain many of the peculiar MS epidemiological data and why no infective agent has been identified for MS and supports recently found MS gut metabolism abnormalities.

During the past 20 years molecular mimicry has been proposed as an explanation for autoimmune side effects of microorganism infections (3–5, 13, 15, 19, 41, 53). The process has involved comparing sequences within the proteins of a microorganism, usually a virus, with known human autoimmune immunogens. The probability of finding a particular sequence is 1 in 20^n , where 20 refers to the 20 amino acids and “ n ” is the number of residues within the sequence under consideration. Finding a duplicate sequence in a microorganism which is identical to a human sequence would appear to be impossible. However, it is not that difficult. First, some amino acids, such as leucine and glycine, are used far more than others, such as tryptophan and histidine. Thus, some sequences are more likely to appear than others. Second, amino acid usage within sequences varies with the species. Third, one does not need to find an exact match to a human immunogen. Each peptide immunogen is composed of amino acids that each contribute differently to the overall immunogenicity of the peptide. Therefore, substitutions can be made in the original immunogen without destroying its activity. For example, it can be easily estimated that 10,000 peptides would satisfy the encephalitogenic requirements for the tryptophan peptide, fswgaegqr, of myelin basic protein (43, 44, 47).

Quite often if a sequence, under consideration, contains some amino acids that match the immunogen in type and location, the sequence is considered a possible mimic. Very seldom is there a concern for the location of the microorganism’s sequence within the cell or within its protein structure itself, i.e., whether the microorganism’s sequence has access to the human immune system. More importantly, the individual contributions to autoimmunity of the individual amino acids of the human immunogen are not considered. Without considering the individual amino acid contributions of the sequence fswgaegqr responsible for autoimmune encephalomyelitis (EAE), for example, which one of the following sequences

would be considered a likely encephalitogen (characters in bold-face represent changes in the sequence from the original fswgae gqr): fswgaigqr, fswaaegqr, or fswgaeger? The correct answer is the first sequence (47). Most would pick the second or the third. The glycine allows an essential bend at the glycine-tryptophan bond (17, 25). The glutamate/glutamine substitution results in negating the positive arginine (50). Therefore, to adequately analyze potential microorganism mimics, one needs to have a totally defined human immunogen. This presents a problem.

In 1970 I synthesized and chemically defined the requirements for EAE induction from the first myelin basic protein encephalitogenic sequence (8, 43, 47). The contribution of each of the nine amino acids to immunity was ascertained. Since then, numerous (Table 1) other short encephalitogenic regions have been discovered within the myelin basic protein and the other myelin proteins. With a few exceptions, the other regions have only been isolated and in some cases synthetically

TABLE 1. Major defined encephalitogenic sequences^a

Encephalitogen	Active EAE species	Reference(s)
Myelin basic protein		
fswgaegqr-tryptophan pep.	Guinea pig, rabbit, monkey	7, 9, 47
tthygslpqqk-mid-peptide	Rabbit, DR rat	31, 33
pqksqrtdenpv-hyperacute	Lewis rat, monkey	45, 52
fklggrdsr	Rabbit, monkey	16, 51
Lipoprotein		
hslgkwlgpdkf	Mouse	39
ntwttcqsiapfsk	Mouse	14
yktticgklsatv	Mouse	38
dyeylinvihafqyv	Mouse	1
Oligodendrocytic glycoprotein		
mevgwyrppfsrvhlyrngk (also 1–22, 43–57, and 92–106)	Mouse	2, 24

^a The decision of what should be included as a “major” encephalitogenic sequence is quite arbitrary. The result depends upon the laboratories testing the sequence and the animal being tested. The purpose of this table is primarily to show that there are several proteins that are encephalitogenic, and within these proteins there are numerous encephalitogenic regions.

* Mailing address: Institute for Disease Research, P.O. Box 890193, Temecula, CA 92589. Phone: (951) 699-5177. Fax: (951) 699-5177. E-mail: fcwestallidr@adelpia.net.

TABLE 2. Potential encephalitogenic regions within proteins from known human bacteria: mid-region encephalitogen from myelin basic protein hygslp

Organism ^a	Description ^b	Sequence ^c	Species
<i>Burkholderia cepacia</i> (-)	Selenophosphate synthase 46317954	vhygslp wla	
<i>Enterococcus faecium</i> (-)	Peptidase M20/M25/M40 family 29374880	yqygtlp vin	
<i>Bacteroides</i> spp. (-)	Hypothetical BT 1743 29347153	vhfgslp tye	<i>B. thetaiotaomicron</i>
	Hypothetical 53715314	fhfgtlp vqe	<i>B. fragilis</i>
	Hypothetical 53714072	shfgalp qsi	<i>B. fragilis</i>
	DNA primase 60681803	glygalp edl	<i>B. fragilis</i>
	Outer membrane-nutrient binding 29341170	drygslp ntg	<i>B. thetaiotaomicron</i>
	Outer membrane-nutrient binding 29341066	skygnlp ns1	<i>B. thetaiotaomicron</i>
<i>Escherichia coli</i> (-)	Hypothetical 75259460	ilygslp vef	
	Hypothetical 26250174	chygslp pvw	
	Intimin 18202007	veygalp vlg	
	ATP-binding D-ribose high-affinity transport rbs A 1790190	vlygalp rts	
	Membrane-permease yicL 26250402	arygtlp vvw	
	Membrane uidC 34396001	ysygslyp rrr	
	K88 fimbrial AC 120423	ifygslp rgs	
<i>Streptococcus</i> spp. (+)	Glycogen synthase 41017195	mrygslyp lvh	<i>S. agalactiae</i>
	SepSi6A 58197483	khfgtlp kvs	<i>S. suis</i>
	LepA 28895013	ryygalp ing	<i>S. pyogenes</i>
	Permease 24379316	kttygtlp sqd	<i>S. mutans</i>
<i>Lactobacillus</i> spp. (+)	Starch synthase 28376995	mhygtlp lvh	<i>L. johnsonii</i>
	Hypothetical 42518201	gyfyslyp tda	<i>L. johnsonii</i>
	Hypothetical 68160961	viygslyp vwqe	<i>L. reuteri</i>
	Aspartokinase 62515517	gyfyslyp ngv	<i>L. delbrueckii</i>
	α -Glucosidase 23003366	gyfyalp ptn	<i>L. gasseri</i>
	DNA helicase 58337296	khfgsl klngk	<i>L. acidophilus</i>
<i>Clostridium</i> spp. (+)	Aspartokinase 28211949	gyfyslyp ngd	<i>C. tetani</i>
	Glycogen synthase 15025239	lrygslyp ivr	<i>C. acetobutylicum</i>
	Acetoacetate decarboxylase 49036684	lkygalp vvt	<i>C. beijerinckii</i>
<i>Bifidobacterium</i> spp. (+)	Hypothetical 23466177	tnygalp gsi	<i>B. longum</i>
<i>Klebsiella</i> spp. (-)	<i>N</i> -Carbamyl-L-amino acid amidohydrolase 38016761	sytygtlp pavd	<i>K. pneumoniae</i>
<i>Fusobacterium nucleatum</i> (+)	Hypothetical 34762382	khyslyp Eks	
<i>Mycobacterium</i> spp. (+)	ABC transport 840831	ayygalp liv	<i>M. tuberculosis</i>
	SecD 41407141	lkygslyp lsf	<i>M. avium</i>
<i>Salmonella enterica</i> serovar Typhimurium (-)	Inner membrane transport yicL 20141848	aqygtlp vvg	
<i>Chlamydomonas reinhardtii</i> (-)	Methionine amino peptidase 6647435	fhygslp fpk	
<i>Haemophilus influenzae</i> (-)	Glycogen synthase 1169909	lyqygtlp ivr	
<i>Bacillus cereus</i> (+)	DEAD/DEAH box helicase 42779931	fhygnlp lii	
<i>Pseudomonas fluorescens</i> (-)	Lipopolysaccharide biosynthesis 70732417	qyyslyp qgy	
<i>Actinobacillus actinomycetemcomitans</i> (+)	Unknown 10880891	thygtlp qdl	

^a Symbols in parentheses: +, gram positive; -, gram negative.

^b Numbers represent access identification numbers on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>).

^c Boldface characters in the sequences indicate amino acids making major contributions to encephalitogenicity.

produced. Even though EAE is by far the most studied autoimmune disease and is considered to be the autoimmune model for multiple sclerosis (MS) and viral neuropathies, few of its immunogens have been adequately defined, i.e., the contribution of each of the amino acids to immunity has not been examined.

The molecular mimicry process has centered on examining an individual microorganism for a sequence which fulfills the requirements for a given human immunogen. I report here the results of the opposite approach. The proteins of a large number of human bacterial and viral nonpathogenic and pathogenic organisms are compared to the three most studied encephalitogenic sequences. The following questions are asked: (i) How plentiful are the matches? (ii) Are these sequences found more often in bacterial or viral organisms? (iii) Is there a particular species that contains these mimics? (iv) Do matches favor a particular encephalitogen? (v) How are these results relevant to multiple sclerosis? (vi) Are these results applicable to searches for other human immunogen mimics?

MATERIALS AND METHODS

Selection of encephalitogenic sites. EAE has for years been used as the autoimmune model for multiple sclerosis (18). This disease is induced when

whole brain, myelin, one of the myelin proteins, or encephalitogenic peptides in an appropriate adjuvant are injected into test animals. The fact that one autoimmune disease, EAE, is initiated even when whole brain is used as the encephalitogen points to the special immunological nature of the myelin proteins. Table 1 lists the major encephalitogenic regions within each of the encephalitogenic myelin proteins. Most of the encephalitogenic regions found in the myelin proteins only have been sequenced. However, the major three encephalitogenic regions of the myelin basic protein have been extensively studied. The encephalitogenic contribution of each of the amino acids from the tryptophan region, fswgaegqr, has been examined thoroughly in the guinea pig (8, 29, 37, 43, 44, 47, 50). The midpeptide, tthygslpqq, has been studied in the DR rat and rabbit (31, 33). The hyperacute EAE site, pqksqrtdenpv, has been analyzed in the Lewis rat (20-22, 45, 52). Furthermore, the tryptophan peptide is encephalitogenic in rabbits (9), guinea pigs, and monkeys (7) but not in Lewis rats. The midpeptide has been found active in rabbits and DR rats but not in guinea pigs. Finally, the hyperacute site is active in Lewis rats and monkeys but not in guinea pigs.

The relevance of any of these sequences to human disease, and particularly to MS, is questionable. The only way to determine whether any of these sequences are encephalitogenic in humans is to inject humans. This, of course, is not feasible. Many of the encephalitogenic regions are capable of reacting with activated lymphocytes from MS patients. However, this is no proof that they can induce disease in humans. Without reviewing this topic, five pertinent papers can be cited. First, Field and Caspary (10) showed that the tryptophan peptide could react with activated lymphocytes from a variety of cancer patients. These patients had neither EAE nor MS. The human cancer cells possess a surface protein that sequentially is similar to the tryptophan peptide. Second, Weizman et al. (42) reported a cell-mediated autoimmune response to human myelin basic protein in

TABLE 3. Potential encephalitogenic regions within proteins from known human bacteria: tryptophan encephalitogen from myelin basic protein fswgaegqr

Organism ^a	Description ^b	Sequence ^c	Species
<i>Bacteroides</i> spp. (–)	Hypothetical 29348610	li wgaegq rx l	<i>B. thetaiotaomicron</i>
	Outer membrane-nutrient binding 29341398	k swgadgn me	<i>B. thetaiotaomicron</i>
	TonB-dependent outer membrane receptor 60492730	y fwg ang qgn	<i>B. fragilis</i>
	Outer membrane-nutrient binding 29341954	f swg vd q sn y	<i>B. thetaiotaomicron</i>
	Outer membrane-nutrient binding 29337665	f twg ag en lp	<i>B. thetaiotaomicron</i>
<i>Enterococcus faecalis</i> (–)	Outer membrane-nutrient binding 29347092	f swg sc sl de	<i>B. thetaiotaomicron</i>
	Hypothetical 29377638	q g wa an qr g	
<i>Lactobacillus johnsonii</i> (+)	Methionyl-tRNA synthetase 29375514	f swg ipl kn d	
	β-Galactosidase 68194203	n swg ad ves p	
	Amino acid transporter 42519891	f n w g af kps f	
<i>Bifidobacterium longum</i> (+)	β-Galactosidase 42518789	d awg ae gt et	
	DNA polymerase III 23464753	v hwg tea q rr	
<i>Escherichia coli</i> (–)	Helicase 23466281	y n w g ae ft k f	
	Sugar phosphate isomerase 26246223	f wg ae ln nr	
<i>Clostridium</i> spp. (+)	Fumarase 75257030	k lwg aq t qr s	
	Hypothetical 18145026	d kwg ae li td	<i>C. perfringens</i>
<i>Streptococcus</i> spp. (+)	Sugar phosphate isomerase 15895864	f wg ae ln le	<i>C. acetobutylicum</i>
	Ribose 5-phosphate isomerase 50589977	f wg ae ln lk	<i>S. suis</i>
	NADH flavin oxidoreductase 28896405	f awg aq yl le	<i>S. pyogenes</i>
	Methionyl-tRNA synthase 55738410	f swg vk vp sd	<i>S. thermophilus</i>
	Ribose transport ATP binding 1172865	t swg ai n w q k	
<i>Haemophilus influenzae</i> (–)	DNA directed RNA polymerase 41017590	d swg ai kan nr	
<i>Helicobacter pylori</i> (–)	TPR repeat 67762886	w rwg q eg q rc	
<i>Burkholderia pseudomallei</i> (–)			

^a Symbols in parentheses: +, gram positive; –, gram negative.

^b Numbers represent access identification numbers on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>).

^c Boldface characters in the sequences indicate amino acids making major contributions to encephalitogenicity.

76% of the autistic children studied. Here again, these children were not suffering from EAE, MS, or cancer. Finally, Spitzer et al. (34, 35) showed that guinea pigs sensitized with encephalitogenic peptides having amino acid sequences different from that in the test protein did not show cellular immunity in vivo or in vitro to myelin basic protein, although the animals developed EAE. Einstein (6a) showed that antigen (MBP) suppression of EAE in guinea pigs did not require an intact tryptophan, and yet the tryptophan is an absolute requirement for disease induction. The specificity for disease induction and activated immune recognition are different.

Three sequences were compared in order that any conclusions presented would reflect all of the sites, not just the characteristic of one region. The tryptophan peptide has been studied by far the most. As part of my Ph.D. thesis (43), I systematically examined in guinea pigs the contribution to encephalitogenicity of each amino acid in the sequence. Further studies in later years also added to this initial work (8, 44, 47, 50).

Of the nine residues of the tryptophan peptide, fswgaegqr, five appear considerably more important. The five are the glutamine, a positively charged amino acid adjacent to the glutamine, the tryptophan located six residues from the glutamine toward the amino-terminal end with the glycine C terminally adjacent to it, and a hydrophobic region (preferably a ring structure) eight residues from the glutamine. The glutamine itself can be replaced by serine or asparagine, but not by glutamic acid. The negative charge of the glutamic acid will negate the positive of the adjacent amino acid. The glutamine appears to be contributing a hydrogen-bonding side chain. The phenylalanine and tryptophan form a hydrophobic pocket, called a molecular sandwich (26, 28, 43). The glycine permits a bend in the molecule at the tryptophan-glycine bond (17, 25).

Using the midpeptide, Smeltz et al. (33) showed by alanine replacement in the DA rat the importance of the “hygslp” sequence. Since only alanine substitutions were used, it is difficult to ascertain the variability acceptable. However, it appears that, unlike the tryptophan-glycine requirement, the tyrosine-glycine shows some flexibility. As stated above, the midpeptide is also active in rabbits. The midpeptide structurally is quite similar to the tryptophan peptide, which is also active in rabbits. Therefore, sequences that satisfy the general “hygslp” requirement and contain a glutamine adjacent to a charged amino acid at the C-terminal end are also noted (46).

The hyperacute sequence, pqksqrtqdenpv, is the least studied of the three encephalitogenic regions. The “tqde” sequence appears to be significant (32). A serine for threonine substitution converts the potency of the molecule from regular EAE to hyperacute EAE (20, 52). The aspartic acid appears to be essential. Although not as specific as the aspartic acid, the serine is important (21, 22). Therefore, small hydrophilic residues were included as acceptable for the replacement of the serine. With respect to the glutamic acid, the “tqde” data

indicate some variability. For this position, glutamic acid, aspartic acid, glutamine, and asparagine were included.

Using the information on the requirements for the three encephalitogenic regions, bacterial and viral sequences were selected as potential encephalitogens.

Analytical process. The BLAST (basic local alignment search tool) program of the National Center for Biotechnology Information was used to ascertain potential encephalitogenic mimics (www.ncbi.nlm.nih.gov/BLAST). Four databases—nr, refseq, Swiss-Prot, and month—were used.

The proteins in the following human bacterial groups were examined for potential encephalitogenic mimics: *Klebsiella*, *Morganella*, *Proteus*, *Serratia*, *Enterococcus*, *Micrococcus*, *Streptococcus*, *Bifidobacterium*, *Lactobacillus*, *Prevotella*, *Bacteroides*, *Fusobacterium*, *Eubacterium*, *Burkholderia*, *Mycobacterium*, *Salmonella*, *Chlamydomydia*, *Haemophilus*, *Bacillus*, *Pseudomonas*, *Actinobacillus*, *Clostridium*, and *Escherichia*.

The proteins in the following human virus groups were examined for potential encephalitogenic mimics: *Morbillivirus*, *Paramyxovirus*, *Rubulavirus*, *Pneumovirus*, *Filoviridae*, *Influenza virus*, *Arenaviridae*, *Bunyaviridae*, *Rotavirus*, *Coltivirus*, *Orthoreovirus*, *Coronavirus*, *Torovirus*, *Flaviviridae*, *Togaviridae*, *Calicivirus*, *Astrovirus*, *Enterovirus*, *Rhinovirus*, *Hepatitis A virus*, *Hepatitis B virus*, *Hepatitis D virus*, *Hepatitis E virus*, *Herpesvirus B*, *Varicella-Zoster virus*, *Herpes simplex virus*, *Herpesvirus*, *Cytomegalovirus*, *Epstein-Barr virus*, *Adenoviridae*, *Parvovirus*, *Polyomavirus*, *Echovirus*, *Bluetongue virus*, and *Papillomavirus*.

Obviously, only the data that have been accumulated to date can be examined. The entire genomes of some microorganisms, including strains, have been ascertained. Others have not. Therefore, as more data are revealed more potential encephalitogenic mimics will be found. Comparative analysis of 16S rRNA sequences amplified from human feces indicated that less than 25% of the molecular species identified corresponded to known organisms (36). Obviously many bacteria in the gut, at least, have yet to be identified.

RESULTS AND DISCUSSION

Tables 2 (midregion), 3 (tryptophan peptide), and 4 (hyperacute site) present the potential encephalitogenic mimics within proteins from known human bacteria. Table 5 gives the same data for the potential encephalitogenic mimics within proteins from known human viruses. No attempt was made in these tables to separate pathogenic from nonpathogenic or-

TABLE 4. Potential encephalitogenic regions within proteins from known human bacteria: HEAE encephalitogen from myelin basic protein pqkshqrtqdenpv

Organism ^a	Description ^b	Sequence ^c	Species
<i>Chlamydophila pneumoniae</i> (-)	Hypothetical Cpn 0483 15618394	llpr nprt edq n -	
<i>Bacteroides</i> spp. (-)	DNA polymerase 60491636	kdldfdef tq dengn	<i>B. fragilis</i>
	Exported 60491363	drhpgg gt eddr pg	<i>B. fragilis</i>
	Hypothetical 60493947	dlekeer ted ef ma	<i>B. fragilis</i>
<i>Fusobacterium nucleatum</i> (+)	Calcium-transporting ATPase 19714607	depkdl tt q de dsy	
	Methyltransferase 34764012	serl sel tq de kfl	
	DNA gyrase A 19705415	evt ede et ede elm	
	Phosphoenolpyruvate protein phosphotransferase 19705098	mekds fp t ede q fe	
<i>Serratia</i> spp. (-)	Piln 38176558	vysvek rt q de q gi	<i>S. entomophila</i>
	Hypothetical 38259461	ryspdf qt q de fak	<i>S. marcescens</i>
	Afp18 48995204	depndn it q de lfr	<i>S. entomophila</i>
	Tn7 transposition 38259433	ealpeal ted ev ll	<i>S. marcescens</i>
<i>Escherichia coli</i> (-)	PTS system 1 21039017	mrd sl pt ede q fq	<i>S. marcescens</i>
	Adenylate cyclase 581058	iqff te et qde ngf	
	FtsA 21321975	assys vl t ede rel	
	ATPase 73853188	ndvas rr t ede rrl	
	Hypothetical 26250231	etgr sr pt ede hmi	
	Peptide synthetase 26248278	ctll nr mt ede ns w	
	FotE 29293010	vwl vq tw ede ns ks	
	Outer membrane usher 15800422	shht ede t ede tfi	
	Ycdy oxidoreductase 15830666	swled q st ede sea	
	Phosphatidylserine synthase 150323686	iarm ck rt ede kl f	<i>C. acetobutylicum</i>
<i>Clostridium</i> spp. (+)	Regulatory recX 18145587	dkl sn id ede ndt	<i>C. perfringens</i>
	Yqe 15024218	sy lp q ql te de iri	<i>C. acetobutylicum</i>
	Hypothetical 28210235	lqr ks er ted eq re	<i>C. tetani</i>
	Glucose-inhibited division B 28209871	kinl ta it ede dii	<i>C. tetani</i>
	Ribose recycling factor 22001946	kkd ns it ede mks	<i>C. acetobutylicum</i>
	ABC transporter-ATP binding 29376690	yeple mv t qde kvi	
	Phenomone binding 29376080	gsmd si ft qde sin	
	Hypothetical 29376056	mvrf sl vt qde tin	
	Hypothetical 29375363	alh km fat qde wgn	
	PrgE 59616089	hav tn fl ted efee	
<i>Enterococcus faecalis</i> (-)	Arginine repressor 39931070	imq qe iet qde lit	
	Conjugal transfer 29377006	ellier l te de lyy	
	V-type sodium ATP synthase F	rknee ev t ede qhk	
	Hypothetical 23465454	aqvl tem t qde snp	
	RpoB 23465772	leket let q de a lv	
<i>Bifidobacterium longum</i> (+)	α-Galactosidase 23464793	dsygt tl te de lla	
	Hypothetical 15674763	rvh yt ft ted dn pk	<i>S. pyogenes</i>
	PTS system II 28896695	ksh im tk ted eak l	<i>S. pyogenes</i>
<i>Streptococcus</i> spp. (+)	Cysteine tRNA synthetase 30316153	elg as gr ted etar	<i>S. pneumoniae</i>
	Hypothetical 58336799	tim nc dv tqde dgk	<i>L. acidophilus</i>
<i>Lactobacillus</i> spp. (+)	Hypothetical 42518235	yfy fd pt qde dyq	<i>L. johnsonii</i>
	RNase 6 62514503	itk ent pt ede kdn	<i>L. casei</i>
	Hypothetical 68160989	iin rq rt ede knk	<i>L. reuteri</i>
	Holliday junction DNA binding resolvase 62515837	yva en l ftede pve	<i>L. delbrueckii</i>
	NADH dehydrogenase 28377235	flq ks l ptede iil	<i>L. plantarum</i>
	FtsY 42519391	esae ev tt ede qer	<i>L. johnsonii</i>
	Glutamyl-tRNA synthetase 38258342	kay es ym ted elsa	<i>L. plantarum</i>

^a Symbols in parentheses: +, gram positive; -, gram negative.

^b Numbers represent access identification numbers on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>).

^c Boldface characters in the sequences indicate amino acids making major contributions to encephalitogenicity.

ganisms. Moreover, no attempt to separate the organisms by location of potential "infection" was made.

The bacteria present 111 potential encephalitogenic mimics: 39 midregion, 23 tryptophan peptide, and 49 hyperacute site. Thus, there are numerous sequences that are potentially encephalitogenic in bacteria. No one immunogen dominates the group. In fact, the differences probably reflect more on the specific requirements imposed on selection. The tryptophan site, which is by far the most studied (and presents the most restrictions), has the fewest mimics but still presents a large selection. The sites are found in most of the bacteria examined. In fact, the number of sites found in a particular bacterium probably reflects more on the amount of data known about the species than any bacterial restriction. Gram stain characteristics seem to have little significance. Finally, the locations of the sites are in proteins located in all parts of the bacterial cell—

inside, outside, etc. The locations of the sites within the proteins are also quite variable.

For molecular mimicry to work the immunogen must be "visible" to the immune system. It has therefore been assumed that it must be located on the surface of a protein that is either excreted by the microorganism or is on its surface. This is not necessarily true. The microorganism itself can initiate an immune response. Cells are hydrolyzed, and their contents are released. A great amount of digestive enzyme activity is present. All this can "expose" potential immunogens (23). It certainly happens within active EAE lesions. These "new" immunogens can be processed by the already-present immune cells.

The one feature of all of the diseases that have been related to molecular mimicry is that the incidence of the primary infection is very high, e.g., measles and influenza. However, the

TABLE 5. Potential encephalitogenic regions within proteins from known human viruses

Section ^a	Virus	Description ^b	Sequence ^c
A	<i>Hepatitis B virus</i>	Polymerase 28812222	hhygt1p nlh
		Polymerase 33468377	gcygslp qgdh
		Reverse transcriptase 76253219	gsygs1p qgeh
		Reverse transcriptase 76253625	hcygt1p slh
Influenza C virus	RNA-directed RNA polymerase 133531	RNA-directed RNA polymerase 133531	ksygs1p elf
		Replication E1 9628545	fkygt1p swv
B	<i>Hepatitis C virus</i>	Polyprotein 1381032	yswganetd
C	<i>Ornithogalum mosaic virus</i>	Genome polyprotein 130497	vdpltgatqdenpl
		Outer capsid 60416215	qreasersqdeikm
	<i>Echovirus type 32</i>	Polyprotein 34485450	kyssnatqdeqym
		<i>Herpesvirus 1</i>	Capsid 59538

^a Sections: A, midregion encephalitogen from myelin basic protein hygslp; B, tryptophan encephalitogen from myelin basic protein fswgaegq; C, HEAE encephalitogen from myelin basic protein pqkshqrtqdenpv.

^b Numbers represent access identification numbers on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>).

^c Boldface characters in the sequences indicate amino acids making major contributions to encephalitogenicity.

incidence of the autoimmune “side effect” is extremely low, e.g., the viral neuropathies incidence is 1/10,000. Any hypothesis that attempts to explain this process must take into account the disparity in incidence rates.

One explanation is taken from experimental autoimmune disease research. In order to induce EAE in animals, both the immunogen and the adjuvant must be injected at the same time. In fact, it has been shown that the two form a complex (27, 28, 48). The need for two separate compounds to be present simultaneously is used to explain the low incidence of viral and postvaccinal neuropathies (49). It has been shown that secondary infections are significantly present during the induction of these neuropathies. The cell wall material of the microorganism responsible for the secondary infection is a source of the adjuvant.

How can this be used as an explanation of multiple sclerosis? In this disease it is hard to find one infection (other than the autoimmune reaction at the lesion), much less two. Tables 2 to 5 present more than potential encephalitogenic sites. They show the probability of finding such sites resides in bacteria and not viruses and, in fact, in the bacteria located in the most concentrated bacterial area of the human anatomy—the gut. Most of the gut bacteria are not pathogenic but form a symbiotic relationship with the individual. Therefore, the requirement for the first “infection” is satisfied by the presence of nonpathogenic bacteria.

What about the need for an adjuvant molecule? Adjuvant molecules are portions of the bacterial cell walls. The simplest is *N*-acetylmuramyl dipeptide (MDP) (6). However, MDP does not aid all encephalitogens in producing EAE. Larger portions apparently are needed for some encephalitogens (28). Fox et al. have shown quite convincingly that adjuvant molecules normally are not in human body fluids or tissue, except the gut (11, 12). So, apparently, the two groups of substances, potential immunogens (mimics) and adjuvant molecules, known to be required for an autoimmune response are normally found to-

TABLE 6. Comparison of urinary 5-HIAA/tryptophan ratios from MS patients in relapse to those not associated with relapse^a

Patient	Relapse		Nonrelapse		% Difference
	Sample no.	5-HIAA/Trp ratio	Sample no.	5-HIAA/Trp ratio	
1	3	0.099	4	0.134	-26
2	4	0.102	1	0.249	-59
3	2	0.084	5	0.117	-28
4	2	0.009	4	0.108	-37
5	4	0.167	4	0.186	-10
6	2	0.149	3	0.387	-23
7	6	0.122	2	0.144	-17
8	1	0.245	4	0.307	-20
9	3	0.077	6	0.091	-15
10	4	0.178	7	0.181	-2
11	3	0.000	3	0.071	-∞
12	2	0.123	2	0.090	+27

^a Relapse average = 0.113; nonrelapse average = 0.172. At each clinic visit, the patients were assigned a “phase” according to the definitions of the Schumacher Committee (30) formulated in 1965 after standard electrophysiological testing. Urine samples were collected periodically for 2 years from the patient’s visits. The first voided urine sample on the day of each clinic visit was collected and stored at -20°C in the dark before analysis. Tryptophan (Trp) and serotonin sulfate contents were determined by means of a Beckman automated amino analyzer. The concentrations of 5-HIAA were measured by the procedure of Udenfriend et al. (40).

gether in the normal human gut. Although it appears that the adjuvant itself cannot cross the gut, the adjuvant-immunogen complex probably can. In fact the MDP-tryptophan peptide complex forms a spherical ball with one hemisphere hydrophobic and the other hydrophilic. This soap-like structure is ideal for membrane penetration (28, 48).

Why is the incidence of MS so low? Although both the immunogen and adjuvant molecules are present, they must be processed in such fashion that the correct units are made in high enough concentration to form the complex. This will depend entirely upon the nature of the gut and its hydrolytic enzymes. The gut is a dynamically evolving biosystem whose bacterial content is changing and therefore the concentrations and types of hydrolytic enzymes continually vary.

There is other evidence that the gut is involved in MS. Serotonin metabolism is greatly altered during multiple sclerosis relapses. This has been seen by measuring the 5-hydroxy indoleacetic acid (5-HIAA)/tryptophan ratio from urine (Table 6). Ninety percent of the serotonin metabolism is derived from the gut and not the nervous system. Furthermore, one of the adjuvant molecules, MDP, is known to mimic serotonin (28).

Rather than study a single organism, the present study examined a large number of nonpathological and pathological human bacteria and viruses in order to ascertain the prevalence of encephalitogenic mimics. There appear to be many potential encephalitogens within bacteria and viral cells. It also seems that the most probable source of these mimics is the normal gut. This information has been applied to MS. However, the procedure used is just as valid for the location of any potential immunogenic mimic. Therefore, it has a broad application to the field of molecular mimicry.

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