Hypotheses

Autoimmunity and the microbiome: T-cell receptor mimicry of "self" and microbial antigens mediates self tolerance in holobionts

The concepts of "holoimmunity" (TcR-mediated tolerance for the holobiont) and "holoautoimmunity" (loss of tolerance for the holobiont) are introduced

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I propose a T-cell receptor (TcR)-based mechanism by which immunity mediates both ''genetic self'' and ''microbial self'' thereby, connecting microbiome disease with autoimmunity. The hypothesis is based on simple principles. First, TcR are selected to avoid strong cross-reactivity with ''self,'' resulting in selection for a TcR repertoire mimicking ''genetic self.'' Second, evolution has selected for a ''microbial self'' that mimics ''genetic self'' so as to share tolerance. In consequence, our TcR repertoire also mimics microbiome antigenicity, providing a novel mechanism for modulating tolerance to it. Also, the microbiome mimics the TcR repertoire, acting as a secondary immune system. I call this TcR-microbiome mimicry ''holoimmunity'' to denote immune tolerance to the ''holobiont self.'' Logically, microbiome-host mimicry means that autoimmunity directed at host antigens will also attack components of the microbiome, and conversely, an immunological attack on the microbiome may cross-react with host antigens producing ''holoautoimmunity.''

Keywords:
antigenic mimicry; autoimmunity; Crohn's disease; diabetes; holobiont; immune tolerance; immunologic mimicry; microbiome; non-self; T-cell receptors

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Introduction: The problem of "microbiome self"-"genetic self" immunological interactions within holobionts

Our understanding of immunological tolerance is challenged by the discovery that most multicellular organisms are "holobionts," consisting not only of a "genetic self" but an equally important "commensal and symbiotic self" represented by the host microbiome. Not only is most of the microbiome acquired after clonal selection or deletion have already shaped the immune system, but the microbiome can change with time, geography and diet, altering the overall antigenic make-up of the holobiont "self" [1, 2]. How can such antigenic alterations be tolerated? The conceptual difficulties are enhanced by increasing numbers of observations that autoimmune diseases are often (perhaps always) associated with significant alterations in the host microbiome, and conversely, that autoimmunity may be induced, modified or even prevented by various manipulations of the host microbiome. In Sjogren's syndrome, tear- and saliva-producing cells are attacked, and patients harbor

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higher numbers and frequencies of *Streptococcus* spp., *Staphylococcus* spp., *Lactobacillus* spp., and *Candida albicans*, and slightly lower proportions of *Fusobactera* and *Prevotella* spp. than unaffected individuals [3]. Many of these microbes, as well as *Escherichia coli*, produce peptides that induce autoantibodies reactive against Sjogren's syndrome autoantigens [4]. Similarly, rheumatoid arthritis patients exhibit decreases in *Haemophilus* spp. and concomitant increases in *Prevotella copri*, *Lactobacilli*, and *Porphyromonas gingivalis*. These microbes exhibit antigens that mimic RA autoantigens [5, 6]. Behcet's syndrome (an autoimmune disease causing inflammation of the circulatory system) is similarly characterized by specific depletion in the genera *Roseburia* and *Subdoligranulum* [7]. And specific alterations in the microbiome that will be reviewed below are present in type 1 diabetes mellitus and Crohn's disease. How can such specific changes in the microbiome be related to autoimmune-disease-related loss of tolerance to "genetic self"?

I suggest the following hypothesis, based upon two well-understood principles of immunology, to explain how the immune system mediates the relationship between "microbiome self" and "genetic self."

First, I assume that clonal selection and/or deletion are carried out as generally accepted, with the result that strongly autoreactive clones are eliminated or tolerized. An unappreciated consequence of eliminating TcR and BcR that are complementary to "self" is that the remaining TcR and BcR will *mimic* "genetic self," and therefore, be tolerant to it.

Second, I assume, following Damian's molecular mimicry theory [8, 9] that microbes evolve to evade the immune system by being selected to mimic the host's "genetic self." Because the microbiome and the TcR, and BcR repertoires are all selected to mimic "genetic self," "microbiome self" will also mirror the host TcR and BcR repertoires. This microbiome-TcR and BcR mimicry provides a possible mechanism by which tolerance for the microbiome (like tolerance for "genetic self") is attained. While the microbiome clearly helps shape the early immune system [10]), one implication of this hypothesis is that TcR and BcR repertoires intrinsically bound or limit the possible compositions of the "microbiome self" to those microbes that can best evade an active immune response.

I call the tolerance of the immune system for the combined "microbiome-genetic host self," or holobiont self, "holoimmunity." "Immunity" is used here broadly to refer not only to the ability of the system to attack and eliminate foreign antigens, but also to carry out house-keeping activities such as monitoring "self," maintaining tolerance, performing cellular debris sampling, and promoting healing. Thus, "holoimmunity" involves not only the elimination of non-commensal and non-symbiotic microbes, but also the maintenance of a healthy and host-appropriate microbiome.

Just as immunity has its correlate in autoimmunity, the concept of "holoimmunity" implies the existence of its correlate, "holoautoimmunity." In autoimmune diseases, the immune system loses tolerance for "genetic self." TcR or BcR become activated against "self" antigens. Because the microbiome has evolved to mimic the host's "genetic self," TcR and BcR that are autoreactive may also attack the components of the microbiome that mimic the targeted "genetic self" antigens. Thus, specific alterations in the microbiome repertoire should characterize each autoimmune disease. Conversely, immunization against components of the microbiome may result in concomitant autoreactivity against corresponding "genetic self" antigens. The concept of holoautoimmunity provides one possible mechanism by which autoimmune diseases produce corresponding changes in the microbiome repertoire, and helps to explain how manipulating the microbiome can both initiate and treat "autoimmune" disease (e.g. [11, 12]).

Tests of the holoimmunity hypothesis

Six tests of the "holoimmunity" and "holoautoimmunity" concepts are reported below using previously published sets of TcR. I use Crohn's disease (CD) and type 1 diabetes mellitus (T1DM) as case studies. Further testable predictions are proposed in the Discussion section.

Test 1 investigates whether TcR mimicry might be due to random matches or database artifacts by comparing various types of control sets.

Test 2 investigates whether TcR mimic the "genetic self," creating a molecular "mirror" of host antigens. TcR mimicry of the "genetic self" may provide a mechanism by which "self" tolerance is achieved.

Test 3 investigates the prediction that TcR sequences in normal human hosts will "mirror" the "commensal and symbiotic self." Such TCR-"commensal and symbiotic self" mimicry could provide a mechanism for host tolerance of the normal microbiome.

Test 4 evaluates the prediction that microbes unrelated to human disease or to the human microbiome will not display similarities to either human "genetic self" or to human TcR.

Tests 2–4 establish the plausibility of the hypothesis that the immune system may mediate the development of the "commensal and symbiotic self" in relation to the "genetic self" by selecting for their immunological compatibility.

Test 5 investigates whether **s**pecific deviations of the TcR mimicry repertoire from "normal" are associated with individual autoimmune diseases and might identify the triggers/targets of the autoimmune/holoautoimmune process.

Test 6 evaluates whether disease-specific alterations in TcR mimicry repertoires produce altered tolerance for specific elements of the microbiome.

Tests 5 and 6 establish the plausibility of the concept of holoautoimmunity resulting in correlated losses of tolerance for "genetic self" and "microbiome self."

Methods

TcR data sets

Three sets of controls were run to establish baseline distributions of TcR mimicry of the microbes listed in Tables 1 and 2. One set was derived from a convenience sample of 101 TcR CD3 V beta/D/J beta regions sequenced from nine "normal" (i.e. disease-free) individuals [13–17]. A second set was

Table 1. Summary of the frequency (in percent) of TcR mimics of 42 genera of bacteria, protozoa, and yeasts characterizing the human microbiome and some of its common pathogens, as well as 11 genera of plant bacteria and fungi

Human pathogen	# Of taxons listed in UniProt	UniProtKB entries × 1,000	Anti TCR% $N = 101$	NOR TCR% $N = 114$	CON TCR% $N = 101$	CD TCR% $N = 103$	T1D TCR % $N = 68$	p-value (χ^2) NOR versus CONT	p-value (χ^2) CD versus NOR	p-value (χ^2) CD versus CON	p-value (χ^2) T1D versus NOR	p-value (χ^2) T1D versus CON	p-value (χ^2) T1D versus CD
B. cereus	411	1,094	40	35	33	31	19						
Bacteroides	738	709	71	58	68	53	44						
Bifidobacteria	184	215	45	32	29	14	25						
B. pertussis	12	51	23	9	4	4	3						
C. jejuni	72	42	2	7	3	6	3						
C. albicans	12	159	9	4	8	17	3						
Cardiobacteria	25	5	12	1	1	0	0						
C. pneumoniae	9	4	1	7	5	6	3						
Clostridium pathogenic	76	127	32	23	28	21	53				<**0.0001**	<**0.0001**	<**0.0001**
Clostridium commensal	498	796	64	40	66	47	11				(<0.0001)	(<0.0001)	(<0.0001)
Coprococcus	19	26	6	6	24	11	1	(0.0004)				(<0.0001)	
Corynebacteria	312	228	61	21	6	27	1	**0.0002**		<**0.0001**	(<0.0001)		(<0.0001)
C. neoformans	29	127	19	0	0	22	3		<**0.0001**	<**0.0001**			(<0.0001)
Entamoeba	57	62	20	9	7	7	3						
Enterobacter	612	595	68	25	2	19	0	<**0.0001**		<**0.0001**			(<0.0001)
E. faecium	162	106	9	21	28	29	13						
E. coli	1,192	1,468	60	30	39	24	21						
Eubacterium	202	123	40	25	34	23	3				(<0.0001)	(<0.0001)	(<0.0001)
Giardia	60	30	24	11	15	13	7						
H. influenzae	43	41	5	5	7	6	1						
H. pyelori	244	417	8	8	17	2	1			(0.0003)		(<0.0001)	
K. pneumoniae	128	190	20	11	16	12	6						
Lactobacilli	673	819	66	50	54	27	34		(0.0001)	(0.0001)			
L. pneumophila	45	23	10	6	12	1	7						
Listeria	122	116	15	9	8	16	4						
M. tuberculosis	211	195	39	16	7	5	7						
Mycobacteria	1,828	989	93	47	27	44	16	**0.0008**			(<0.0001)		(<0.0001)
Mycoplasma	329	74	11	16	7	11	3						
Neisseria	318	143	24	7	9	7	1						
Prevotella	500	240	51	49	60	44	10				(<0.0001)	(<0.0001)	(<0.0001)
P. aeruginosa	473	337	44	12	11	48	12		<**0.0001**	<**0.0001**			(<0.0001)
Salmonella.	890	503	34	14	12	11	7						
S. marcescens	34	54	13	7	10	6	3						
S. dysenteriae	18	36	5	6	1	6	4						
Staphylococcus	834	238	28	14	16	15	9						
Streptococcus group A	19	90	48	17	9	5	7						
Streptococcus (all)	1,334	950	88	41	36	30	13				(0.0001)		(0.0002)
T. gondii	70	96	53	26	29	25	21						
T. pallidum	16	6	0	3	2	0	0						
T. vaginalis	11	50	33	17	15	21	7						
T. cruzi	15	59	31	14	15	17	8						
Plant bacteria													
Agrobacterium	1,731	537	71	6	13								
A. radiobacter and tumefaciens	2	12	24	3	6								
Fusarium	1,922	460	74	44	64								
F. oxysporum	263	301	54	32	45								
Janthinobacteria	29	42	35	20	31								
J. lividum	1	11	4	12	10								

(*Continued*)

Table 1. (*Continued*)

Human pathogen	# Of taxons listed in UniProt	UniProtKB entries × 1,000	Anti TCR% $N = 101$	NOR TCR% $N = 114$	CON TCR% $N = 101$	CD TCR% $N = 103$	T1D TCR % $N = 68$	*p*-value (χ^2) NOR versus CONT	*p*-value (χ^2) CD versus NOR	*p*-value (χ^2) CD versus CON	*p*-value (χ^2) T1D versus NOR	*p*-value (χ^2) T1D versus CON	*p*-value (χ^2) T1D versus CD
Leuconostoc	71	37	12	4	7								
L. mesenteroides and ventriculitis	2	2	4	2	4								
Pantoea	132	113	54	8	13								
P. anthophila, ananatis, dispersa	3	8	16	5	7								
Pectobacterium	68	77	21	4	9								
Phytoplasma	25	14	2	3	2								
Thielavia	28	19	36	17	16								
T. terrestris	2	19	24	15	12								
Ustilago	52	15	44	28	26								
U. maydis	8	7	10	10	17								
Xanthomonas	610	394	71	37	48								
X. campestris	139	65	9	25	14								
Xylella	24	24	9	4	3								

Plant bacterial and fungal species that are commensal or pathogenic for human beings are shaded. The number (N) of TCR screened for each group (Antisense, Normals, Controls, Crohn's Disease [CD], and type 1 diabetes mellitus [T1DM]) are provided. Statistically significant differences between groups are provided in the columns to the right: the *p* values of those that are significantly greater (by $\chi 2$ analysis) than the comparison group are in bold, underlined; those that are significantly less, are shown within parentheses. If no *p*-value is provided, then any differences were found to have a *p*-value greater than 0.0012 (the cutoff value for significance after a Bonferroni correction for the 42 bacterial categories tested). No significant correlations (by linear regression) were found for the number of TcR similarities and either the number of taxons or the number of proteins entries in the UniProtKB database (see text for details). Shaded boxes indicate plant bacteria and fungi species that have become human commensal organisms or are known to cause human disease. The unshaded plant bacteria and fungi data are the sum of the "humanized" and non-humanized rates of mimicry, so that the rate of non-humanized mimicry can be calculated by subtracting out the "humanized" rate.

derived from a convenience sample of 109 CD3 V beta/D/J beta regions of TcR sequences from 13 people after uncomplicated mono-infections with streptococcal [18], staphylococcal [19], influenza A virus [20–23], HTLV-1 [24, 25], Epstein-Barr virus [26, 27], and tuberculosis [28–30] infections. All 210 control TcR sequences were analyzed for similarity to human "self" antigens. The third set of randomized controls was derived from the second by transforming each TcR sequence into its antisense sequence [31] utilizing the following substitutions: A to R; C to T; D to L; E to L; F to I or M; G to P; H to V; I to F; K to Y; L to E or D; M to F; N to I; P to G; Q to I; R to A or S; S to R or S; T to W or C; V to H; W to T; Y to K.

The first two sets of controls were compared with 103 CD3 V beta/D/J beta TCR sequences from six people with CD [32, 33]. The controls were also compared with 68 CD3 V beta TCR sequences from eight people with T1DM [34–39].

All TCR sequences are provided in the Supplementary Material.

Proteonomics

The TcR sequences were used as search strings in BLAST2.0 (www.expasy.org) for comparison with the entire UniProtKB database and screened for bacterial, fungal, yeast, and protozoal similarities by keyword search. A separate search was made of the UniProtKB virus database and yet another separate search on the *Homo sapiens* database. For microbe searches, the BLAST parameters were set on an E threshold (1,000), number of best scoring sequences to show (1,000), and number of best alignments to show (1,000); gapped similarities were permitted and the low complexity filter was turned off. The PAM-30 Matrix was employed because of the short length of the TcR sequences. An example of the type of data generated is shown in Fig. 1.

For the *Homo sapiens* search, the BLAST parameters were set on an E threshold of 100, number of best sequences to show 100, and number of best alignments to show 100. The rest of the parameters were the same as in the microbe searches.

Table 2. Summary of the frequency (in percent) of TcR mimics of 38 viruses capable of infecting human beings as well as eight common classes of plant viruses

Human pathogen	# Of taxons listed in UniProt	UniProtKB entries × 1,000	Anti TCR% $N = 101$	NOR TCR% $N = 114$	CON TCR% $N = 101$	CD TCR% $N = 103$	T1D TCR % $N = 68$	*p*-value ($\chi 2$) NOR versus CON	*p*-value (χ^2) CD versus NOR	*p*-value (χ^2) CD versus CON	*p*-value (χ^2) T1D versus NOR	*p*-value (χ^2) T1D versus CON	*p*-value (χ^2) T1D versus CD
Adenovirus	199	12	19	16	13	6	15						
Astrovirus	107	2.5	3	2	2	0	0						
Bocavirus	41	3	2	0	0	0	1						
Cardiovirus	29	0.8	3	0	0	0	1						
Coronavirus	195	3	6	3	16	0	1			(<0.001)		(<0.001)	
Coxsackie A	79	17	4	5	8	3	5						
Coxsackie B	28	17	0	3	7	0	34				<**0.0001**	<**0.0001**	<**0.0001**
CMV	117	18	19	21	21	22	26						
Echoviruses	60	10	3	10	10	6	3						
Enteroviruses	542	21	4	10	11	5	3						
EBV	27	65	11	6	8	9	10						
HAV	59	230	0	1	1	1	0						
HBV	119	167	33	8	8	2	10						
HCV	204	238	44	18	29	15	24						
HEV	40	137	22	1	4	3	3						
HHV1	17	12	25	5	8	1	4						
HHV2	10	6	11	3	4	2	9						
HHV6	5	3	7	6	7	1	1						
HHV8	4	2	13	5	6	1	3						
HTLV	14	3	11	1	10	0	0						
Infl A Virus	266	583	34	24	24	11	24						
Infl B virus	56	368	7	1	2	1	1						
Infl C virus	21	356	2	0	2	0	1						
Jap enc virus	25	6	1	2	2	1	1						
Measles virus	15	12	14	6	5	0	0						
Mumps virus	36	1	6	1	1	0	0						
Norovirus	3	32	5	5	6	4	6						
Papilloma virus	217	12	43	15	21	14	12						
Parainfluenza	48	2	4	1	2	0	0						
Polio virus	25	9	0	0	1	1	0						
Polyoma virus	3	7	1	2	3	2	1						
Reovirus	126	5	3	3	0	5	1						
RSV	56	0.06	7	0	1	1	0						
Rhinovirus	292	8	3	3	4	3	6						
Rotaviruses	508	28	19	9	8	4	4						
Rubella	41	31	6	2	0	0	3						
Varicella zoster	2	4	8	3	10	1	3						
Plant viruses													
Curl	730	13	25	0	0								
Mosaic	873	29	70	0	1								
Mottle	175	4	51	0	2								
Rattle	7	0.3	3	3	1								
Spot	86	6	33	3	6								
Stunt	54	3	15	0	1								

(*Continued*)

Table 2. (*Continued*)

Human pathogen	# Of taxons listed in UniProt	UniProtKB entries × 1,000	Anti TCR% $N = 101$	NOR TCR% $N = 114$	CON TCR% $N = 101$	CD TCR% $N = 103$	T1D TCR % $N = 68$	*p*-value ($\chi 2$) NOR versus CON	*p*-value (χ^2) CD versus NOR	*p*-value (χ^2) CD versus CON	*p*-value (χ^2) T1D versus NOR	*p*-value (χ^2) T1D versus CON	*p*-value (χ^2) T1D versus CD
Wilt	22	3	9	4	3								
Woodiness	5	0.05	0	0	0								

The number (N) of TCR screened for each group (Antisense, Normals, Controls, Crohn's Disease [CD], and type 1 diabetes mellitus [T1DM]) are provided. Statistically significant differences between groups are provided in the columns to the right: the *p* values of those that are significantly greater (by $\chi 2$ analysis) than the comparison group are in bold, underlined; those that are significantly less, are shown within parentheses. If no *p*-value is provided, then any differences were found to have a *p*-value greater than 0.0013 (the cutoff value for significance after a Bonferroni correction for the 38 virus categories utilized in the study). No significant correlations (by linear regression) were found for the number of TcR similarities, and either the number of taxons nor the number of protein entries in the UniProtKB database (see bold, underlined and shaded). CMV, cytomegalovirus; EBV, Epstein–Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HHV, human herpes virus; HTLV, human T cell lymphoma viruses; Inf, influenza; Jap Enc, Japanese encephalitis virus; RSV, respiratory syncytial virus.

The resulting BLAST results were screened by the names of the microbes listed in Tables 1 and 2. In all, 41 specific genera of human-associated bacteria, protozoa, and yeast were included in the survey and 38 types of pathogenic viruses. As negative controls for whether microbiome-associated microbes are specifically mimicked by human TcR, 11 genera of plant-specific bacteria and fungi, and eight classes of plant-specific viruses were screened as well (Tables 1 and 2). This exercise turned out to be more complicated than expected as a number of plant bacteria and fungi have become human commensal organisms (listed in the shaded boxes at the bottom of Table 1). Such "humanized" plant microbes are predicted to mimic human TcR and were analyzed separately from "non-humanized" species. A PubMed search was conducted on each species of microbe to determine whether it was a human commensal, symbiont or pathogen, or had no known human relationship. Distinctions between pathogenic and non-pathogenic (or opportunistic) forms of *Clostridioforme* bacteria were made on the basis of published criteria [40], and Streptococcal species were divided into group A *Streptococci* and viridans groupings according to recent convention (http://viridans.emlsa.net/).

The *Homo sapiens* search data were screened for TcR-related sequences, which were discarded as being irrelevant to the current study, since the V- and J-beta subunits are widely shared.

Only sequence similarities in which at least six amino acids were identical in a 10-amino acid sequence were considered to be significant similarities. Using the T1DM TcR used here, and a subset of the control TCR [41], I have previously demonstrated that the number-of-identities criterion fits experimental results involving antigenic cross-reactivity better than measures such as the Waterman-Eggert score or E value from the BLAST search [41–45]. In the event, the vast majority of the similarities found during this study exceeded this cutoff (Figs. 1–3).

Statistics

Linear regression (R^2) values (http://vassarstats.net/index.html) were calculated to determine whether the number of taxons or the number of protein entries in the UniProtKB database significantly skewed the results of the similarity searches.

A chi squared test (http://www.quantpsy.org/chisq/chisq.htm) was used to compare the observed frequencies of the microbial similarities to TcR between the control and the CD groups; the control and the T1DM groups; and the CD and T1DM groups. Because multiple chi squared tests were run on the same sets of data, a Bonferonni correction was employed (http://www.winsteps.com/winman/bonferroni.htm).

Kolmogorov-Smirnov (http://www.wessa.net/rwasp_Reddy-Moores%20K-S%20Test.wasp) and Mann-Whitney (http://vassarstats.net/index.html) tests were used to determine whether the patient control TcR and the antisense patient control TcR derived from them showed significantly different distributions of microbial similarities.

Results

Test 1: The distribution of microbial mimics of TcR sequences is not random or a database artifact

Linear regression (R^2) values were calculated to examine whether the number of taxons or the number of protein entries associated with each microbe is a factor in determining the number of similarities found. They are not: Bacteria normal controls versus taxons, $R^2 = 0.12$; Bacteria normal controls versus UniProtKB entries, $R^2 = 0.37$; Bacterial patient controls versus taxons, $R^2 = 0.21$; Bacterial patient controls versus UniProtKB entries, $R^2 = 0.34$; Virus normal controls versus taxons, $R^2 = 0.02$; Virus normal controls versus UniProtKB entries, $R^2 = 0.11$; Virus patient controls versus taxons $R^2 = 0.02$; Virus patient controls versus UniProtKB entries $R^2 = 0.06$.

The distribution of microbial mimics of the *antisense* TcR controls was more significantly related to number of taxons and protein entries: Bacteria antisense controls versus taxons, $R^2 = 0.54$; Bacteria antisense controls versus UniProtKB entries, $R^2 = 0.52$; Virus antisense controls versus taxons, $R^2 = 0.32$;

Hypotheses

CASSESENSPLHFGNG (CD Patient B Active [30])

HUMAN

A0A0J9YWX3_HUMAN **Protein TRBJ1-6** (Fragment) OS=Homo sapiens
TCR 8 NSPLHFGNG 16
NSPLHFGNG
A0A0J 4 NSPLHFGNG 12

Q5ZGK1_HUMAN **BV01S1J1.6 protein** (Fragment) OS=Homo sapiens
TCR 1 CASSESENSPLHFGNG 16
CAS NSPLHFGNG
Q5ZGK1 55 CASQGVGNSPLHFGNG 70

A4D1A8_HUMAN **Piccolo protein (Aczonin)** OS=Homo sapiens
TCR 3 SSESENSP 10
SSESENSP
A4D1A8 392 SSESENSP 399

Q9Y3T9 (NOC2L_HUMAN) **Nucleolar complex protein** 2 homolog OS=Homo sapiens
TCR 4 SESENSP 10
SESENSP
NOC2L 30 SESENSP 36

Q6ZMY6 (WDR88_HUMAN) **WD repeat-containing protein** 88 OS=Homo sapiens
TCR 3 SSESENSP 10
SSE ENSP
WDR88 457 SSERENSP 464

Q9UL58 (ZN215_HUMAN) **Zinc finger protein** 215 OS=Homo sapiens
TCR 4 SESENSP - LHFGN 15
SE + N P LHFGN
ZN215 447 SEDSNNPTLHFGN 459

O15018-2 (PDZD2_HUMAN) **PDZ domain-containing protein 2** OS=Homo sapiens
TCR 2 ASSESENSPL 11
ASS ENSPL
PDZD2 1503 ASSAMENSPL 1512

P51826 (AFF3_HUMAN) **AF4/FMR2 family member 3** OS=Homo sapiens
TCR 3 SSESENS -PLHF 13
SSESE S P+HF
P51826 442 SSESEGSKPPHF 453

Q14CR0 (Q14CR0_HUMAN) **ATP-binding cassette, sub-family G**, OS=Homo sapiens
TCR 3 SSESENSPLHF 13
SSES+NS L+F
Q14CR0 29 SSESDNS-LYF 38

BACTERIA

W7BR89_LISGR - **Short-chain alcohol dehydrogenase** Listeria grayi FSL F6-1183
TCR5 ESENSPLHF 13
ESEN PLHF
W7BR89 ESENAPLHF 241

A0A099BUR6_9BACT - **Dolichyl-phosphate-mannose-protein mannosyltransferase** Prevotella sp. S7 MS 2
TCR7 ENSPLHFGN 15
EN+ PLHFGN
9BACT406 ENAPLHFGN 414

Figure 1. Example of sequence similarities between a Crohn's disease patient T-cell receptor CD3 V beta/D/J beta region, human proteins, and microbial proteins. (BLAST 2.0 search of the UniProtKB database using the PAM-30 Matrix with E-value set to 100 for human proteins and 1,000 for microbial proteins). Data such as these are the basis for Tables 1 and 2.

Virus antisense controls versus UniProtKB entries, $R^2 = 0.02$.

In sum, antisense TcR mimic microbes on a random basis determined by taxon and microbial protein entry number; normal human TcR do not. The difference between the two sets is statistically significant according both Kolmogorov-Smirnov and Mann-Whitney tests: Bacterial TcR (sense vs. antisense), Kolmogorov-Smirnov Test statistic, 0.31148, $p = 0.005$; Mann-Whitney Test $U_A = 117.5$, $z = -2.56$, $p = 0.005$. Viral TcR (sense vs. antisense) Kolmogorov-Smirnov Test statistic 0.30769, $p = 0.049$; Mann-Whitney Test $U_A = 1570.5$, $z = -2.21$, $p = 0.014$. In contrast, no significant differences were found between the patient control TcR and the normal control TcR by the Kolmogorov-Smirnov or Mann-Whitney tests. Thus, antisense TcR behave significantly differently than patient or normal control TcR in terms of microbial mimicry.

Test 2: TcR mimic "genetic self" antigens

The standard view of the immune system is that it produces T- and B-cell receptors, as well as antibodies, that are *complementary to* host, commensal, and pathogen antigens. This complementary response is weak, and controlled by tolerizing mechanisms to the host, and commensal antigens, but strong and actively promoted against pathogen antigens. One unexpected observation from the current study is that all TcR other than the randomized antisense ones, mimicked many human antigens. It is not possible to present all of the relevant data here, even in tabular or graphical form, because every human TcR mimicked at least a dozen different human proteins, yielding thousands of significant similarities. Typical examples are provided in Figs. 1–3.These data, in aggregate, demonstrate that TcR selection by the immune system creates a set of lymphocytes that mirror a significant portion (if not all of) the antigenic diversity of the host itself.

Test 3: TcR mimic human microbiome antigens

Another surprising result is that TcR frequently *mimic* microbiome antigens.

A0A033UGE5_STAAU - **Uncharacterized protein** Staphylococcus aureus C0673
TCR7 ENSPLHFGN 15
ENSPLHF N
STAAU52 ENSPLHFVN 6

S5NHH2_SALBN - **Type III secretion transcriptional regulator HilD** Salmonella bongori
TCR1 CAS - - SESENSPLHF 13
C+S SESE SPL F
S5NHH2 CTSCHSESEGSPLDF 174

D8H6A8_BACAI - **Uncharacterized protein** Bacillus cereus (strain CI)
TCR5 ESENSPLH 12
+SENSPLH
D8H6A8 DSENSPLH 26

A0A031GCY4_9PSED - **AraC family transcriptional regulator** Pseudomonas sp. RIT288
TCR8 NSPLHFG 14
NSPLHFG
9PSED181 NSPLHFG 187

A2ER08_TRIVA - **Ankyrin repeat protein, putative** Trichomonas vaginalis
TCR5 ESE -NSPLHF 13
ESE N+PLHF
A2ER08 ESEGNTPLHF 902

FUNGI AND PROTOZOA

H8WZA9_CANO9 - **Faf1 protein** Candida orthopsilosis (strain 90-125) (Yeast)
TCR3 SSESENSPL 11

SSESENS L
H8WZA9 SSESENSSL 98

A0A0D2TU41_CRYGA - **DNA phosphorothioation-dependent restriction protein DptG** Cryptococcus gattii NT-10
TCR2 ASS - - - ESENSPLHF 13
ASS ESEN+ LHF
CRYGA438 ASSAELESENTRLHF 452

A0A086L9Z0_TOXGO - **RecF/RecN/SMC N terminal domain-containing protein** Toxoplasma gondii FOU

TCR5 ESENSP- LHFG 14
E+E+SP LHFG
TOXGO553 ETEDSPLLHFG 563

VIRUSES

G9IU55_HHV3 **Tegument protein** OS=Human herpesvirus 3 (Vericella-zoster virus)
TCR 3 SSESENS 9
SSESENS
G9IU55 2656 SSESENS 2662

Figure 1. Continued.

In other words, the immune system appears either to adapt to its microbiome, by selecting TcR that *mirror* the microbiome itself, or selects from available microbes a microbiome that is compatible with the existing TcR repertoire. Table 1 demonstrates that, on average, the probability that any set of human TcR will mimic key constituents of the gut microbiome such as *Bacteroides*, *Bifidobacterium*, *E. coli*, *Bifidobacteria*, *Coprococcus*, *Corynebacteriuim*, *Clostridia*, *Enterobacteria*, *Eubacteria*, *Lactobacilli*, or *Prevotella* is 36.0%. Every TcR of every patient, without exception, mimicked more than one of these commensal microbes. In contrast, the probability that any given TcR mimicked the remaining microbes in Table 1 is much lower (13.6%) and approaches zero for infectious agents such as *Bacillus pertussis*, *Campylobacter jejuni*, *Cardiobacteria*, *Chlamydia pneumonia*, *Shigella dysenteriae*, and *Haemophilus influenza*. Pathogens, in short, do not appear to mimic host TcR at anywhere near the rate that microbes making up the microbiome do.

Table 2 similarly demonstrates that TcR mimics are relatively rare with regard to human viruses, with the exceptions of those viruses that can induce chronic, persistent infections such as adenoviruses, enteroviruses, cytomegalovirus, hepatitis B and C viruses, papillomaviruses, rotaviruses, and varicella zoster virus. On average, 13.8% of TcR mimic these chronic viruses as compared with 4.2% of TcR that mimic viruses associated with acute disease. Chronic or persistent infection may therefore, be facilitated by having an unusually high degree of TcR mimicry, resulting in antigenic tolerance.

Antigenic exposure to a microbe can be ruled out as a causative factor in TcR mimicry of microbes: Despite almost universal vaccination against *B. pertussis*, polio, measles, mumps, and rubella viruses, TcR rarely showed similarities to these microbes.

Overall, these results suggest that the greater the number of TcR that mimic a particular microbial genera or species, the more likely that genera or species is to be tolerated as part of the microbiome.

Test 4: Human TcR rarely mimic plant microbes but "randomized" TcR do

If TcR and BcR are selected to mimic or mirror host antigens, it follows that pathogens will be characterized by being antigenically complementary to TcR and BcR, while commensal and symbiotic microbes will camouflage themselves by looking like "genetic self." However, there is a third possibility as well, which is that antigens may exist that are neither complementary nor similar to the host's "genetic self," or to its TcR and BcR. Such "non-antigens" would be unable to interact with host proteins, and therefore, have no effect on the host.

Hypotheses

CASSGGGNEKLFFGSG (Crohn's Disease, Patient B [30])

A0A024R3K6_HUMAN Potassium inwardly-rectifying channel, subfamily J, Homo sapiens

F5GZ56_HUMAN Intraflagellar transport protein 172 homolog OS=Homo sapiens

P47534SYA_MYCGE - Alanine--tRNA ligase Mycoplasma genitalium

```
A0A024R3K6 237          GNENLFF  243
                        GNE+ LFF
TCR  6                  GGNEKLFF  13
                        GGNEK+FF
F5GZ56 375              GGNEKYFF  382
                        GGN+K+F
P47534 SYA_MYCGE 873    GGGNDKLFRGS      883
                        GGGN+KLF  GS
TCR  5                  GGGNEKLFFGS  15
                        GGG+ E  LFF
Q96ST3  459             GGGTESLFF  467
```

Q96ST3 (SIN3A_HUMAN) Paired amphipathic helix protein Sin3a OS=Homo sapiens

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F5GX87_HUMAN Cytochrome b ascorbate-dependent protein 3 (Fragment) OS=Homo sapiens

A2GW17_TRIVA - Putative uncharacterized protein Trichomonas vaginalis

```
F5GX87  88              NEKLFF  93
                        NEKLFF
A2GW17_TRIVA186         NEKLFF    202
                        NEKLFF
TCR  3                  NEKLFF  13
                        NEKLFF
Q4VX54 51               NEKLFF  67
```

Q4VX54_HUMAN Death domain-associated protein 6 (Fragment) OS=Homo sapiens

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P47534SYA_MYCGE - Alanine--tRNA ligase Mycoplasma genitalium

!

```
P47534 SYA_MYCGE 873    GGG - - NDKLFRGS      883
                        GGG    N+KLF  GS
TCR  4                  SGGG - - NEKLFFGS  15
                        SG  G    NEKLF
A0A024R3G4  281         SGNGCKNEKLF  291
                        SG  G     NEKLF
R2RGV7_9ENTE394         SG - GFGNEKLF      403
                        SG   G GNEKLF
TCR4                    SG – G-GNEKLF      12
```

A0A024R3G4_HUMAN Sterol-C5-desaturase OS=Homo sapiens

R2RGV7_9ENTE - Uncharacterized protein Enterococcus malodoratus ATCC 43197

Figure 2. Examples of sequence similarities between T-cell receptor CD3 V beta/D/J beta regions derived from Crohn's disease patients demonstrating simultaneous similarities between the TcR sequences, human sequences, and microbial sequences. Many additional similarities to individual human and microbial antigens that do not cluster also exist for these TcR, but are not shown (see Fig. 1 for a more complete example).

Plant-infecting microbes might be expected to display such "non-antigens." Indeed, human TcR almost never mimic plant viruses (Table 2): While 13.8% of TcR mimic chronic human viruses; 4.2%, acute human viruses; only 1.5% mimic plant viruses. And while 36% of TcR mimic human microbiome bacteria, only 6.4% of TcR mimic plant bacterial or fungal genera unrelated to human disease (Table 1). Exceptions "prove the rule": 14.9% of plant bacteria and fungi that have become "humanized" and are able to live as commensal organisms or to cause human disease mimic TcR (Table 1, shaded entries).

Antisense control TcR mimic plant microbes at far higher rates than do control TcR. An average of 41.4% of antisense TcR mimic plant bacteria and fungi; "humanized" ones account for only 18.1%. Additionally, 25.8% of antisense TcR mimic plant viruses.

Overall, the differences between control TcR and antisense TcR mimicry of plant microbes is significant by both the Kolmogorov-Smirnov (statistic 0.35714; $p = 0.05$) and Mann-Whitney ($U_A = 218$, $z = 2.84$; $p = 0.002$) tests. No differences were found by these tests between patient control TcR and normal control TcR.

In short, TcR sequences are non-random, such that they mimic microbes that can effectively interact with the host but these TcR do not mimic "non-antigens" from plant microbes. Randomized antisense TcR, in contrast, mimic these "non-antigen" plant microbes at rates commensurate with their appearance in the UniProt database (see Test 1 above).

Test 5: Evidence for disease-specific altered TcR mimicry distributions in CD and T1DM

To test whether distributions of microbe-TcR mimicry are modified by specific autoimmune diseases, control TcR were compared with TcR from CD and T1DM patients.

With regard to CD, Table 1 shows that TcR derived from CD patients are significantly more likely to mimic *C. albicans*, *Corynebacterium*, *Enterobacteriaciae*, *Mycobacteria*, and *Pseudomonas* species than are control TcR. These increases are accompanied by significant decreases in TcR mimicry of *Lactobacilli* among the CD TcR. No significant differences in viral mimicry were observed between control and CD TcR (Table 2).

With regard to T1DM, Table 1 shows that alterations in T1DM TCR mimicry of bacteria are significantly different from the CD or control population TcR mimicries. T1DM TcR display significantly increased mimicry only to pathogenic *Clostridia*, accompanied by significant

P39358 (YJHG_ECOLI) Uncharacterized protein YjhG OS=Escherichia coli (strain K12)

P39358 (YJHG_ECOLI) 226 CASSGGG 232
CASSGGG
TCR 1 CASSGGGN 8
ASSGGGN
A0A0D3RS46 30 ASSGGGN 36

A0A0D3RS46_HUMAN SIX homeobox 3 (Fragment) OS=Homo sapiens

OR8B4_HUMAN Olfactory receptor 8B4 OS=Homo sapiens

C4ICE8C4ICE8_CLOBU - Transcriptional regulator Clostridium butyricum !

TCR 11 LFFGSG 16
LFFGSG
Q96RC9 249 LFFGSG 254
LF+GS
C4ICE8 C4ICE8_CLOBU6 NEKLFYGS 13
NEKLF+GS
TCR 7 GNEKLFFGS 15
G+E LFFG
A0A087WTM7 2840 GSEMLFFG 2847

A0A087WTM7_HUMAN Apolipoprotein B-100 OS=Homo sapiens

Figure 2. Continued.

decreases in mimicry to commensal *Clostridia*, *Eubacteria*, *Mycobacteria*, *Prevotella*, and *Streptococcus viridans* species.

No significant alterations in TcR mimicry were observed with viruses in CD compared with the control groups, but the T1DM TcR showed a significant increase in mimicry of coxsackie B viruses (Table 2).

In sum, TCR mimicry of both commensal and pathogenic microbes changes significantly as a result of autoimmune disease in ways that suggest both enhanced activation against, and enhanced tolerance to, different microbes in the host microbiome. Thus, the triggering of both CD and T1DM appear to be accompanied by disease-specific shifts in TcR mimicry of the microbiome.

Test 6: Alterations in TcR repertoire mirror changes in microbiome constituents in T1DM and CD that may reflect their causative agents

In general, microbiome diversity tends to decrease in people with autoimmune diseases, including T1DM [46] and CD [47, 48]. This decrease, sometimes statistically significant, is also apparent in the TcR mimicries reported in Tables 1 and 2. It appears that activation of peripheral blood TcR reflects the distribution of the microbiome, and that as that microbiome changes, so does the repertoire of circulating TcR.

TcR alterations in T1DM correlate with probable causative agents

Only two sets of microbial-TcR mimicry are significantly altered in T1DM according to the data summarized in Tables 1 and 2: Coxsackie B viruses and Clostridium species. This evidence is consistent with evidence that coxsackie B viruses are triggers of T1DM (e.g. [49–51]). However, coxsackieviruses have repeatedly proven unable to induce T1DM in animal models [52]. While no one has yet experimentally explored the possibility that *Clostridia* are causative agents of T1DM, *Clostridia* are among the types of gut bacteria (including *Bacteroides*, *Lactobacilli*, and *Bacilli* such as *Streptococci*) that are known to be seriously disturbed during diabetes [53–58]. A BLAST search on the UniProtKB bacterial database using human insulin A and B chains – the major antigenic targets in T1DM [38, 59–61] – as the search strings found that *Clostridia* and *Lactobacilli* are the only human bacteria to appear in the top 10 matches in each case (data not shown). Whether Clostridia can cause T1DM alone, or rather work in conjunction with coxsackieviruses, has not been tested, but would be consistent with experiments demonstrating that virally induced T1DM in an animal model can be prevented or cured with antibiotics [11]. Indeed, Filippi and von Herrath [62] have recently suggested that, "This could be explained by the fact that viral association with T1D will likely be multifactorial." Perhaps T1DM is actually caused by a *combined* coxsackievirus-*Clostridium* infection, a testable prediction of holoautoimmunity.

TcR alterations in Crohn's disease correlate with probable causative agents

As with T1DM, the cause or causes of CD are not known [63], but antigens expressed by an imbalanced microbiome have been implicated in eliciting the immune response that drives gut inflammation [64, 65]. Viruses do not appear to be among these, and many have explicitly been excluded (including all hepatitis viruses, enteroviruses including coxsackie types A and B, Epstein–Barr virus, measles virus, human herpes virus types 1, 6, and 8, varicella-zoster virus, mumps virus, rubella virus, rotavirus, norovirus, and adenovirus [reviewed in [66–68]). Notably, there is no significant change in this study of CD TcR mimicry of any virus (Table 2).

Significant increases in some bacteria-TcR mimicries are, however, evident in Table 1, and as in T1DM, these involve microbes associated epidemiologically and pathologically with the development of CD. These include increases in *Enterobacteriaceae*, including *E. coli*, [69–72]; *Pseudomonas* species [73–75]; atypical *Mycobacteria* [76–78]; and cryptococcal infections [79–81]. TcR mimics of all of these putative triggers of CD are significantly increased in the data analyzed here (Table 1). *Corynebacteria* mimicry of TcR also increases, but I can find no evidence that this group of bacteria is (or is not) associated with CD pathogenesis, providing a testable prediction from the current data. In addition, *Salmonella* flagellin has been implicated as a possible antigenic trigger of anti-TLR5 autoimmunity in CD [82], but many other

TCR Diabetic 3: YFCAVGALAGTASKLTG [36]

```
TCR DIA 3: 1                              YFCAVGALA
                                          +FCA+GALA
Clostridium sp.  G5FAL6 : 198             FFCA IGALA

TCR DIA 3: 1                              CAVGALAGTA
                                          C  VGAL+GTA
M. tuberculosis NDH-1  P95175 : 205       CPVGALTGTA
                                            VGALTGTA
HUMAN  Zinc transporter SLC39A7 293         VGALAGTA

TCR DIA 3: 6                              GALAGTASK
                                          GALAGTA+K
E. coli NAG kinase Q1R3V2: 78             GALAGTANK
Salmonella typhimurium E1WEH7             GALAGTANK

HCV polyprotein Q9DIN5: 28                  GAAARTASRLTG
                                            GA+A  TAS+LTG
TCR DIA 3: 5                              AVGALAGTASKLTG
                                          AVGALAGTA
HUMAN  Zinc transporter SLC39A7 293       AVGALAGTA
                                            VGALAGT
HCV polyprotein  Q4PQV0: 941              VGALAGT
                                          VGALAGT
TCR DIA 3: 7                              AVGALAGTAS
                                              ALA  TAS
Rubella Q98652: 125                           ALAETAS

Adenovirus B protein V Q6RK89: 315              TASKLT
                                                TASKLT
TCR DIA 3: 11                             GALAGTASKLTG
                                           G LAGTA  KL  G
HUMAN Alpha-mannosidase H0YA68 850  GDLAGTAPKLPG  861

CMV Capsid UL94 P16800: 281     YLCAVG
                                Y+CAVG
TCR DIA 3: 1                    YFCAVGALAGTASKL
                                        AGT  SKL
CMV glycoprot UL37 Q92MD0 : 56          AGTESKL
```

TCR Dia 4 : CASSLATSGGGSDTQYFGP [36]

```
HUMAN Insulin receptor substrate-2 Q8TF73 60   ATAGGGSAPQ
                                               AT+GGGS   Q
TCR DIA 7                                      ATSGGGSDTQYFGP
                                                       DTQYFGP
CVB4  Q86887   834                             DTQYFGP
CMV UL28 P16847: 111                           DTQYFG
CMV UL29 Q6SWA3: 432                           DTQYFG

CVB3  P03313   676                          SNEGSGTQVFG
                                            S   GS TQ  FG
TCR DIA 10                                  SGGGSDTQYFG

HUMAN EGF Fibulin-like protein Q580Q6  104   ASSMATSG
                                             ASS+ ATSG
TCR DIA 4                                    ASSLATSGGG
                                             AS  LA+ +G  G
CVB3  P03313   2334                          ASLLAEAGKG
CVB4  Q86887  2334                          ASLLAEAGKG
```

Figure 3. Examples of sequence similarities between T-cell receptor CD3 V beta/D/J beta regions derived from type 1 diabetes mellitus patients demonstrating simultaneous similarities between the TcR sequences, human sequences, and microbial sequences. Many additional similarities to individual human and microbial antigens that do not cluster also exist for these TcR, but are not shown (see Fig. 1 for a more complete example).

bacteria have also been found to carry antigenically similar flagellins, including some, such as *E. coli*, implicated above in as CD triggers [83, 84]. Table 1 also suggests that rather than *S. cerevisiae*, which is commonly used in the diagnosis of CD [85–88], the responsible agent may be *C. albicans*, which also produces positive results in the anti-*S. cerevisiae* antibody (ASCA) test [88–90].

As with T1DM, the TcR mimicry data provided here (Table 1) support a multifactorial trigger for CD, in agreement with recent opinion [91], and the requirement for CD diagnosis that evidence of multiple bacteria and yeast infections be present [85–87, 92–94]. Another testable prediction from the TcR data are therefore, that a combination of such agents should be able to trigger CD holoautoimmunity.

In sum, significant alterations in TcR mimicry from normal distributions may identify combinations of infectious agents responsible for triggering autoimmune/holoautoimmune diseases. Unlike current unifactorial approaches, the TcR data implicate specific *sets* of infections as the triggers of such diseases.

Discussion

The results of the six tests performed here establish the plausibility of the holoimmune hypothesis that TcR are selected to mimic the "genetic self," thereby, constraining possible microbiome constituents to those that also mimic both "genetic self" and "self"-defining TcRs. The concept of holoimmunity also provides a framework for understanding holoautoimmunity, in which both "genetic self" and "microbiome self" are attacked simultaneously. Specific alterations in TcR mimicry of microbiome constituents may provide evidence concerning the (multifactorial) triggers of holoautoimmune diseases. Undoubtedly other mechanisms are also at work in determining potential tolerance and antigenicity, including activation of T-regs, MHC-, and CD1-antigen display [95, 96], TcR α-β-chain pairing [97], innate immune pathways [1, 2, 11, 98–100] and idiotype-anti-idiotype networks (see Figure 4). The current hypothesis is compatible with these other mechanisms and undoubtedly works in concert with them. Key tests will involve whether TcR mimicry can accurately predict tolerance and "non-antigenicity," and whether the putative "triggers" of holoautoimmunity can be used

TCR DIA 1: CASSLWGSNQPQH (DIABETES TCR: [35]	
J3QSZ3_HUMAN Glycogenin-2 161	CANSPLGSNQP
	CA+S / GSNQP
TCR DIA 1: 3	CASSLWGSNQP
	SSL GSNQP
B7AEC0 Bacteroides eggerthii: 86	SSLSGSNQP
	SSL GS QP
J3KQL8_HUMAN Apolipoprotein L2 27	SSLRGS IQP
H9KVB3_HUMAN Otogelin 37	LWGSAEPQ
	LWGS +PQ
TCR DIA 1: 5	LWGSNQPQ
	LWGS+QP
C0D7T1 Clostridium asparigiforme: 148	LWGSDQP
E5RIK9_HUMAN Transcription termination factor 3 64	ASSLWNSSQ
	ASSLW S+Q
TCR DIA 1: 2	ASSLWGSNQ
	ASSLWG+N
H7FWZ5 Lactobacillus salivarius: 192	ASSLWGAN

Figure 3. Continued.

to set up viable animal models of disease.

Further tests of the holoautoimmunity hypothesis

Many testable predictions follow from the hypothesis. One is that autoimmune diseases such as rheumatoid arthritis, lupus, myocarditis, idiopathic thrombocytopenia purpura, thyroiditis, etc. should display disease-specific alterations in the microbiome that are reflected in TcR mimicry, and identify possible antigenic triggers. The TcR data should also be reflected in alterations in BcR and antibodies, so that, for example, IgA antibodies will mediate the microbiome and holoautoimmunity in ways directly analogous to those described here for TcR.

A second set of testable predictions is that the immune system has evolved only to "care about" foreign materials that can interact effectively with the host through molecularly complementary interactions. Specifically, pathogens or toxins are only of potential significance to the holobiont if they are be molecularly complementary to host antigens (e.g. cellular receptors or enzymes), and use that complementarity to recognize and interact effectively with host molecular processes. Figure 4A illustrates the concept. The hypothesis makes the testable prediction that plant viruses or most plant bacterial and fungal antigens that are neither similar nor complementary to TcR and BcR have no ability to interact effectively with human physiological functions. An expanded version of this idea could help to explain what the immune system views as "antigenic" or not.

Figure 4B illustrates how "immunological mirroring" applies to understanding holoautoimmunity. The model incorporates evidence that anti-TcR antibodies and anti-idiotype TcR exist in many autoimmune diseases [101–105]. Whether such antibodies and TcR are pathogenic or are elicited as part of a Jerne-type idiotype-anti-idiotype regulatory network is subject for debate [101–108]. A novel prediciton is that these anti-TcR antibodies and anti-idiotype TcR will also target the host and microbiome as well as immune system (TcR) antigens. No one has yet studied such anti-TcR antibodies or anti-idiotypic TcR to determine whether they also participate in recognition of host or microbiome antigens associated with autoimmune disease. Further investigation of this fascinating topic might reveal the deeper mechanisms by which the microbiome mediates, and is mediated by, the host immune system, thereby, playing a networked role in immune regulation.

A further logical consequence of "immunological mirroring" illustrated in Fig. 4A is that the "microbiome self" will be molecularly complementary to some pathogens (left), permitting the "microbiome self" to act as a secondary "immune system" for the host. Such immune-like function would explain (beyond producing antibiotics and maintaining a stable microbiome ecosystem) how a healthy microbiome mediates against infectious disease. Some anti-pathogen activity may reside literally in the host-like molecular composition of the microbiome. This prediction is supported by evidence that helminths can modulate both the constituents of the bacterial microbiome and the host immune system in ways that can be beneficial [109–111].

It follows from TcR-host-microbiome mimicry that the microbiome will also be affected by many of the same pathogens and toxins as the host. For example, *C. difficile* infection causes specific alterations in the microbiome that may be responsible for the chronic nature of the infection [112, 113]. Reconstituting a healthy microbiome by fecal transplant often restores the health of the holobiont as is does in cases of inflammatory bowel disease [114, 115]. Thus, the holobiont may be viewed as an integrated ecology.

Because the host and its microbiome share integral elements of a common ecology, any invasive species that affects one is likely to affect the other. The risk of holoautoimmunity will therefore, be a function of how well the overall holobiont ecosystem is balanced. Any factor that can modify a key element of that ecosystem may also alter the rest. For example, recent research demonstrates that a functional microbiome is established partly at birth, as a result of exposure of the infant to its mother's microbiome while passing through the vagina. Infants born by ceasarean section do not have such exposure. Significant differences in susceptibility to allergic and autoimmune diseases have been associated with caesarean births [116–117]. Another factor is diet: Spicy foods can contain compounds with antibiotic properties, such that the ingestion of them can increase susceptibility to holoautoimmunity [118, 119]. Antibiotics themselves can alter the risk of autoimmunity by altering the microbiome. And recent studies suggest that cancer therapies are mediated by the gut microbiome, implying that cancer risk may be mediated similarly [120]. Such cross-talk may help to explain the effectiveness of microbially derived adjuvants in cancer therapy and

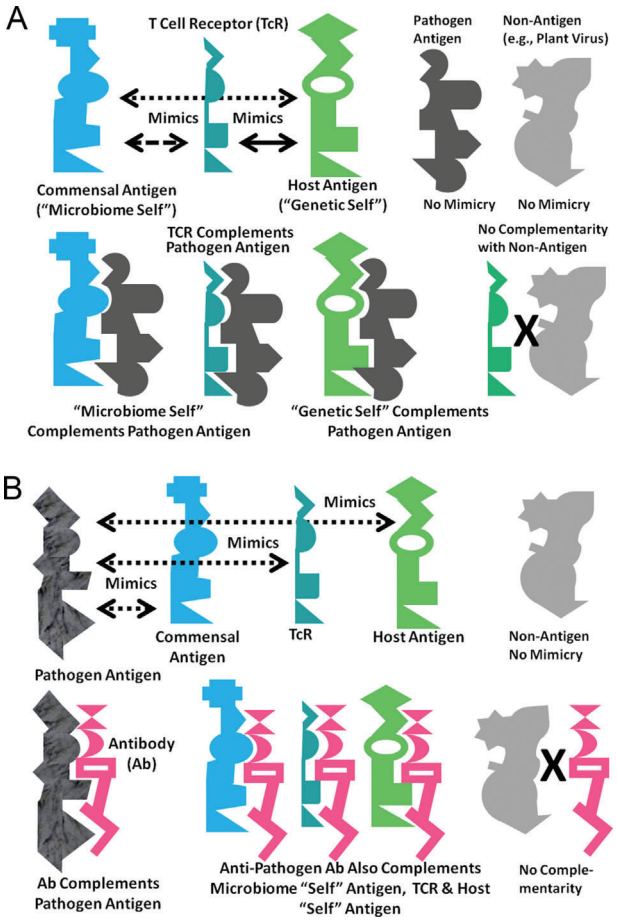

Figure 4. A, top: A visual summary of the concept of holoimmunity. At the top, T-cell receptors (TcR) are selected to mimic host antigens (the "genetic self") and produce a molecular or antigenic "immunological mirror" of the host. Commensal and symbiotic microbes making up the microbiome of the host are tolerated by the immune system in part because they express a significant number of antigens mimicking both host antigens and the TcR "immunological mirror" (Tables 1 and 2). The result is simultaneous tolerance for a "microbiome self" and "genetic self" that overlap. Some potential antigens, such as plant viruses (upper right) neither mimic nor are complementary to human hosts, and interact with neither their cells nor their TcR. The bottom part of the figure illustrates how pathogens can be defined by their molecular complementarity to the host antigens. Only microbes able to interact with host physiological processes can become pathogenic (center). TcR mimic host antigens and prevent or counter infections by pathogens by means of molecular complementarity to antigens on the pathogen (right). Thus, agents that are not molecularly complementary to the host are of no physiological or immunological importance (in keeping with the plant bacteria, fungi, and virus data presented here [Tables 1 and 2]). **B, bottom:** A visual summary of the concept of holoautoimmunity. At the top, some pathogenic microbes express antigens that mimic antigens of the microbiome and of the host, as well as host TcR (Figs. 1–3). In consequence, (below), antibodies induced against pathogenic mimics may recognize, through molecular complementarity, not only the pathogenic antigen, but also, to a greater or lesser degree, the "microbiome self" antigen, the "host self" antigen, and the TcR that the pathogenic antigen mimics. Thus, any autoimmune disease will affect not only the host, but its microbiome counterpart to produce "holoautoimmunity." Antigens that neither mimic nor are complementary to host antigens (e.g. plant viruses) play no role in holoautoimmunity (right).

the apparent effectiveness of Coley's toxins for some cancer types. Thus, specific deviations of the microbiome from normal may precede and predict particular holoautommune diseases.

Conclusions and outlook

To summarize, my hypothesis is that "genetic self"-"microbiome self" compatibility in mammalian holobionts is mediated by TcR and BcR mimicry of both, simultaneously (holoimmunity). The microbiome is not determined by the immune system, but is highly constrained by that to which the immune system is tolerant. Loss of tolerance to "genetic self" or "microbiome self" leads to holoautoimmunity, affecting both "selves" simultaneously. In consequence, every autoimmune disease is accompanied (and perhaps preceded) by characteristic alterations in particular portions of the microbiome corresponding, by molecular mimicry, to the host target of the disease. Conversely, what appears to be an autoimmune disease against the host may, in fact, be initiated against microbiome constituents that mimic the host antigens targeted in disease. Manipulating the host microbiome will therefore, affect host immunity and susceptibility to autoimmunity. The six tests carried out in this paper provide initial data confirming the plausibility of the hypothesis, but far more data-intensive tests are clearly required to validate the details. Most importantly, the hypothesis provides a rationale for using alterations in TcR repertoires to identify possible multifactorial microbial triggers of holoautoimmune processes, from which new animal models might be developed, and treatment strategies (perhaps involving microbiome manipulation) devised.

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