Sequence Similarity between Commensal Bifidobacterium and Cytotoxic T Lymphocyte Epitope Peptides against Human Tumor-Associated Antigens

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Article

Sequence Similarity between Commensal Bifidobacterium and Cytotoxic T Lymphocyte Epitope Peptides against Human Tumor-Associated Antigens

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Abstract: The precise molecular basis of anti-tumor immunity is not yet fully understood, although commensal Bifidobacterium (c-BIF) is expected to be one of the key players in cancer control via antigenic mimicry. We investigated the sequence similarity between c-BIF and cytotoxic T lymphocyte (CTL) epitope peptides against human tumor-associated antigens to better understand the molecular basis of antigenic mimicry. We used two different similarity analyses for this purpose, a linear sequence analysis and a similarity analysis of T cell receptor (TCR)-mediated recognition of CTL epitope peptides on antigen-presenting cells. The linear sequence analysis revealed 3,900 positive numbers of similarity sites between them with 126 mean per peptide and 107 median per peptide, while the TCR-mediated recognition analysis revealed 5,018 positive numbers with a mean of 162 and median of 132 per peptide. These results demonstrated the existence of durable and abundant sequence similarities between c-BIF and CTL epitope peptides, suggesting that the former play a pivotal in inducing cellular and humoral immunity against the latter in the absence of cancer cells via antigenic mimicry.

Keywords: anti-tumor immunity; antigenic mimicry; CTL epitope peptides; human tumor-associated antigens; intestinal microbiota; linear sequence analysis; TCR-mediated recognition analysis

1. Background

Over the past two decades, immune checkpoint inhibitors (1,2), gene-modified T cell therapy (3), and target therapies using various types of monoclonal antibodies (4) have led to remarkable success in cancer therapy and cancer immunoprevention. Further, Sivan et al. reported that commensal Bifidobacterium (c-BIF) not only promoted anti-tumor immunity but also enhanced the efficacy of immune checkpoint inhibitors in a mouse model (5), potentially suggesting a new treatment modality for clinical use. Zitvogel et al. hypothesized that gut microbial proteins might be sufficiently similar to human tumor antigens to be capable of eliciting tumor-specific T lymphocytes and antibodies that can recognize future tumor cells via "antigenic mimicry" (6). Mitsuoka provided an overview of the mechanisms by which intestinal microbiota influence host immunity and cancer prevention (7). We previously reported that the antibodies reactive to each of 31 different CTL epitope peptides not only were detectable in a majority of healthy donors (8), but also inhibited tumor growth in association of activation of dendritic cells and suppression of T regulatory cells at the tumor site in a mouse model (9), suggesting a potential new treatment modality.

Despite this wide body of research, however, the molecular basis of antigenic mimicry in not yet well understood. In the present study, therefore, we investigated the sequence similarity between c-BIF and the 31 CTL epitope peptides against tumor-associated antigens to understand whether c-BIF plays a role in the induction of cellular and humoral immunity in the absence of tumor cells via antigenic mimicry. We also conducted TCR-mediated recognition similarity analysis between commensal clostridium, a pathogenic bacterium, and CTL peptides.

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2. Materials and Methods

We employed two different similarity analyses: (1) a linear sequence similarity analysis between c-BIF and each of the 31 CTL epitope peptides shown in Table 1, and (2) a similarity analysis of T cell receptor (TCR)-mediated recognition of c-BIF and each of the 31 CTL epitope peptides on antigen-presenting cells shown in Table 1. These analyses were conducted based on the information from GenPept.Graphics Next Previous Descriptions, as shown in Supplemental Information 1. We also conducted TCR-mediated recognition similarity analysis between commensal clostridium and CTL peptides based on the information from GenPept.Graphics Next Previous Descriptions shown in Supplemental Information 2.

Table 1. Information on the 31 peptides CTL epitope peptides against human tumor associated antigens used for.

Symbol for peptide	HLA type	Origin protein	Position of peptide	Amino acid sequence	(Refs.)
CypB-129	A2, A3sup	Cyclophilin B	129-138	KLKHYGPGWV	Jpn J Cancer Res 2001,92(7):762-7.
Lck-246	A2	p56^{lck}	246-254	KLVERLGAA	Int J Cancer 2001 94(2):237-42.
Lck-422	A2, A3sup	p56^{lck}	422-430	DVWSFGILL	Int J Cancer 2001 94(2):237-42.
ppMAPkkk-432	A2, A26	ppMAPkkk	432-440	DLLSHAFFA	Cancer Res 2001 61(5):2038-46.
WHSC2-103	A2, A3sup, A26	WHSC2	103-111	ASLDSDPWV	Cancer Res 2001 61(5):2038-46.
HNRPL-501	A2, A26	HNRPL	501-510	NVLHFFNAPL	Cancer Res 2001 61(5):2038-46.
UBE2V-43	A2	UBE2V	43-51	RLQEWCSVI	Cancer Res 2001 61(5):2038-46.
UBE2V-85	A2	UBE2V	85-93	LIADFLSGL	Cancer Res 2001 61(5):2038-46.
WHSC2-141	A2	WHSC2	141-149	ILGELREKV	Cancer Res 2001 61(5):2038-46.
HNRPL-140	A2	HNRPL	140-148	ALVEFEDVL	Cancer Res 2001 61(5):2038-46.
SART3-302	A2	SART3	302-310	LLQAEAPRL	Int J Cancer 2000 88(4):633-9.
SART3-309	A2	SART3	309-317	RLAEYQAYI	Int J Cancer 2000 88(4):633-9.
SART2-93	A24	SART2	93-101	DYSARWNEI	J Immunol 2000 164(5):2565-74.
SART3-109	A24, A3sup, A26	SART3	109-118	VYDYNCHVDL	Cancer Res 1999 59(16):4056-63.
Lck-208	A24	p56^{lck}	208-216	HYTNASDGL	Eur J Immunol 2001 31(2):323-32.
PAP-213	A24	PAP	213-221	LYCESVHNF	J Urol 2001 166(4):1508-13.
PSA-248	A24	PSA	248-257	HYRKWIKDTI	Prostate 2003 57(2):152-9.
EGFR-800	A24	EGF-R	800-809	DYVREHKDNI	Eur J Cancer 2004 40(11):1776-86.
MRP3-503	A24	MRP3	503-511	LYAWEPSFL	Cancer Res 2001 61(17):6459-66.
MRP3-1293	A24	MRP3	1293-1302	NYSVRYRPGL	Cancer Res 2001 61(17):6459-66.
SART2-161	A24	SART2	161-169	AYDFLYNYL	J Immunol 2000 164(5):2565-74.
Lck-486	A24	p56^{lck}	486-494	TFDYLRSVL	Eur J Immunol 2001 31(2):323-32.
Lck-488	A24	p56^{lck}	488-497	DYLRSVLEDF	Eur J Immunol 2001 31(2):323-32.
PSMA-624	A24	PSMA	624-632	TYSVSFDSL	Cancer Sci 2003 94(7):622-7.
EZH2-735	A24	EZH2	735-743	KYVGIEREM	Prostate 2004 60(4):273-81.
PTHrP-102	A24	PTHrP	102-111	RYLTQETNKV	Br J Cancer 2004 91(2):287-96.
SART3-511	A3sup	SART3	511-519	WLEYYNLER	Cancer Immunol Immunother 2007 56(5):689-98.
SART3-734	A3sup	SART3	734-742	QIRPIFSNR	Cancer Immunol Immunother 2007 56(5):689-98.
Lck-90	A3sup	p56^{lck}	90-99	ILEQSGEWWK	Br J Cancer 2007 97(12):1648-54.

A3sup, HLA-A3 supertypes (A3, A11, A31, and A33); HLA, human leukocyte antigen; CypB, cyclophilin B; EGFR, epidermal growth factor-receptor ; HNRPL, heterogeneous nuclear ribonucleoprotein L; Lck, p56^{lck} ; MRP3, multidrug resistance-associated protein 3; PAP, prostatic acid phosphatase; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; PTHrP, parathyroid hormone-related peptide; SART2, squamous cell carcinoma antigen2; SART3, squamous cell carcinoma antigen 3; UBE2V, ubiquitin-conjugated enzyme variant Kua: WHSC2. Wolf-Hirshhorn syndrome critical region.

In order to avoid any possible biases, a sequence was considered to be positive for linear sequence similarity if it consisted of at least 5 identical amino acids between c-BIF and each of 31 CTL epitope peptides.

T lymphocytes recognize complexes of 9 to 10 length peptides and antigen-presenting cells through TCRs. TCRs contain a single binding site for antigens, i.e., the complementarity determining region (CDR) 3 within one of the 4th to 7th positions' amino acids, and also two different binding sites for major histocompatibility complex (MHC), CDR 1 within one of the positions of 2nd or 3rd amino acid, and CDR2 within one of the positions of 8th to 9th amino acids, respectively, whereas an amino acid at 1st position of CTL epitope peptide is scarcely involved in the binding to TCR, respectively (10). Then, the definition of positive sequence similarity was evaluated between 8 amino acids of the CTL epitope peptide from the 2nd~9th position and the corresponding c-BIF in the case of peptides of 9 amino acids, or both 2nd ~9th and 3rd ~10th amino acids in the case of peptides of 10 amino acids.

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Positive sequence similarity was defined as follows: (1) a positive sequence consists of at least one identical amino acid from CDR1, CDR2, and CDR3, (2) a positive sequence consists of at least 5 identical amino acids, and (3) the majority (≥75%) of amino acids of CTL epitope peptides were identical to those of c-BIF-derived peptide to avoid any possible biases. It also adapted to commensal clostridium.

3. Results

Linear sequence similarity analysis

The linear sequence similarity between c-BIF and each of 31 CTL epitope peptides against tumor-associated antigens was investigated and the results are shown in Table 2. The analysis revealed 4,002 positive numbers of similarity sites between them with 129 mean per peptide ranging from 84 to 443. Median positive numbers were 107.

Table 2. Linear sequence similarity analysis between commensal BIF and each of the cytotoxic T lymphocyte epitope peptides against human tumor-associated antigen.

Peptide name	Original protein	Position	Sequence	Sequence for analyis	numbers of 8 amino acid identity	numbers of 7 amino acid identity	numbers of 6 amino acid identily	numbers of 5 amino acid identity	total
Lck-486	p56 lck	486-494	TFDYLRSVL	TFDYLRSVL	0	54	13	44	111
WHSC2-141	WHSC2	141-149	ILGELREKV	ILGELREKV	0	17	72	1	90
Lck-246	p56 lck	246-254	KLVERLGAA	KLVERLGAA	4	24	182	6	216
Lck-422	p56 lck	422-430	DVWSFGILL	DVWSFGILL	0	11	39	47	97
ppMAPkkk-432	ppMAPkkk	432-440	DLLSHAFFA	DLLSHAFFA	0	18	32	46	96
WHSC2-103	WHSC2	103-111	ASLDSDPWV	ASLDSDPWV	0	16	27	64	107
UBE2V-43	UBE2V	43-51	RLQEWCSVI	RLQEWCSVI	0	0	13	71	84
UBE2V-85	UBE2V	85-93	LIADFLSGL	LIADFLSGL	0	46	80	4	130
HNRPL-140	HNRPL	140-148	ALVEFEDVL	ALVEFEDVL	1	7	24	76	108
SART3-302	SART3	302-310	LLQAEAPRL	LLQAEAPRL	5	20	66	4	95
SART3-309	SART3	309-317	RLAEYQAYI	RLAEYQAYI	1	7	65	17	90
SART2-93	SART2	93-101	DYSARWNEI	DYSARWNEI	0	4	4	91	99
Lck-208	p56 lck	208-216	HYTNASDGL	HYTNASDGL	0	11	90	9	110
PAP-213	PAP	213-221	LYCESVHNF	LYCESVHNF	0	0	101	0	101
MRP3-503	MRP3	503-511	LYAWEPSFL	LYAWEPSFL	9	7	39	50	105
SART2-161	SART2	161-169	AYDFLYNYL	AYDFLYNYL	0	2	3	185	190
SART3-734	SART3	734-742	QIRPIFSNR	QIRPIFSNR	0	16	16	71	131
SART3-511	SART3	511-519	WLEYYNLER	WLEYYNLER	0	14	16	59	89
PSMA-624	PSMA	624-632	TYSVSFDSL	TYSVSFDSL	0	26	97	21	144
EZH2-735	EZH2	735-743	KYVGIEREM	KYVGIEREM	0	14	39	46	99
PTHrP-102	PTHrP	102-111	RYLTQETNKV	RYLTQETNKV	0	26	95	322	443
Lck-488	p56 lck	488-497	DYLRSVLEDF	DYLRSVLEDF	0	53	11	78	142
CypB-129	Cyclophilin B	129-138	KLKHYGPGWV	KLKHYGPGWV	0	1	55	46	102
SART3-109	SART3	109-118	VYDYNCHVDL	VYDYNCHVDL	1	14	16	71	**102**
HNRPL-501	HNRPL	501-510	NVLHFFNAPL	NVLHFFNAPL	1	7	28	64	119
PSA-248	PSA	248-257	HYRKWIKDTI	HYRKWIKDTI	3	31	75	167	276
EGF-R-800	EGF-R	800-809	DYVREHKDNI	DYVREHKDNI	0	38	76	0	116
MRP3-1293	MRP3	1293-1302	NYSVRYRPGL	NYSVRYRPGL	18	3	27	56	104
Lck-90	p56 lck	90-99	ILEQSGEWWK	ILEQSGEWWK	0	10	32	59	101
Lck-449	p56 lck	449-458	VIQNLERGYR	VIQNLERGYR	1	44	19	44	108
PAP-248	PAP	248-257	GIHKQKEKSR	GIHKQKEKSR	0	1	46	52	97
Totall									4002
Mean									129
Median									105

Details for the Lck486-94 peptide are shown as a representative case among the 31 peptides, since the Lck486-94 peptide was expressed on both metastatic tumor cells and activated T cells, and then one of the suitable peptides used for the study (11). The Lckprotein, a member of the proto-*oncogene* tyrosine-protein kinase family, is essential for T cell activation and also for anchor-independent proliferation of tumor cells. Furthermore, a monoclonal Lck486-94 peptide antibody inhibited tumor growth in a mouse model in association with activation of dendritic cells and suppression of T regulatory cells (9). There are no eight amino acid identity site, 54 seven amino acid identity sites, 13 six amino acid identity sites, and 44 five amino acid identity sites. Collectively, a total of 111 amino acid identity sites existed between c-BIF and the LCK486-94 peptide (Table 2).

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Similarity analysis of TCR-mediated recognition

A similarity analysis of TCR-mediated recognition between c-BIF and each of the 31 CTL epitope peptides was conducted. The results are shown in Table 3. The sequence analysis clarified 5,018 positive numbers of similarity sites between them with 162 mean per peptide ranging from 25 to 677. Median peptide numbers were 132.

Table 3. TCR-mediated sequence similarity analysis between commensal BIF and each of the cytotoxic T lymphocyte epitope peptides against human tumor-associated antigen.

Peptide name	Original protein	Position	Sequence	Sequence for analyis	numbers of 8 amino acid identity	numbers of 7 amino acid identity	numbers of 6 amino acid identily	numbers of 5 amino acid identity	total of c-BIF	total of clostridium
Lck-486	p56 lck	486-494	TFDYLRSVL	TFDYLRSVL	0	59	10	95	164	218
WHSC2-141	WHSC2	141-149	ILGELREKV	ILGELREKV	0	18	16	472	506	673
Lck-246	p56 lck	246-254	KLVERLGAA	KLVERLGAA	0	17	149	28	194	1950
Lck-422	p56 lck	422-430	DVWSFGILL	DVWSFGILL	0	5	22	21	48	453
ppMAPkkk-432	ppMAPkkk	432-440	DLLSHAFFA	DLLSHAFFA	0	0	36	56	92	694
WHSC2-103	WHSC2	103-111	ASLDSDPWV	ASLDSDPWV	0	4	4	135	143	15
UBE2V-43	UBE2V	43-51	RLQEWCSVI	RLQEWCSVI	0	0	12	24	26	59
UBE2V-85	UBE2V	85-93	LIADFLSGL	LIADFLSGL	0	45	1	0	46	733
HNRPL-140	HNRPL	140-148	ALVEFEDVL	ALVEFEDVL	0	2	8	31	41	822
SART3-302	SART3	302-310	LLQAEAPRL	LLQAEAPRL	0	13	25	86	134	786
SART3-309	SART3	309-317	RLAEYQAYI	RLAEYQAYI	0	0	164	110	274	704
SART2-93	SART2	93-101	DYSARWNEI	DYSARWNEI	0	0	7	18	25	51
Lck-208	p56 lck	208-216	HYTNASDGL	HYTNASDGL	0	11	54	152	217	106
PAP-213	PAP	213-221	LYCESVHNF	LYCESVHNF	0	0	105	2	107	79
MRP3-503	MRP3	503-511	LYAWEPSFL	LYAWEPSFL	0	10	17	53	80	12
SART2-161	SART2	161-169	AYDFLYNYL	AYDFLYNYL	0	0	1	29	30	148
SART3-734	SART3	734-742	QIRPIFSNR	QIRPIFSNR	0	18	41	48	107	81
SART3-511	SART3	511-519	WLEYYNLER	WLEYYNLER	0	2	24	10	36	699
PSMA-624	PSMA	624-632	TYSVSFDSL	TYSVSFDSL	0	6	56	103	165	129
EZH2-735	EZH2	735-743	KYVGIEREM	KYVGIEREM	0	0	14	77	91	109
PTHrP-102	PTHrP	102-111	RYLTQETNKV	RYLTQETNKV	0	3	93	226	322	68
Lck-488	p56 lck	488-497	DYLRSVLEDF	DYLRSVLEDF	0	0	71	606	677	998
CypB-129	Cyclophilin B	129-138	KLKHYGPGWV	KLKHYGPGWV	0	0	2	47	49	130
SART3-109	SART3	109-118	VYDYNCHVDL	VYDYNCHVDL	0	1	28	4	33	59
HNRPL-501	HNRPL	501-510	NVLHFFNAPL	NVLHFFNAPL	5	5	46	73	129	545
PSA-248	PSA	248-257	HYRKWIKDTI	HYRKWIKDTI	0	0	136	197	333	128
EGF-R-800	EGF-R	800-809	DYVREHKDNI	DYVREHKDNI	0	9	43	115	167	125
MRP3-1293	MRP3	1293-1302	NYSVRYRPGL	NYSVRYRPGL	0	2	13	122	137	72
Lck-90	p56 lck	90-99	ILEQSGEWWK	ILEQSGEWWK	0	11	23	188	222	299
Lck-449	p56 lck	449-458	VIQNLERGYR	VIQNLERGYR	0	45	30	214	289	87
PAP-248	PAP	248-257	GIHKQKEKSR	GIHKQKEKSR	0	1	51	82	134	88
Total									5018	11120
Mean									162	359
Median									134	129

Details for the Lck486-94 peptide are shown as a representative case among the 31 peptides. The first position of amino acid (T in Lck486-94 peptide) could not be involved in binding to either MHC molecules or TCR, whereas FD at the 2nd or 3rd position could bind to MHC, YLRS at 4~7 position to TCR, or VL at 8th or 9th position bind to MHC, respectively. We therefore analyzed the sequence similarity between the Lck486-94 peptide beside T (FDYLRSVL) and peptides within c-BIF. There were no eight amino acid identity sites. Seven amino acids (FDYLRSVL) beside Y (under lined letter) at the 4th position of the Lck486-94 peptide were identical to one site at the 50~57 position, or 56 different sites at the 77~84 positions of Bifidobacterium longum (Lacl family transcriptional regulator). Similarly, seven amino acids 84~91(FDELRSVL) beside E (underlined) at the 4th position of Bifidobacterium longum or 82~89 of Bifidobacterium longum infantes (Lacl family transcriptional regulator). A total of 59 different sites were defined as the positive sequence similarity (Table 3). It is of note that these peptides work as transcriptional regulators to regulate the conversion of DNA to RNA, thereby orchestrating gene activity.

Six amino acids at the 2nd to 7th positions (FDYLRS) of the Lck486-494 peptide were identical to the position of 318~323 (hypothetical protein) or 324~329 (1,4-beta-xylanase) of Bifidobacterium longum, respectively. Six amino acids (FDYLRSV) beside R at the 6th position were identical to the

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position of 372~378 beside Q of Bifidobacterium longum (polysaccharide pyruvyl transferase). Six amino acids (FDYLRSVL) besides R and S at the 4th and 5th positions were identical to the position of 166~173 of Bifidobacterium adolescentis (hypothetical protein). A total of 10 different sites were defined as the positive sequence similarity (Table 3). Five amino acids (FDYLR) were all identical to the positions of 173~177 (preprotein translocase subunit SecA) of Bifidobacterium longum, to the positions of 183~187 (preprotein translocase subunit SecA) of each of the five different commensal Bifidobacteria (adolescentis, Bifidobacterium, bifidum, breve, or longum), 206~210 of breve, 207~211 of longum, 221~225 of adolescentis, and 230~234 of adolescentis protein) or 324~329 (1,4-beta-xylanase) of Bifidobacterium longum, respectively. A total of 95 different sites were defined as the positive sequence similarity (Table 3). Notably, these peptides mostly play a central role in coupling the hydrolysis of ATP to the transfer of proteins into and across the cell membrane.

Collectively, a total of 164 amino acid identity sites existed between c-BIF and Lck486-94 peptide (Table 3).

We also conducted a TCR-mediated recognition similarity analysis between commensal clostridium and the CTL peptides. The results showed 11,120 positive numbers with 359 mean and 129 median per peptide, respectively (furthest to the right column of Table 3). It is of note that more than half of these peptides belonged to hypothetical protein. A few of them function as either RNA processing protein RimM, which is essential for processing of 16S rRNA, or DNA gyrase subunit A, which belongs to the type II topoisomerases and which negatively supercoils closed circular double-stranded DNA.

4. Discussion

We employed both a linear sequence analysis and a similarity analysis of TCR-mediated recognition of CTL epitope peptides on antigen-presenting cells. This is mainly because the antibody recognition patterns are different from TCR-mediated recognition. Antibody interacts with antigen in the extracellular space of antigen-presenting cells. Antibodies produced by activated B lymphocytes generally recognize only a small region on the surface of a peptide or protein. In contrast, TCR recognizes the peptide antigen presented in the form of a complex of antigen bound to the MHC molecule. Further, B lymphocyte differentiation into plasma cells to produce a subclass of antibodies requires the assistance of antigen-specific helper T lymphocytes. Accordingly, linear sequence analysis may contribute to a better understanding of antibody-mediated antigenic mimicry, while similarity analysis of T cell receptor (TCR)-mediated recognition may contribute to a better understanding of T lymphocyte-mediated antigenic mimicry. Regardless of those assumption, the results showed the existence of durable and abundant sequence similarities between c-BIF and CTL epitope peptides by either linear sequence analysis or TCR-mediated recognition similarity analysis.

We performed the function of c-BIF peptides showing positive sequence similarity to the Lck486-94 peptide as one representative case among the 31 peptides. Seven amino acids (FDYLRSVL) beside Y (underlined letter) at the 4th position of the Lck486-94 peptide were identical to one site of as large as 57 different sites of 77~84 positions of Bifidobacterium longum (LacI family transcriptional regulatory). Transcriptional regulators regulate diverse genes and complex regulars in bacteria and eukaryotes, which in turn might be important to better understand how c-BIF plays a role in the induction of cellular and humoral immunity in the absence of tumor cells via antigenic mimicry.

In contrast, commensal clostridium might make almost no contribution to the antigenic mimicry, since there is a very low percentage of commensal clostridium in stool (<one millionth) with little immune activation of tumor immunity (7). Further, more than half of these peptides belonged to hypothetical protein. A few of them function either as the RNA-processing protein RimM, which is essential for processing of 16S rRNA, or the DNA gyrase subunit A, which belongs to type II topoisomerase and negatively supercoils closed circular double-stranded DNA.

Mitsuoka described how intestinal microbiota influenced host immunity and cancer prevention, and mentioned that Bacteroides, eubacterium and c-BIF promote health, including by promoting the maintenance of a normal immune response (7). He also noted that commensal clostridium is mostly involved in many types of diseases as pathogenic bacteria. These results suggest that commensal

clostridium rather disturbs the induction of cellular and humoral immunity in the absence of tumor cells via antigenic mimicry.

It might seem reasonable to expect that sequence similarity between c-BIF and human-immunodeficiency virus (HIV)-specific virus via antigenic mimicry also exists, as suggested by Su et al. (12). They showed the existence of memory T lymphocytes specific to HIV and c-BIF. Therefore, the sequence similarity analyses conducted in this study might lead to an improved understanding of whether c-BIF plays a role in the induction of cellular and humoral immunity reactive to HIV in the absence of viral infection via antigenic mimicry.

In this manuscript we have demonstrated that robust and abundant amino acid similarities exist between c-BIF and CTL epitope peptides against tumor-associated antigens, indicating that c-BIF could be a key player capable of inducing cellular and humoral immunity in the absence of cancer cells via antigenic mimicry.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Authors contribution: K.Itoh provided basic concept of the manuscript. S.Schichijo provided the information from GenPept.Graphics Next Previous Descriptions in Supplemental Information 1 and 2. S. Suekane provided information of intestinal microbiota influence host immunity and cancer prevention. All authors were involved this manuscript preparation with acceptance for the submission.

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