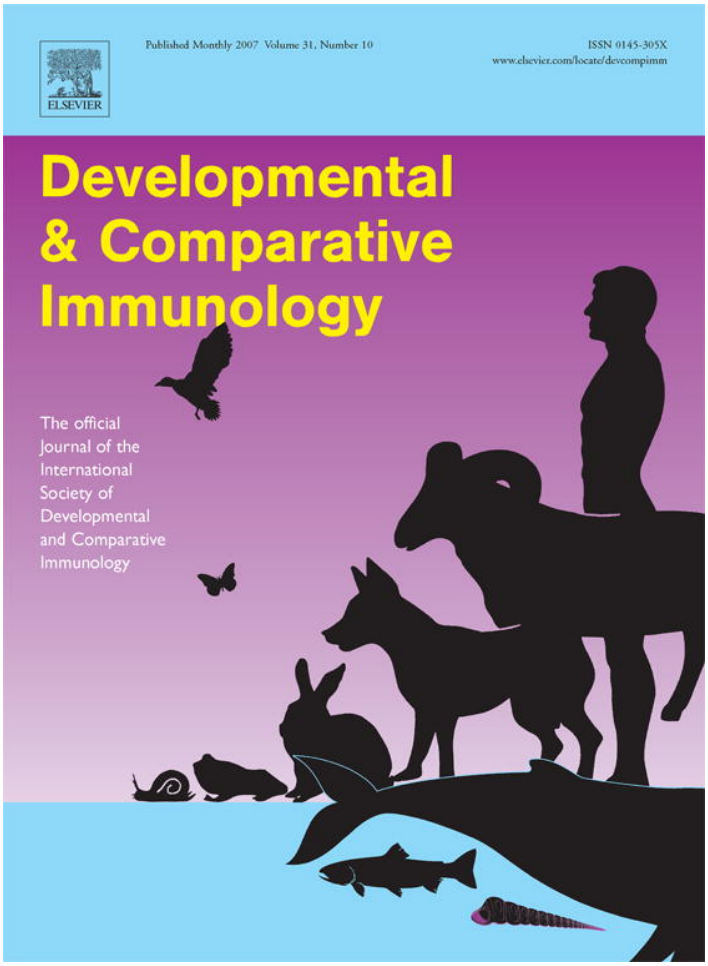

Available online at www.sciencedirect.com
ScienceDirect
Developmental and Comparative Immunology 31 (2007) 1050–1072
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

IMGT Locus in focus

IMGT Colliers de Perles and IgSF domain standardization for T cell costimulatory activatory (CD28, ICOS) and inhibitory (CTLA4, PDCD1 and BTLA) receptors

Vijay Phani Garapati[a], Marie-Paule Lefranc[a,b,*,1]

[a]*IMGT®, The International ImMunoGeneTics Information System®, Laboratoire d'ImmunoGénétique Moléculaire, LIGM, Institut de Génétique Humaine, IGH, UPR CNRS 1142, 141 rue de la Cardonille 34396 Montpellier Cedex 5, France*
[b]*Institut Universitaire de France, France*

Received 22 December 2006; received in revised form 24 January 2007; accepted 26 January 2007
Available online 5 March 2007

Abstract

T cell activation depends on the specific recognition by their T cell receptors (TR) of antigenic peptides bound to major histocompatibility complex (pMHC). Optimal T cell responses occur when T cells not only receive antigen-specific signals through the TR but also non-antigen-specific costimulatory activatory or inhibitory signals through costimulatory receptors. The activatory CD28/B7-1 (or B7-2), inhibitory CTLA4/B7-1 (or B7-2), activatory ICOS/B7H2 and inhibitory PDCD1/B7H1 (or B7DC) pathways involve the interaction of the V-LIKE-DOMAIN of the receptor with a B7 family member. The BTLA/HVEM pathway involves the interaction of the BTLA receptor C-LIKE-DOMAIN with HVEM, a TNFR family member. The human and mouse CD28, CTLA4, ICOS, PDCD1 and BTLA genes, alleles and alternative transcripts and the IMGT Colliers de Perles of the IgSF domains, based on the IMGT unique numbering, are described according to the IMGT-ONTOLOGY concepts of IMGT®, the international ImMunoGeneTics information system®, http://imgt.cines.fr).

Keywords: IMGT; CD28; CTLA4; ICOS; PDCD1; BTLA; IgSF; IMGT Collier de Perles

1. Introduction

Mounting an appropriate immune response depends on the careful regulation of lymphocyte activation. Lymphocytes require two independent signals to become fully activated. The first one is an antigen-specific signal. The T cell receptors (TR) on T cells or the immunoglobulins (IG) on B cells specifically bind the antigen and the signal is transmitted to the cell interior through the coreceptors which comprise for the TR the CD3 gamma (CD3G), CD3 delta (CD3D), CD3 epsilon (CD3E)

Abbreviations: aa, amino acid; kb, kilobase; bp, base pair

*Corresponding author. IMGT®, The International ImMunoGeneTics Information System®, Laboratoire d'ImmunoGénétique Moléculaire, LIGM, Institut de Génétique Humaine, IGH, UPR CNRS 1142, 141 rue de la Cardonille 34396 Montpellier Cedex 5, France. Tel.: +33 4 99 61 99 65; fax: +33 4 99 61 99 28.
E-mail address: Marie-Paule.Lefranc@igh.cnrs.fr (M.-P. Lefranc).

[1]IMGT®: http://imgt.cines.fr

0145-305X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dci.2007.01.008

and alternatively spliced CD3 zeta and CD3 eta (CD3Z) proteins organized in CD3$\gamma\varepsilon$, $\delta\varepsilon$, and $\zeta\zeta$ or $\zeta\eta$ dimers, and for the IG, CD79A and CD79B [1,2]. A second signal, termed 'costimulation' is critical for full activation of a naive lymphocyte. The costimulatory signal is independent of the antigen receptor and has no stimulatory capacity on its own, but is required to allow full activation [3].

The first cell surface protein shown to function as a costimulatory receptor on T lymphocytes was CD28 [4]. Since the identification of CD28, the number of proposed costimulatory receptors has grown significantly [5]. They are classified into activatory and inhibitory receptors depending on the resulting positive or negative signal on T cell activation, which follows ligand binding. These proteins possess at least one domain with a V-LIKE or C-LIKE fold and thus belong to the immunoglobulin superfamily (IgSF) [6–8] (Table 1). They are type I transmembrane proteins.

The CD28 family includes three closely related members: CD28, CTLA4 (cytotoxic T-lymphocyte-associated protein 4) and ICOS (inducible T-cell costimulator). Programmed cell death 1 (PDCD1) represents a more distantly related protein. The extracellular region of the CD28 family member (CD28, CTLA4, ICOS) proteins and of the PDCD1 protein comprises one V-LIKE-DOMAIN [9]. Their ligands expressed on antigen-presenting cells (APC) are members of the B7 family [10,11]. CD28, an activatory receptor [4,12], and CTLA4, an inhibitory receptor [13–15], share the same ligands B7-1 (CD80) and B7-2 (CD86) [16,17]. ICOS, an activatory receptor [18,19] binds B7H2 (ICOSL, B7RP1) [20,21]. PDCD1, an inhibitory receptor [22], binds B7H1 (PD-L1, B7-H1) and B7DC (PD-L2, B7-DC) [23]. A fifth receptor, B and T lymphocyte attenuator (BTLA) comprises one C-LIKE-DOMAIN [9]. BTLA, an inhibitory receptor, binds herpes virus entry mediator (HVEM), a member of the TNF receptor superfamily [24–26] although it was first suggested that it may also interact with B7H4 [27]. The costimulatory receptors bind their ligands via their extracellular IgSF domain (V-LIKE-DOMAIN for CD28, CTLA4, ICOS and PDCD1 and C-LIKE-DOMAIN for BTLA) and the signal is exerted and delivered to the T cells through the cytoplasmic region of the receptors.

In humans, the CD28, CTLA4, ICOS and PDCD1 genes are localized on chromosome 2 (Table 2, Fig. 1). The CD28, CTLA4 and ICOS genes are closely linked at 2q33.3 and oriented in the forward (FWD) orientation [28,29]. They form an immunological costimulatory receptor locus which spans 380 kilobases (kb). CD28 and CTLA4 are separated by 30 kb [30]. Human chromosome 2q33 is considered as an immunologically important region based on the linkage of numerous autoimmune diseases to the CTLA4 locus [31]. PDCD1 is localized on chromosome 2 at 2q37.3 [32] and BTLA on chromosome 3 at 3q13.2, both genes being in reverse (REV) orientation [24].

In this review, we provide a standardized description of the human and mouse CD28, CTLA4, ICOS, PDCD1 and BTLA genes and proteins according to IMGT-ONTOLOGY [33] and to the IMGT Scientific chart rules [34–36].

Table 1
Characteristics of the human CD28 family member (CD28, CTLA4, ICOS), PDCD1 and BTLA proteins

Protein name	Chain association	Monomer molecular weight (in kDa)	Extracellular IgSF domain		Number of N-glycosylation sites[a]	Costimulatory signal[b]	Cell expression	Inducible
			Number	Type				
CD28	Homodimer	25	1	V	5	+	T cells	Yes
CTLA4	Homodimer	24	1	V	2	–	T cells	Yes
ICOS	Homodimer	22	1	V	3	+	T cells	Yes
PDCD1	Monomer	31	1	V	4	–	T, B and myeloid cells	Yes
BTLA	Monomer	32	1	C	3	–	T and B cells	Yes

kDa: kilodalton.
These proteins are membrane type I proteins (N-terminal end is extracellular and C-terminal end is intracellular).
[a]All the N-glycosylation sites are localized in the extracellular IgSF (V-LIKE-DOMAIN for CD28, CTLA4, ICOS and PDCD1) and C-LIKE-DOMAIN for BTLA.
[b](+) indicates an activatory signal and (–) indicates an inhibitory signal.

Table 2
Chromosomal localization of the *Homo sapiens* and *Mus musculus* CD28 family member (CD28, CTLA4, ICOS), PDCD1 and BTLA genes

IMGT and HGNC gene name	Chromosomal localization		Gene orientation on chromosome		Gene aliases
	Human	Mouse	Human	Mouse	
CD28	2q33.3	1C1.3 (30.1 cM)	FWD	FWD	Tp44
CTLA4	2q33.3	1C1.3 (30.1 cM)	FWD	FWD	CD152, CTLA-4
ICOS	2q33.3	1C1.3 (32.0 cM)	FWD	FWD	CD278, AILIM, CRP-1
PDCD1	2q37.3	1D	REV	REV	CD279, PD-1
BTLA	3q13.2	16B5 (29 cM)	REV	FWD	CD272, BTLA1

FWD (forward orientation) and REV (reverse orientation) are according to the concept of ORIENTATION of genes and locus on chromosome (IMGT Index, http://imgt.cines.fr).

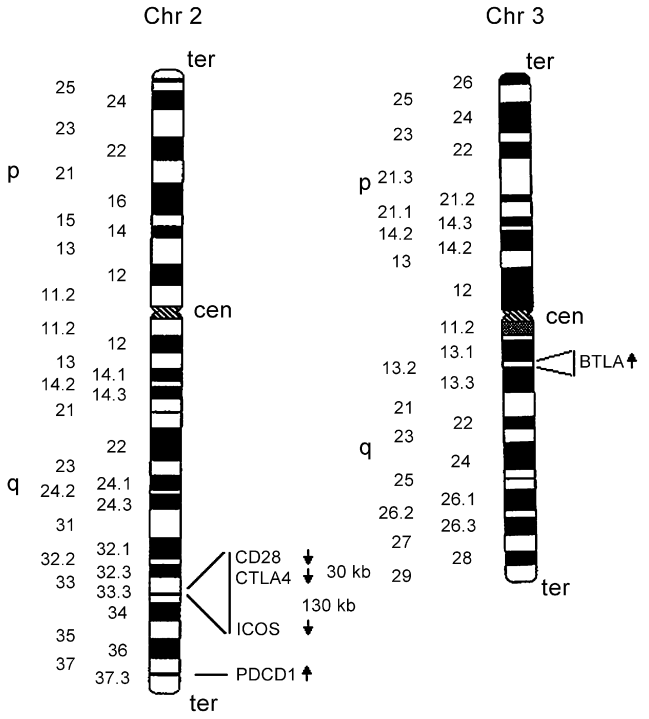

Fig. 1. *Homo sapiens* chromosomal localization of the CD28 family members (CD28, CTLA4, ICOS), PDCD1 and BTLA genes. CD28, CTLA4, ICOS are localized on chromosome 2 at band 2q33.3 in FWD orientation, PDCD1 on chromosome 2 at band 2q37.3 in REV orientation, and BTLA on chromosome 3 at band 3q13.2 in REV orientation. CD28 is at 30 kb from CTLA4 and CTLA4 is at 130 kb from ICOS (IMGT Repertoire > RPI, http://imgt.cines.fr). Gene orientation is shown by arrows. FWD and REV orientation are according to the concept of ORIENTATION of genes and locus on chromosome (IMGT Index, http://imgt.cines.fr).

We provide two-dimensional (2D) graphical representations or IMGT Collier de Perles of the IgSF domains based on the IMGT unique numbering [6,7]. The IMGT Colliers de Perles allow to bridge the gap between sequences and 3D structures in IMGT®, the international ImMunoGeneTics information system® [34], as this can be demonstrated with the three-dimensional (3D) structures for CD28, CTLA4, PDCD1 and BTLA, which are available in PDB [37] and in IMGT/3Dstructure-DB [38]. The IMGT Colliers de Perles also allow to compare IgSF domains between non-mammalian species as recently shown in teleost [9].

2. CD28

2.1. CD28 activatory receptor

CD28 is a 25 kDa homodimeric glycoprotein present on T cells that interacts with B7-1 (CD80) and B7-2 (CD86) expressed on the APC [4,12,39]. A cysteine in the connecting region allows the homodimer formation. CD28 costimulation is essential for T cell proliferation, survival, interleukin (IL2) production and T helper type 2 (Th2) development [40]. The activation signalling pathway which follows the CD28/B7-1 and CD28/B7-2 interactions has been widely recognized as the major costimulation activatory pathway for naive T cell activation [41,42]. This pathway plays a central role in immune responses against pathogens, in autoimmune diseases and in graft rejection. CD28 intensifies and prolongs biochemical signals that are normally generated by the TR-CD3 complex [40]. CD28 mediates the production of IL2, that in turn allows the proliferation, differentiation and survival of activated T cells [43].

The possible mechanisms by which human and mouse CD28 initiate costimulation is by recruiting the phosphatidylinositol-3-kinase (PI3K), following phosphorylation of the "tyrosine–methionine–asparagine–methionine" ^{13}YMNM^{16} motif in their cytoplasmic region [43] (Fig. 2). Phosphorylation of Y^{13} allows the recruitment of PI3K, growth factor receptor-bound protein 2 (GRB2) and Grb2-related adaptor downstream of SHC (GADS) via their src-homology region 2 (SH2) domains. This tyrosine is considered to uncouple the signals required for proliferation and survival [44]. Proline-rich motifs ^{18}PRRP^{21} and ^{30}PYAP^{33} adjacent to the ^{13}YMNM^{16} motif in the cytoplasmic region recruit the interleukin-2 tyrosine kinase (ITK) (a pleckstrin homology and SH3 domain-containing T-cell-specific tyrosine kinase) and the Src family lymphocyte-specific protein tyrosine kinase (LCK) via their SH3 domain, which leads to their activation. These proline-mediated interactions with SH3 domains are critical for downstream signalling [45,46]. The interaction pattern of CD28 with B7-1 (CD80)

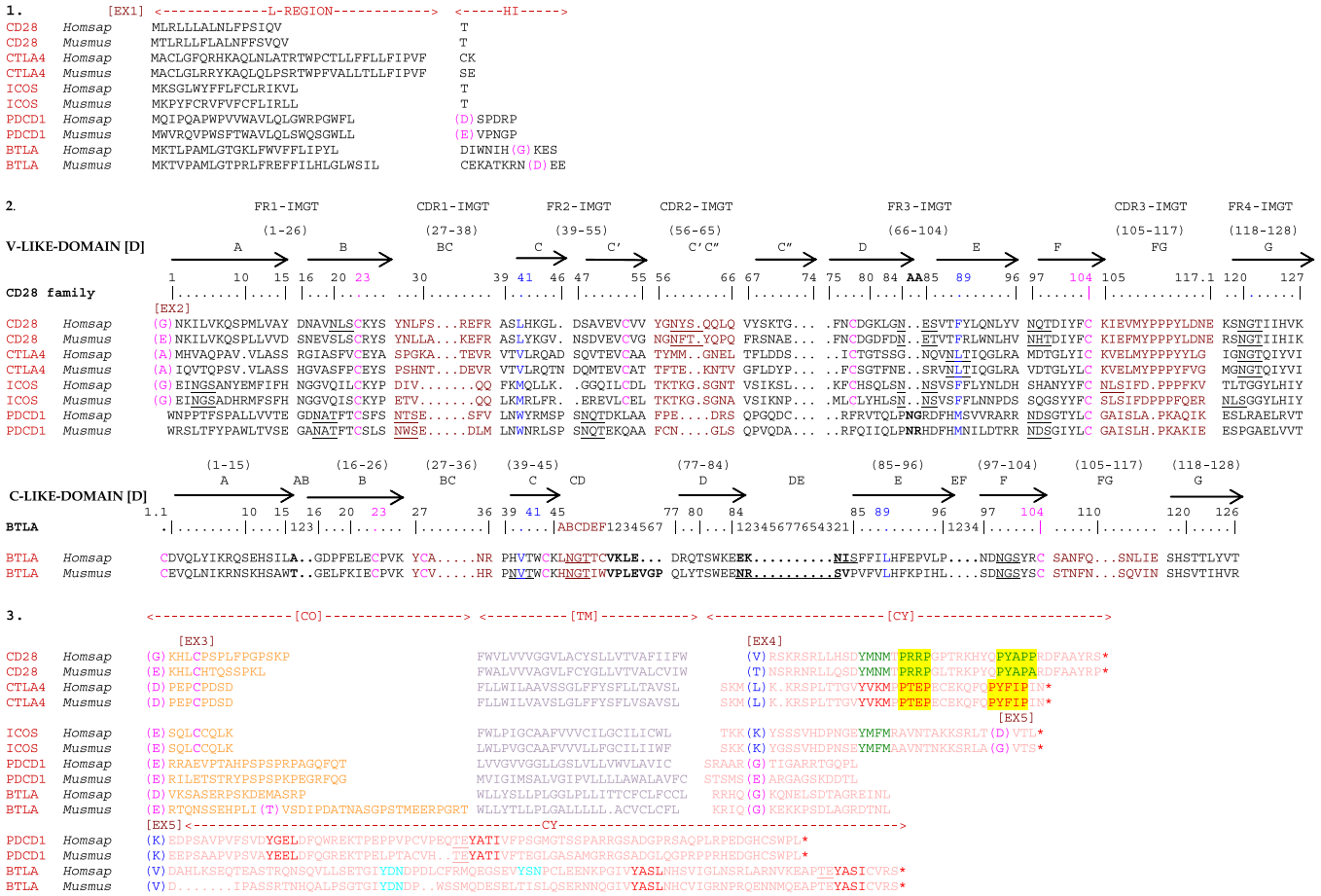

Fig. 2. IMGT Protein display of CD28 family member (CD28, CTLA4, ICOS), PDCD1 and BTLA proteins from human (*Homo sapiens*) and mouse (*Mus musculus*). All amino acid sequences are translations from alleles *01. Accession numbers are available in Fig. 3 legend. The IMGT Protein display is according to the IMGT unique numbering for V-LIKE-DOMAIN [6] and for C-LIKE-DOMAIN [7]. The V-LIKE-DOMAINs and the C-LIKE-DOMAINs are designated with the IMGT labels (IMGT Scientific chart, http://imgt.cines.fr). Beta strands of the extracellular IgSF domain are shown by horizontal arrows. Dots indicate gaps according to IMGT unique numbering [6,7]. Putative N-glycosylation sites are underlined. Amino acids resulting from a splicing with a preceding exon are shown between parentheses and the splicing frames are indicated in corresponding IMGT colors (IMGT Scientific chart, http://imgt.cines.fr). Splicing frame 1 in purple, Splicing frame 0 in blue. PI3K binding domain "Y-M-x-M" is in green, the proline-rich motifs are in yellow with letters in green for ITK (SH3) binding domain and with letters in red for phosphatase 2A (PP2A) binding domain, ITIM are in red (amino acids 'TE' of ITSM are underlined). The GRB2 binding domains "Y-x-N" are in cyan. The cysteine in the connecting region of CD28, CTLA4 and ICOS involved in the homodimer disulfide bond is in magenta. Gene names (symbols) are according to IMGT Nomenclature Committee (IMGT-NC) [33] and to the HUGO Nomenclature Committee (HGNC). Homsap*: Homo sapiens;* Musmus*: Mus musculus*. L-REGION, HI: HYDROPHILIC-REGION, CO: CONNECTING-REGION, TM: TRANSMEMBRANE-REGION and CY: CYTOPLASMIC-REGION.

suggests that CD28 has a dual role as an adhesion and a signalling molecule. B7-1 binds with low affinity to CD28 and with high affinity for CTLA4 and these interactions might hold the APC and the T cell at uniform distance that aids proper interaction of TR with antigenic peptides bound to major histocompatibility complex (pMHC) [16]. Both B7-1 and B7-2 are able to induce the association of PI3K with CD28 following the phosphorylation of the ^{13}YMNM^{16} motif. However, engagement of CD28 with B7-1 and with B7-2 have probably different functional consequences. Rapid dissociation of B7-2 from CD28 may not permit the robust tyrosine phosphorylation that the prolonged binding of B7-1 induces [17].

The signals generated by CD28 integrate with those of the TR-CD3 and activate the mitogen-activated protein kinase (MAPK) JNK. The JNK protein kinase phosphorylates transcription factors that activate the IL2 gene. These stimulations lead to the production of cytokines by T cells [47].

2.2. CD28 gene exon–intron organization

The human CD28 gene localized on chromosome 2 at 2q33.3 spans 28.2 kb from the EX1 initiation codon (ATG) to the stop codon. The coding region is organized in four exons (EX1–EX4) encoding 220 amino acids (aa) [48] (Fig. 3). The EX1 (52 bp) encodes the L-REGION (17 aa), EX2 (357 bp) encodes the extracellular V-LIKE-DOMAIN [D] (119 aa), EX3 (125 bp) encodes the CONNECTING-REGION (CO) (16 aa) and the TRANSMEMBRANE-REGION (TM) (26 aa), and EX4 (126 bp) encodes the CYTOPLASMIC-REGION (CY) (42 aa) (Table 3, Fig. 4). Different transcripts that result from alternative splicing have been described, five of them lack either the complete EX2 or a large part of it which affects the ligand binding extracellular V-LIKE-DOMAIN [D] (Table 4, Fig. 5). Two transcripts lack EX3 or a large part of it which results in TM absence or truncation, and may encode potential soluble isoforms (Fig 5) [49].

The mouse CD28 gene is localized on chromosome 1 at band C (C1.3, 30.1 cM). The mouse CD28 gene has four exons (EX1–EX4) encoding 218 aa [50]. The murine CD28 gene shares 61% nucleotide identity and the murine CD28 protein shares 68% aa identity with the human CD28 gene and protein, respectively. One allele has been observed in mouse but no splice variant has been described so far. The cytoplasmic region demonstrates high interspecies

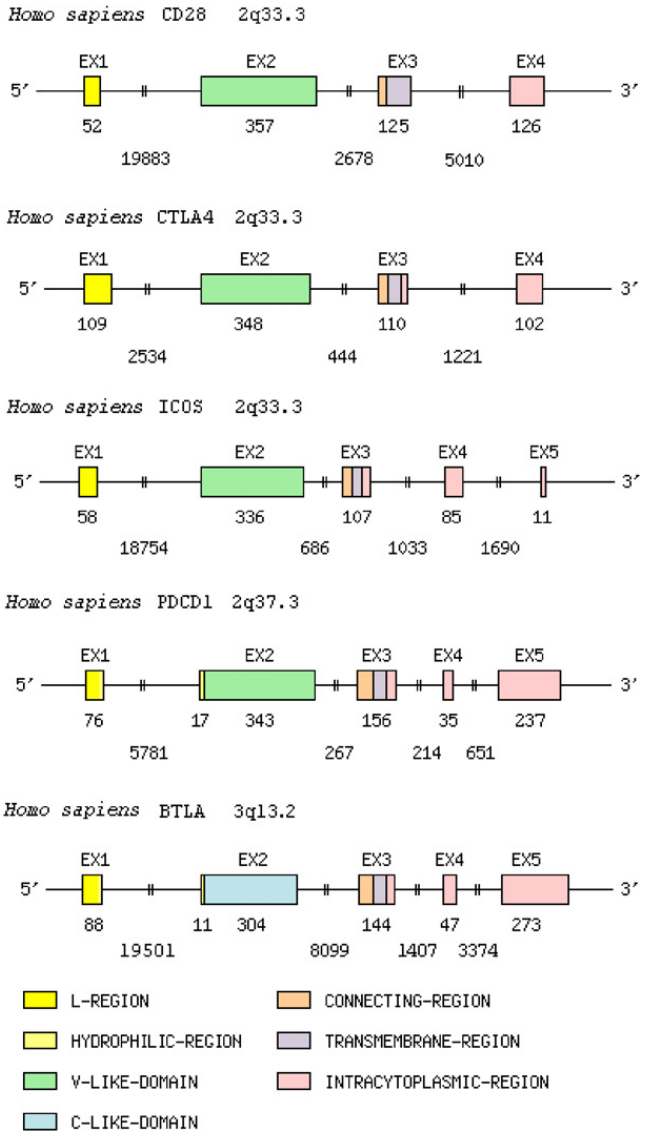

Fig. 3. Gene exon/intron organization of the *Homo sapiens* CD28 family member (CD28, CTLA4, ICOS), PDCD1 and BTLA genes. Intron and exon lengths are in base pairs (bp) (EMBL/GeneBank/DDBJ accession numbers, CD28*01 (M37812, M37813, M37814, M37815), CTLA4*01 (M74363, M3243, M37244, M37245), ICOS*01 (AJ535718), PDCD1*01 (AF363458) and BTLA*01 (AJ717664). Introns indicated with (‖) are not at scale. Colors are according to the IMGT color menu for regions and domains (http://imgt.cines.fr).

conservation. The motif ^{13}YMNM^{16} which allows the recruitment of PI3K following phosphorylation of the tyrosine and the proline-rich motifs are conserved in all vertebrate species including teleost [9].

2.3. CD28 V-LIKE-DOMAIN IMGT Collier de Perles

The extracellular CD28 region comprises a typical V-LIKE-DOMAIN which has the structure

Table 3
Exon lengths of the human CD28 family member (CD28, CTLA4, ICOS), PDCD1 and BTLA genes and corresponding translated regions

Gene and protein name	Protein length	EX1 bp (L-HY)	SF	EX2 bp (L-D)	SF	EX3 bp (CO-TM-CY)	SF	EX4 bp (CY)	SF	EX5 bp (CY)
CD28	220	52 (17) (16-1)	1	357 (119) (0-119)	1	125 (42) (16-26-0)	0	126 (42)	—	—
CTLA4	223	109 (36) (34-2)	1	348 (116) (0-116)	1	110 (37) (9-25-3)	0	102 (34)	—	—
ICOS	199	58 (19) (18-1)	1	336 (112) (0-112)	1	107 (36) (9-24-3)	0	85 (28)	1	11 (4)
PDCD1	288	76 (25) (25-0)	1	360 (120) (6-114)	1	156 (52) (23-24-5)	1	35 (12)	0	237 (79)
BTLA	289	88 (29) (23-6)	1	315 (105) (4-101)	1	144 (48) (18-26-4)	1	47 (16)	0	273 (91)

EMBL/GeneBank/DDBJ accession numbers: CD28*01 (M37812–M37815), CTLA4*01 (M74363, M37243–M37245), ICOS*01 (AJ535718), PDCD1*01 (AF363458) and BTLA*01 (AJ717664). Exon lengths are in base pairs (bp) with between parentheses, the number of encoded amino acids for the corresponding translated regions. For EX1, EX2 and EX3 the amino acids (aa) numbers are shown for both the complete exon and per region.
SF: splicing frame type (see IMGT Aide-mémoire > splicing sites, http://imgt.cines.fr). The amino acid that results from the splicing in splicing frame 1 is included in the aa number of the downstream (3′) exon.
IMGT label abbreviations: L: L-REGION, HI: HYDROPHILIC-REGION, D: DOMAIN [V-LIKE-DOMAIN for the CD28 family member (CD28, CTLA4, ICOS) and PDCD1 proteins and C-LIKE-DOMAIN for the BTLA protein], CO: CONNECTING-REGION, TM: TRANSMEMBRANE-REGION, CY: CYTOPLASMIC-REGION.

of a β-barrel with two layers formed by the antiparallel ABED and GFCC′C″ β-strands, respectively [6] (Fig. 6). The lengths of the strands and loops of the V-LIKE-DOMAIN are reported in Table 5 [6]. Positions are according to the IMGT unique numbering for V-DOMAIN and V-LIKE-DOMAIN [6,8]. The strand A has 15 aa (positions 1–15), strand B has 11 aa (positions 16–26), BC loop (CDR1-IMGT) has 9 aa (positions 27–38, positions 32–34 being unoccupied), strand C has 7 aa (positions 39–46, position 46 being unoccupied), strand C′ has 8 aa (positions 47–55), C′C″ loop (CDR2-IMGT) has 9 aa (position 56–65, position 61 being unoccupied), strand C″ has 6 aa (positions 66–74, positions 72–74 being unoccupied), strand D has 9 aa (positions 75–84, position 75 being unoccupied), strand E has 12 aa (positions 85–96), strand F has 8 aa (positions 97–104), FG loop (CDR3-IMGT) has 14 aa (position 105–117, with an addition aa at 117.1), strand G has 10 aa (positions 118–128, position 128 being unoccupied). The CD28 V-LIKE-DOMAIN has four cysteines, the 1st-CYS (C23) and 2nd-CYS (C104) involved in the classical intradomain bond, and cysteine (C53) and cysteine (C78) involved in a second additional intradomain bond. There are five N-glycosylation sites: N20 (strand B), N58 (CDR2-IMGT), N84 (DE turn), N97 (EF turn) and N120 (strand G). The cysteines and the N-glycosylation sites (except for N20) are also conserved in the mouse CD28 V-LIKE-DOMAIN. From mutation studies, the ^{109}MYPPPY^{114} conserved motif at the tip of the FG loop in CD28, and also found in CTLA4 (Fig. 2), is considered as the core-binding site for the B7-1 and B7-2 ligands. The *cis–trans–cis* main conformation of the three prolines brings the side chains of the prolines and tyrosines towards the ligands B7-1 and B7-2 [51]. The ^{109}MYPPPY^{114} motif is conserved in most of the organisms including *Gallus gallus* [52]. Mutations of the LDN residues of the ^{109}MYPPPYLDN^{117} motif of CD28 reduced both B7-1 and B7-2 binding, whereas mutations of Y^{110} and Y^{114} selectively reduced B7-2 binding without impacting upon B7-1 binding [53].

3. CTLA4

3.1. CTLA4 inhibitory receptor

The cytotoxic T-lymphocyte-associated protein 4 (CTLA4), the second member of the CD28 family, is an inhibitory receptor expressed by activated T cells. CTLA4 is expressed at very low levels by naive T cells compared to CD28, but following stimulation its expression increases dramatically [54,55]. CTLA4 plays a fundamental role in controlling T cell reactivity to self-antigens. As CD28, CTLA4 also interacts with B7-1 and B7-2 but CTLA4 transmits an inhibitory signal to T cells. CTLA4 engagement inhibits TR binding to the CD3 zeta (CD3Z) chain in the CD3 complex thereby inhibiting CD3Z phosphorylation and subsequent TR-CD3 signal transduction [15,56]. CTLA4 and CD28 differ in their affinities towards B7-1 and B7-2. The higher affinity of CTLA4 for B7-1 and B7-2 allows the CTLA4 inhibitory receptor to compete with the

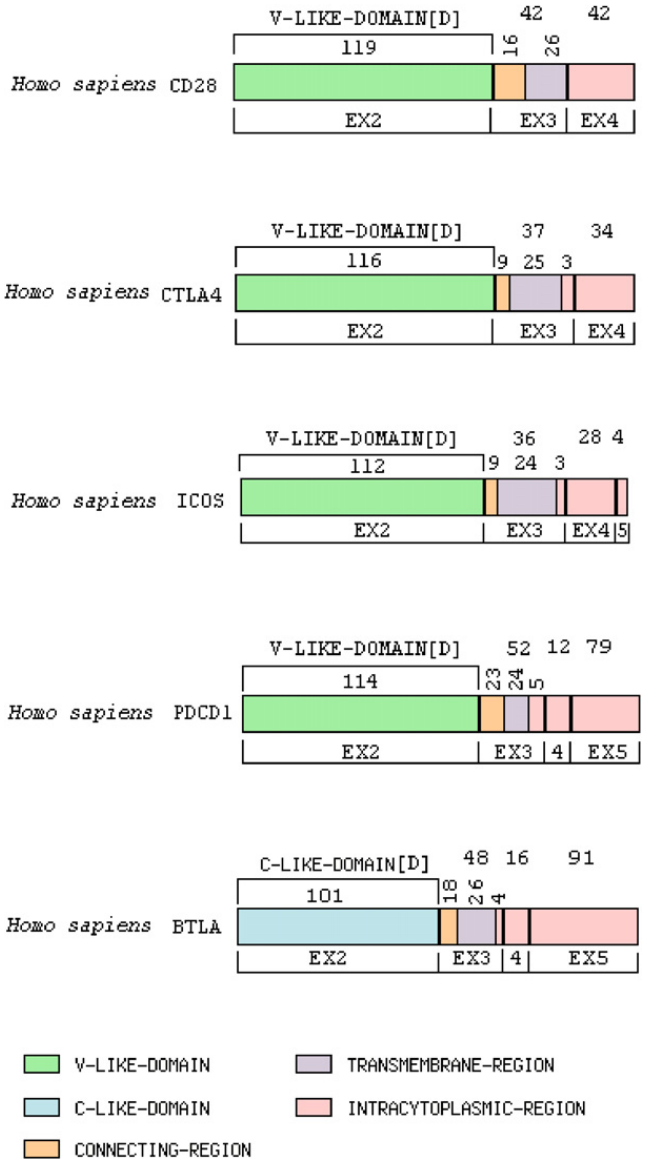

Fig. 4. Regions and domains of the *Homo sapiens* CD28 family member (CD28, CTLA4, ICOS), PDCD1 and BTLA proteins. Lengths of the domains are in number of amino acids. In CD28 family member and in PDCD1 genes, EX2 encodes a V-LIKE-DOMAIN [D] [6] whereas, in BTLA, EX2 encodes a C-LIKE-DOMAIN [D] [7]. Colors are according to IMGT Color menu for regions and domains (http://imgt.cines.fr).

CD28 activatory receptor for these shared ligands [16]. CTLA4 plays an important role in maintaining tolerance to self-antigens, both as a negative regulator of the T cell activation and as an inducer of clonal anergy [56].

3.2. CTLA4 gene exon–intron organization

The human CTLA4 gene localized on chromosome 2 at 2q33.3 spans 7 kb (7195 bp). The coding region is organized in four exons (EX1–EX4) encoding 223 aa [13,14,28] (Fig. 3). CTLA4 maps on the same locus as CD28 and is separated by 30 kb [57]. The chromosomal proximity of CD28 and CTLA4 and their close structural relationship suggests that these two genes which encode proteins sharing 31% aa identity resulted from duplication of a common ancestor [13,14,28]. The EX1 (109 bp) encodes the L-REGION (36 aa), EX2 (348 bp) encodes the extracellular V-LIKE-DOMAIN [D] (116 aa), EX3 (110 bp) encodes the CO (9 aa), TM (25 aa) and part of the CY (3 aa), EX4 (102 bp) encodes most of the CY (34 aa).

The mouse CTLA4 gene localized on chromosome 1 at band C (C1.3, 30.1 cM) spans 10.64 kb and is also organized in four exons (EX1–EX4) [13,57]. As in humans, the mouse CTLA4 gene is closely linked to the CD28 gene. Sequence comparison between the mouse and human CTLA4 genes revealed a high degree of sequence conservation both in the noncoding regions and the coding regions (78% identity), with an overall score of 71% over the entire length of the two genes [14,57]. The mouse CTLA4 protein shares 73% aa sequence identity with the human protein. The cytoplasmic region of human and mouse CTLA4 contains the ^{12}YVKM^{15} motif that recruits the Src homology 2 (SH2) domain, tyrosine phosphatase 2 (SHP2) [58] and the proline-rich motifs ^{17}PTEP^{20} and ^{28}PYFIP^{32} that bind the protein phosphatase 2A (PP2A) [59].

3.3. CTLA4 V-LIKE-DOMAIN IMGT Collier de Perles

The CTLA4 V-LIKE-DOMAIN has the classical topology. The lengths of the strands and loops are reported in Table 5 and the IMGT Collier de Perles is shown in Fig. 7. The conserved hexamer ^{109}MYPPPY^{114} motif in the FG loop and the ^{120}NGT^{122} motif in the G strand, also observed in CD28, are well conserved in all vertebrates including fish [9]. CTLA4 engagement with B7-1 induces a lattice structure that results in an alternating network of CTLA4 and B7-1 homodimers [60]. This lattice structure limits the B7-1 interaction with CD28. This model offers some explanation as to how limiting levels of CTLA4 expression can be so effective in preventing immune response [60]. There are two N-glycosylation sites at N88 (E-STRAND) and N120 (G-STRAND) in both human and mouse V-LIKE-DOMAIN, however the second site N120

is only present in human allele *02, as discussed in next paragraph. This N120 site is also conserved in human and mouse CD28 V-LIKE-DOMAIN (Fig. 2).

3.4. CTLA4 polymorphism and diseases

Many association studies of human autoimmune diseases linked to 2q33 mainly focused on CTLA4 gene polymorphisms. In human, three CTLA4 alleles have been identified (Table 6). The CTLA4*02 allele has a single nucleotide transition in EX2 (g364>a) leading to an aa change (A122>T) in the V-LIKE-DOMAIN (position according to the IMGT unique numbering for V-DOMAIN and V-LIKE-DOMAIN) [6]. This mutation leads to the loss of the glycosylation of N120 in the motif N-x-S/T. The CTLA4*03 allele has a single nucleotide transition which leads to an aa change in EX1 (a49>g, T17>A) [61]. This a49>g polymorphism has been linked to different autoimmune diseases such as Graves' disease, type 1 diabetes, coeliac disease, systemic lupus erythromatosus. There are more Graves disease patients with g/g or a/g at nucleotide 49 of the CTLA4 domain and significantly fewer with the a/a compared with normal controls [61–64].

4. ICOS

4.1. ICOS activatory receptor

The inducible T-cell co-stimulatory (ICOS), an homodimeric protein of 22 kDa, matching CD28 in potency, is the third member of the CD28 family [18]. ICOS gene is expressed in detectable levels on resting T cells and increases upon T cell activation and CD28 costimulation [65]. ICOS interacts with B7H2 (ICOSL, B7RP-1), a member of the B7 family expressed on the APC [20]. The *in vitro* experiments have verified that the interaction between ICOS and B7H2 stimulates the proliferation of T cells and production of Th2 cytokines, preferentially IL10 secretion. In humans, the expression of ICOS on the

Table 4A
Alternative spliced transcripts of the human CD28, ICOS, BTLA and mouse BTLA

Species	Gene name	IMGT spliced transcript type[a]	Accession numbers	Molecule type	Transcript length (codons or amino acids)	Number of missing codons	Positions of missing codons	New codons (or amino acids)[b]
Homo sapiens	CD28	A	J02988	cDNA	220			
		B	(1)		136	84	40–123	Y124
		C	AF222341	cDNA	123	97	40–136	W137
		D	AF222342	cDNA	101	119	18–136	
		E	AF222342	cDNA	80	140	40–123, 152–207	Y124
		F	AJ295273	cDNA	55	165	40–123, 137–178	Y124 G179, E180, E181*
Homo sapiens	ICOS	A	AJ277832	cDNA	199			
		B	BC028006	cDNA	168	31	168–195	M196*
Homo sapiens	BTLA	A	AJ717664	cDNA	289			
		B	DQ198368	cDNA	241	48	135–182	
Mus musculus	BTLA	A	AY29325	cDNA	306			
		B	AK041334	cDNA	305	1	156	I157

(1) no accession number, but described in PMID:2162892 [48] and mentioned in M37812–M37815.

[a]IMGT spliced transcript A is the complete transcript as deduced from genomic DNA, the human CD28 IMGT spliced transcripts types B to F result from alternative splicing that affects EX2 and/or EX3 and/or EX4 (Table 4B and Fig. 5). The CD28 resulting transcripts lack a part or the totality of the extracellular V-LIKE-DOMAIN [D] and/or part of the totality of the TRANSMEMBRANE-REGION. The human ICOS and BTLA spliced transcript of type B lack the EX4 (CY) and EX3 (CO-TM-CY), respectively.

[b]New codons (or amino acids) that result from the alternative splicing are numbered according to the downstream acceptor splice site and by comparison to the IMGT spliced transcript A. An asterisk (*) indicates a premature stop codon resulting from frameshift.

Table 4B
Exons affected by the alternative splicing

Species	Gene name	IMGT spliced transcript type[a]	Transcript length (codons or amino acids)	EX1	EX2	EX3	EX4	EX5	EX6
Homo sapiens	CD28	A	220	1–17	(18)–136	(137)–178	179–220		
		B	136	1–17	(18)–39 and (124)—136 (Y)	(137)–178	179–220		
		C	123	1–17	(18)–39	(137)–178 (W)	179–220		
		D	101	1–17	No EX2	(137)–178	179–220		
		E	80	1–17	(18)–39 and (124)—136 (Y)	137–151	208–220		
		F	55	1–17	(18)–39 and (124)–136 (Y)	No EX3	179–181		
Homo sapiens	ICOS	A	199	1–19	20–131	132–167	168–195	196–199	
		B	168	1–19	20–131	132–167	No EX4	196 (M)	
Homo sapiens	BTLA	A	289	1–29	30–134	135–182	183–198	199–289	
		B	241	1–29	30–134	No EX3	183–198	199–289	
Mus musculus	BTLA	A	306	1–36	37–143	144–155	156–208	209–224	225–306
		B	305	1–36	37–143	144–155	157–208	209–224	225–306

Description of the affected exons in alternative spliced transcripts. The position of codons present in the transcripts are indicated. Positions of the new codon resulting from the splicing and encoded amino acid are indicated between parentheses. *Homo sapiens* CD28 transcript B (136 codons) lacks codons 40–123 replaced with a tyrosine (Y) codon, transcript C (123 codons) lacks codons 40–136 replaced with a tryptophan (W) codon. Transcript D (101 codons) has no EX2, transcript E (80 codons) lacks codons 40–123 replaced with a (Y) codon and lacks codons 152–207, transcript F (55 codons) lacks codons 40–123 replaced with a (Y) codon and lacks 137–178. Transcript F has no EX3 and, owing to the alternative splicing, the EX4 reading frame is shifted which leads to a premature stop codon. *Homo sapiens* ICOS transcript B lacks codons 168–195 replaced with a methionine (M) codon. As a result from the alternative splicing, the EX5 reading frame is shifted which leads to a premature stop codon. *Homo sapiens* BTLA transcript B has no EX3 and lacks codon 135–182. *Mus musculus* BTLA transcript B, lacks the first codon of EX4 while preserving the reading frame 1. Blank cells indicate no codons.

activated Th cells is enhanced by the cytokines IL12 and IL23 [18,65]. The ICOS protein has a high degree of functional similarity in human and mouse. ICOS has a ^{16}YMFM^{19} motif in the cytoplasmic region which binds PI3K but not GRB2 whereas the ^{13}YMNM^{16} motif of CD28 binds PI3K and GRB2. It has been hypothesized that the reason that CD28 signalling, but not ICOS signalling, induces IL2 production is its ability to bind GRB2. An ICOS mutant, containing the CD28 YMNM motif showed that the difference of a single aa N > F defines the functional difference between ICOS and CD28 [66].

4.2. ICOS gene exon–intron organization

The human ICOS gene is localized on chromosome 2 at 2q33.3 and separated from the CTLA4 gene by 130 kb. The human ICOS gene has five exons (EX1–EX5) that encode 199 aa and spans 22,758 bp from the EX1 initiation codon (ATG) to EX5 termination codon (stop codon) [19] (Fig. 3). EX1 (58 bp) encode the L-REGION (18 aa) and a short HI (1 aa), EX2 (336 bp) encodes the V-LIKE-DOMAIN (112 aa), EX3 (107 bp) encodes the CO (9 aa) and the TM (24 aa) and a part of the CY (3 aa), EX4 (85 bp) and EX5 (11 bp) encode most of the CY (32 aa). The ICOS protein shares 24% and 17% identity, with the CD28 and CTLA4 proteins, respectively. An alternative splicing product with three exons (EX1–EX3) that potentially encodes 168 aa has been described (Table 4, Fig. 5).

The mouse ICOS gene is localized on chromosome 1 at band C (C1.3, 32.0 cM) (Table 2) and is

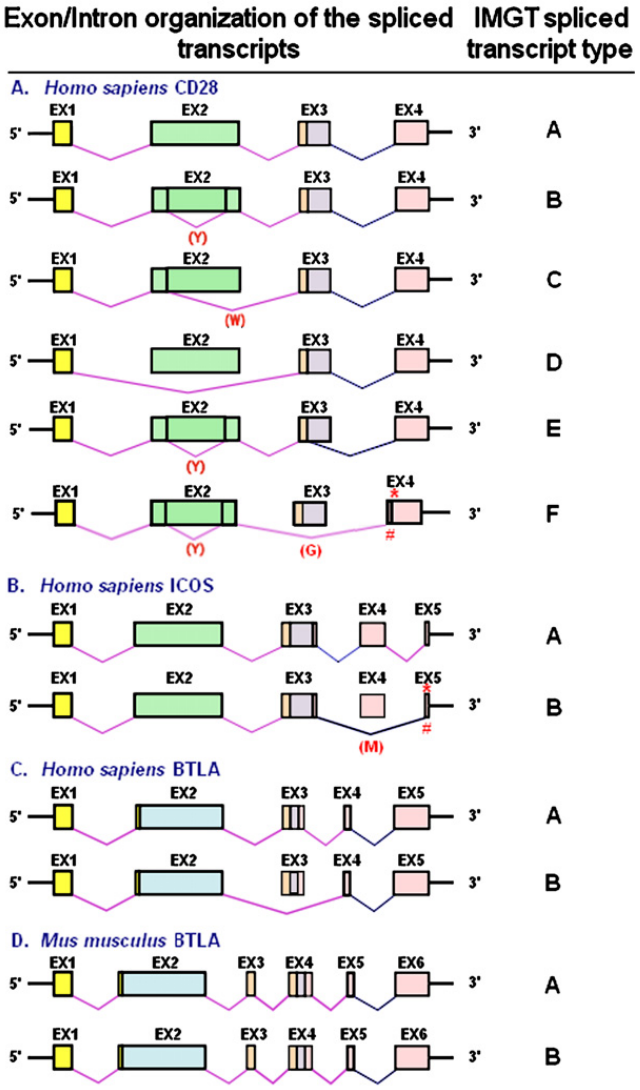

Fig. 5. IMGT spliced transcript types for *Homo sapiens* CD28, ICOS and BTLA and *Mus musculus* BTLA. IMGT spliced transcript A is the complete transcript as deduced from genomic DNA, the other IMGT spliced transcript types (B to F) result from alternative splicing that affects EX2 and/or EX3 and/or EX4. The resulting transcripts lack part or the totality of the extracellular V-LIKE-DOMAIN [D] and/or part of the totality of the TRANSMEMBRANE-REGION. Amino acids encoded by the new codon created by the alternative splicing are shown in red and between parentheses. A hash (#) indicates a frameshift. An asterisk (*) indicates a premature stop codon. The lines connecting the exons are colored according to IMGT splicing frame type. Purple: splicing frame 1, Blue: splicing frame 0. (IMGT/Aide-mémoire, http://imgt.cines.fr).

encoded by five exons (EX1–EX5) [21]. The mouse ICOS gene shares 78% nucleotide identity and 69% aa identity with the human gene. Two alleles have been identified in mouse ICOS gene (Table 7). The allele ICOS*02 has a single nucleotide transition leading to an aa change in EX1 (g20>a, R7>H).

4.3. ICOS V-LIKE-DOMAIN IMGT Collier de Perles

Lengths of the strands and loops of the ICOS V-LIKE-DOMAIN are indicated in Table 5 and the IMGT Collier de Perles is shown in Fig. 8. There are three N-linked glycosylation sites, two of them are at conserved positions in human and mouse N4 (A-STRAND) and N84 (D-STRAND). The third one is at N105 (CDR3-IMGT) in human and at N118 (G-STRAND) in mouse. The ligand interacting conserved motif ^{109}MYPPPY^{114} seen in the CD28 and CTLA4 is not present in ICOS and is replaced by ^{109}F.DPPPF^{115} (Fig. 2) which indicates that the ligand for ICOS is different from those of CD28 and CTLA4 [18]. Moreover, the FG-LOOP (CDR3-IMGT) of ICOS is shorter of one (for mouse) or two (for human) aa than those of CD28 and CTLA4.

4.4. ICOS polymorphism and diseases

Autosomal recessive ICOS deficiency, a form of common variable immunodeficiency identified in some patients corresponds to an homozygous deletion of EX2 and EX3 in the ICOS gene [67]. ICOS is a regulator of Th2 development and effector function and therefore represents an attractive candidate for Th2-mediated diseases like asthma and allergy. It has been shown that the ICOS gene is polymorphic with the presence of single nucleotide polymorphisms (SNPs) in the first intron and in the 3′ untranslated region, but no variation in the coding sequence was detected [68]. Different SNPs were also observed in the 5′ untranslated region and in the introns 2, 3 and 4. Variants in the potential promoter region (−1413 g/a) are significantly associated with allergic sensitization and serum IgE levels [69].

5. PDCD1

5.1. PDCD1 inhibitory receptor

The PDCD1 is functionally similar to CTLA4 and exerts an inhibitory signal on T cell activation [22,70–72]. The PDCD1 protein is expressed as a monomer by activated T cells, B cells and myloid cells, in contrast to the restricted expression of CD28 and CTLA4 on T cells. PDCD1 lacks the ^{109}MYPPPY^{114} motif seen in CD28 and CTLA4 and the cysteine in the connecting region (CO).

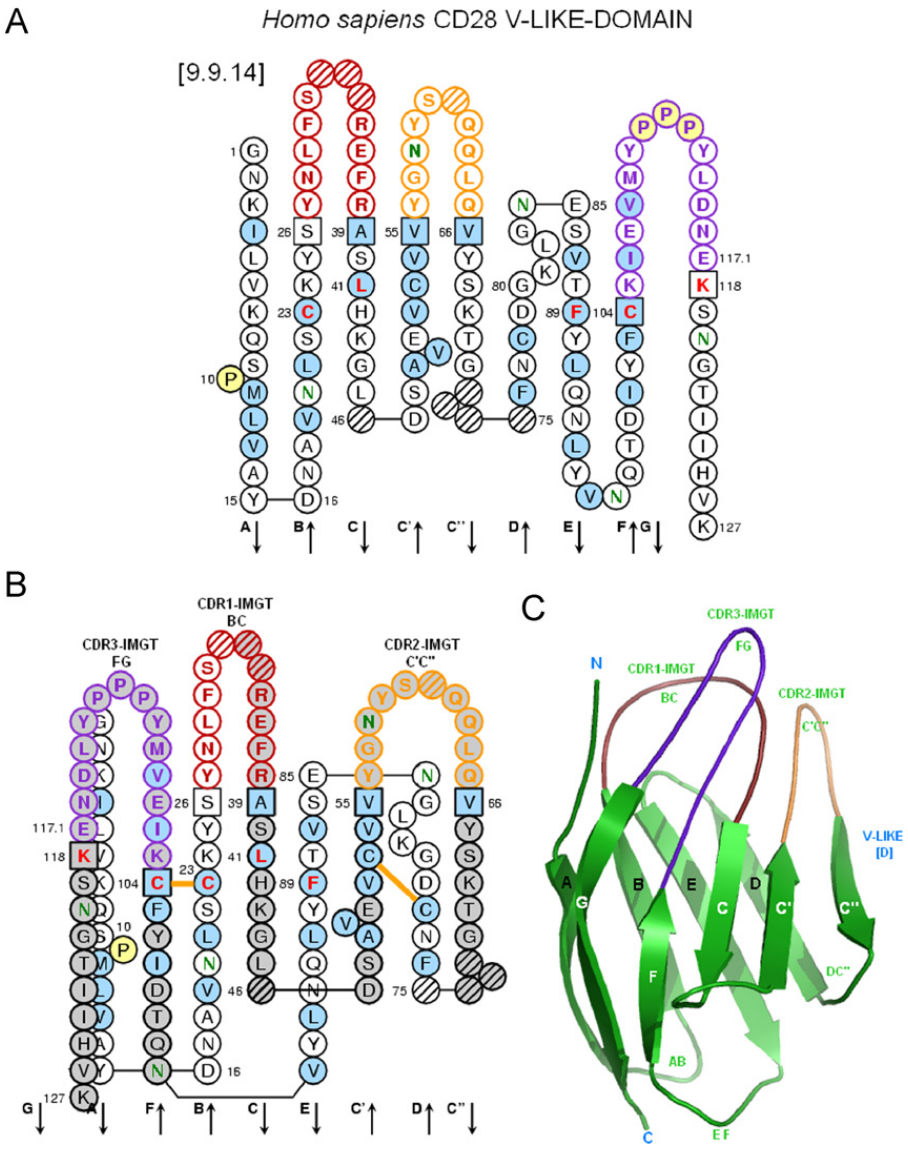

Fig. 6. IMGT Colliers de Perles and three-dimensional (3D) structure of the *Homo sapiens* CD28 V-LIKE-DOMAIN. (A) IMGT Collier de Perles on one layer. (B) IMGT Collier de Perles on two layers (C) 3D structure. (A) and (B) The CD28 V-LIKE-DOMAIN (119 aa) comprises nine antiparallel beta sheets [6]. Amino acid numeration is according to IMGT unique numbering for V-DOMAIN and V-LIKE-DOMAIN [6]. Hatched circles correspond to missing positions according to that numbering. Asparagines (N) that belong to potential N-glycosylation sites are in green (N20, N58, N84, N97 and N120). BC loop (CDR1-IMGT) is in red, C′C″ loop (CDR2-IMGT) in orange and FG loop (CDR3-IMGT) in purple. IMGT Collier de Perles was obtained from IMGT/3Dstructure-DB [38] (http://imgt.cines.fr). (C) Ribbon representation was obtained with PyMOL (http://pymol.sourceforge.net/). PDB code and IMGT/3Dstructure-DB entry ID: 1yjd.

PDCD1 binds the ligands B7H1 and B7DC with different affinity [23,70]. The interaction of PDCD1/B7DC exhibited a 2–6-fold higher affinity and had different association/dissociation kinetics compared with the interaction of PDCD1/B7H1 [23]. The cytoplasmic region of PDCD1 has two immunoreceptor tyrosine-based inhibitory motifs (ITIM) ^{31}YxxL^{34} and ^{56}YxxI^{59} separated by 21 bp (in human) or 19 bp (in mouse). The interaction of PDCD1 with its ligands leads to the phosphorylation of the tyrosines in the ITIM and to the rapid recruitment and activation of SHP2 and therefore to the suppression of the PI3K/AKT activation [71]. This results in the dephosphorylation of downstream proteins and to the G0/G1 cell cycle arrest. The C-terminal ITIM is part of another regulatory motif what has been described as immunoreceptor tyrosine-based switch motif (ITSM) with the consensus sequence TxYxx(V/I) [9,73]. The ITSM of PDCD1 was essential to block B cell receptor

Table 5
Lengths of the strands and loops for the V-LIKE-DOMAIN of the CD28 family member (CD28, CTLA4, ICOS) and PDCD1 proteins

IMGT labels		V-LIKE-DOMAIN strands and loops		CD28	CTLA4	ICOS	PDCD1
		Numbering	Length	[D] [9.9.14]	[D] [9.8.13]	[D] [5.9.12]	[D] [7.6.12]
A-STRAND	FR1-IMGT	1–15	15	15	14 (−10)	15	15
B-STRAND		16–26	11	11	11	11	11
BC-LOOP	CDR1-IMGT	27–38	12	**9**	**9**	**5**	**7**
C-STRAND	FR2-IMGT	39–46	8	7 (−46)	8	7 (−46)	8
C′-STRAND		47–55	9	9	9	8 (−47)	9
C′C″-LOOP	CDR2- IMGT	56–65	10	**9**	**8**	**9**	**6**
C″-STRAND	FR3- IMGT	66–74	9	6 (−72, 73, 74)	6 (−72, 73, 74)	6 (−72, 73, 74)	6 (−72, 73, 74)
D-STRAND		75–84	10	9 (−75)	8 (75, 76)	9 (−75)	8 (−75, 76)
DE-TURN		84A,84B					+2
E-STRAND		85–96	12	12	12	12	12
F-STRAND		97–104	8	8	8	8	8
FG-LOOP	CDR3- IMGT	105–117		**14** (+117.1)[a]	**13**	**12** (−111)	**12** (−111)
G-STRAND	FR4- IMGT	118–128	11	10	10	10	10
		Total length	128	119	116	112	114

Numbering is according to the IMGT unique numbering for V-DOMAIN and V-LIKE-DOMAIN [6]. The delimitations of the strands and loops for the V-LIKE-DOMAIN are identical to those of the IG and TR V-DOMAINs [8]. Lengths of the BC (CDR1-IMGT), C′C″ (CDR2-IMGT) and FG (CDR3-IMGT) loops for each V-LIKE-DOMAIN [D] are shown between brackets (and in bold in the coloumn). Blank cells indicate no amino acids. A plus (+) sign indicates additional positions. A minus (-) sign indicates the absence of amino acids. IMGT Protein display (amino acid sequences with IMGT numbering) is shown in Fig. 2 and IMGT Colliers de Perles are shown in Figs. 6–9.

[a]Additional aa in CDR3-IMGT of CD28 has been assigned to position 117.1 (instead of 112.1) to maintain an identical numbering for the motif at the tip of the loop.

mediated Ca2+ flux and tyrosine phosphorylation of downstream effector molecules. A point mutation of the ITIM had little effect on PDCD1 signalling or functional activity. In contrast, mutation of the ITSM abrogated the ability of PDCD1 to block cytokine synthesis and to limit T cell expansion [73].

5.2. PDCD1 gene exon–intron organization

The PDCD1 gene is localized on chromosome 2 at 2q37.3 in REV orientation and spans 9.8 kb (9821 bp) [32]. The coding region comprises 864 nucleotides that encode 288 aa. The PDCD1 protein comprises one extracellular V-LIKE-DOMAIN [D]. The PDCD1 gene is encoded by five exons (EX1–EX5). The EX1 (76 bp) encodes the L-REGION (25 aa) followed by an intron of 5,781 bp. EX2 (360 bp) encodes a short HI (6 aa) and the extracellular V-LIKE-DOMAIN (120 aa) and is separated by an intron of 267 bp of EX3 (156 bp) that encodes the CO (23 aa), the TM (24 aa) and part of the CY (5 aa). The EX4 (35 bp) and EX5 (237 bp) separated by an intron of 651 bp encode most of the CY (12 aa and 79 aa, respectively). Five human PDCD1 alleles have been identified. The details of the polymorphisms are listed in Table 6.

The mouse PDCD1 gene, localized on chromosome 1 (1D) in REV orientation, has also five exons EX1–EX5 [22]. No alleles have been described so far in mouse. The human and mouse PDCD1 genes share 70% sequence similarity at the nucleotide level and 59% identity at the aa level.

5.3. PDCD1 V-LIKE-DOMAIN IMGT Collier de Perles

The lengths of the strands and loops of the V-LIKE-DOMAIN are reported in Table 5 and the IMGT Collier de Perles is shown in Fig. 9. In contrast to the members of the CD28 family, PDCD1 only possesses the classical intrachain disulfide bond [72]. In human and mouse PDCD1, four N-glycosylation sites are conserved at positions N18 (B-STRAND), N27 (BC-LOOP), N48 (C′-STRAND) and N97 (F-STRAND) in the V-LIKE-DOMAIN.

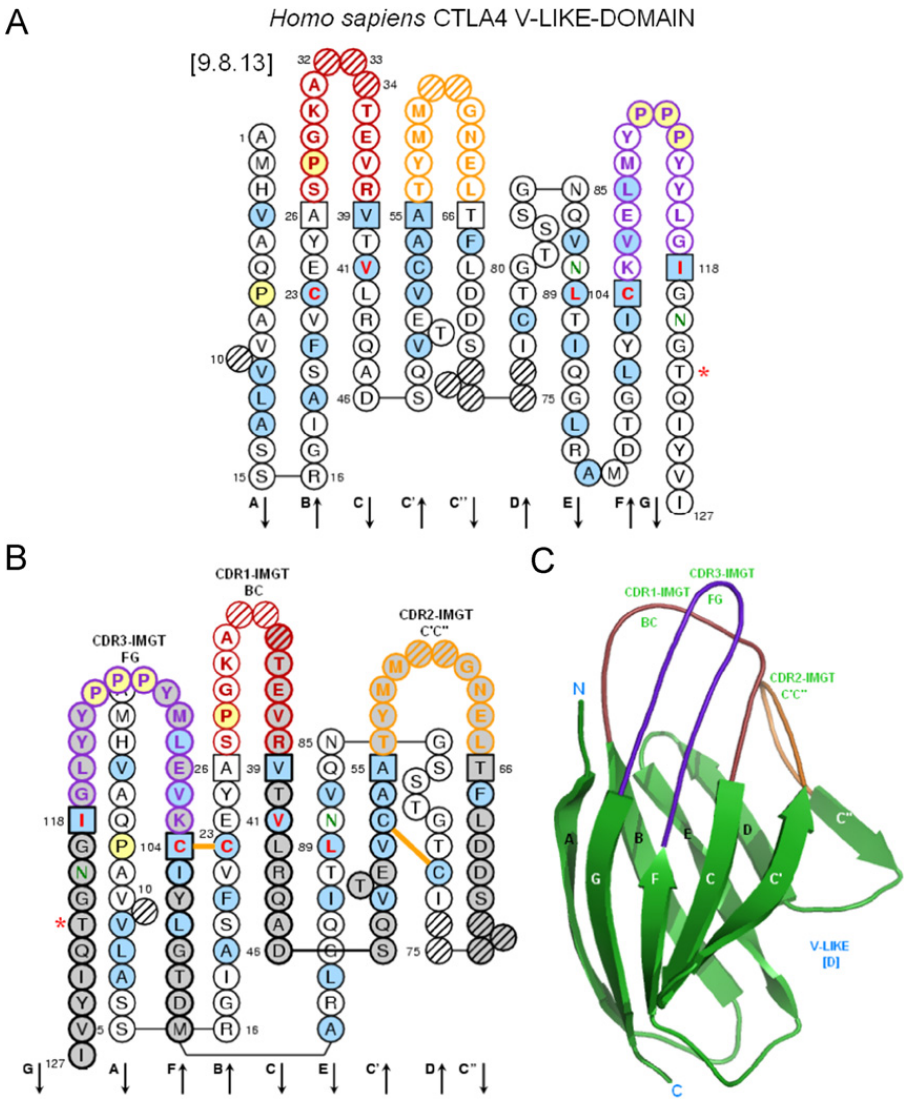

Fig. 7. IMGT Colliers de Perles and three-dimensional (3D) structure of the *Homo sapiens* CTLA4 V-LIKE-DOMAIN. (A) IMGT Collier de Perles on one layer. (B) IMGT Collier de Perles on two layers. (C) 3D structure. (A) and (B) The CTLA4 V-LIKE-DOMAIN (116 aa) comprises nine antiparallel beta sheets [6]. Amino acid numeration is according to IMGT unique numbering for V-DOMAIN and V-LIKE-DOMAIN [6]. Hatched circles correspond to missing positions according to that numbering. Asparagines (N) that belong to potential N-glycosylation sites are in green (N88 and N120). An asterisk indicates a polymorphic amino acid (A122>T in CTLA4*02). BC loop (CDR1-IMGT) is in red, C′C″ loop (CDR2-IMGT) in orange and FG loop (CDR3-IMGT) in purple. IMGT Collier de Perles was obtained from IMGT/3Dstructure-DB [38] (http://imgt.cines.fr). (C) Ribbon representation was obtained with PyMOL (http://pymol.sourceforge.net/). PDB code and IMGT/3Dstructure-DB entry ID: 1i85.

5.4. *PDCD1 polymorphism and diseases*

A number of susceptibility loci for systemic lupus erythromatus (SLE) has been suggested in different populations. Systemic lupus erythematosus susceptibility 2 (SLEB2), one of the susceptibility locus for Nordic multicase families, and the PDCD1 gene within this locus, has been considered the strongest candidate for the disease [74]. Seven SNPs were identified in the PDCD1 gene within the SLEB2 locus. On the two SNPs which are localized in the coding region (in EX5), one leads to an aa change (c17>t, A6>V) whereas the second one is silent (c177>t, A60). The other SNPs are in the noncoding region, one in the promoter (-531 bp upstream from the translation ATG start site), one in intron 2 (position 6438), one in intron 4 (position 7146) and another one in the 3′ UTR (position 8738) [74,75]. The SNPs in the noncoding regions may disrupt the binding of transcription factors and lead to the aberrant regulation of PDCD1, contributing to the disregulated self-tolerance and to the chronic

Table 6
Homo sapiens CD28 family member (CD28, CTLA4, ICOS), PDCD1 and BTLA genes, alleles and IMGT spliced transcript types

IMGT gene names	IMGT allele names[a]	Fct	EX	IMGT reference sequences			Allelic differences				
				Accession numbers[c]	Molecule type	IMGT spliced transcript type[b]	Exons	Nucleotide positions in		Amino acid and codon positions in	
								Exons	cDNA	Exons	cDNA
CD28	CD28*01	F	EX1–4	M37812–M37815	gDNA	A					
				J02988	cDNA	A					
				(1)		B					
				AF222341	cDNA	C					
				AF222342	cDNA	D					
				AF222343	cDNA	E					
				AJ295273	cDNA	F					
CTLA4	CTLA4*01	F	EX1–4	M74363 M37243–M37245	gDNA						
	CTLA4*02			AC010138	cDNA	A	EX2	g364>a	439	A122>T	147
	CTLA4*03			AF411058	cDNA	A	EX1	a49>g	49	T17>A	17
							EX2	g364>a	439	A122>T	147
ICOS	ICOS*01	F	EX1–5	AJ535718	gDNA	A					
				AJ277832	cDNA	A					
				BC028006	cDNA	B					
PDCD1	PDCD1*01	F	EX1–5	AF363458	gDNA	A					
	PDCD1*02			AY238517	cDNA	A	EX5	c177>t	804	A59	268
	PDCD1*03			BC074740	cDNA	A	EX2	c165>t	243	A55	81
	PDCD1*04			L27440	cDNA	A	EX3	c49>t	484	P17>S	162
							EX5	c177>t	804	A59	268
	PDCD1*05			U64863	cDNA	A	EX2	c20>t	113	S7>F	38

Table 6 (*continued*)

IMGT gene names	IMGT allele names[a]	Fct	EX	IMGT reference sequences			Allelic differences				
				Accession numbers[c]	Molecule type	IMGT spliced transcript type[b]	Exons	Nucleotide positions in		Amino acid and codon positions in	
								Exons	cDNA	Exons	cDNA
BTLA	BTLA*01	F		AJ717664	cDNA	A					
	BTLA*01			DQ198368	cDNA	B					
	BTLA*02			NM_181780	cDNA	A	EX2	t273>c	306	F91	102
	BTLA*03			AK131204 [d]	cDNA	A	EX2	t273>c	288	F91	96
							EX5	t62>c	638	L21>P	213
	BTLA*04			AY293286	cDNA	A	EX2	g280>a	313	V94>M	105
							EX3	a10>g	412	S4>G	138
								a40>g	442	M14>V	148
								t111>g	535	C37>W	179
							EX4	c45>t	591	N15	197
							EX5	t21>c	615	S7	205
								a73>g	667	T25>A	223
								a134>g	728	Y45>C	243

gDNA: genomic DNA; cDNA: complementary DNA; Fct : Functionality ; EX : exons.

[a]Alleles are numbered starting from allele *01 that corresponds to the IMGT reference sequence.

[b]IMGT spliced transcript types for *Homo sapiens* CD28, ICOS and BTLA are described in Table 4 and displayed in Fig. 5. (1) IMGT spliced transcripts A and B described in PMID: 2162892 and mentioned in M37812-M37815 [48].

[c]EMBL/GenBank/DDBJ nucleotide accession numbers, except for NM_181780 (Entrez gene, NCBI).

[d]There are two potential ATG codons separated by 18 nt (6 aa) in EX1. The mutations are described with the numbering of the largest transcript. Mutations are described according to the IMGT Scientific chart rules (http://imgt.cines.fr/textes/IMGTScientificChart/). Nucleotide positions, amino acid and codon positions are given in the exons and in cDNA. For EX2, positions are according to the IMGT unique numbering for V-LIKE-DOMAIN [6].

Table 7
Mus musculus CD28 family member (CD28, CTLA4, ICOS), PDCD1 and BTLA genes alleles and IMGT spliced transcript types

IMGT gene names	IMGT allele names[a]	Strain	Fct	IMGT reference sequences			Allelic differences				
				EX	Accession numbers[b]	Molecule type	IMGT spliced transcript type[c]	Exons	Nucleotide positions in		Amino acid and codon positions in	
									Exons	cDNA	Exons	cDNA
CD28	CD28*01		F	EX1–4	AL672024	gDNA	A					
	CD28*01		F		M34563 [d]	cDNA	A					
CTLA4	CTLA4*01	129/SvJ	F	EX1–4	AF142145	gDNA	A					
	CTLA4*02		F		X05719	cDNA	A	EX3	a88>t	544	T30>S	182
	CTLA4*03	C57BL/6NCr	F		BC052683	cDNA	A	EX2	a368>g	443	Q123>R	148
ICOS	ICOS*01	129/Ola	F	EX1 EX2–5	AF327184 AF327185	gDNA	A					
	ICOS*02	BALB/c	F		AF257230	cDNA	A	EX1	g20>a	20	R7>H	7
PDCD1	PDCD1*01		F	EX1–5	X67914	cDNA	A					
BTLA [78]	BTLA*01	129/SvEv	F		AY293285	cDNA	A					
	BTLA*02		F		BC108964	cDNA	A	EX2	a117>c	220	N40>H	74
	BTLA*03		F		BC108963	cDNA	B	EX4	g55>a52	517	E19>18K	173
	BTLA*04	C57BL/6	F		AK041334	cDNA	B	EX2	g1>c	121	E1>P	41
									a2>c	122		
									a14>c	134	N5>T	45
									a20>c	140	K7>T	47
									c36>g	156	H12>Q	52
									t43>a	163	W15>R	55
									g64>c	187	E22>Q	63
									g135F>t	255	W45F>C	85
									a135.4>g	267	E45.4	89
									g133.6>a	271	G45.6>S	91
									t135.6>c	273		
									g251.2>a	305	R84.2>Q	102
									t270>c	330	H90	110
									c297>t	357	N99	119
									g380>c	428	R127>T	143

Fct: Functionality ; gDNA: genomic DNA; cDNA: complementary DNA.
[a]Alleles are numbered starting from allele *01 that corresponds to the IMGT reference sequence.
[b]EMBL/GenBank/DDBJ nucleotide accession numbers.
[c]IMGT spliced transcript types for *Mus musculus* BTLA and their characteristics are described in Table 4 and displayed in Fig. 5.
[d]Nucleotide sequence M34563 has a deletion (a557>del#) and an insertion (566^567>ins^c) ($SDY^{187-189}>VTT^{187-189}$) which most probably result from sequencing or typing errors. So this sequence is not considered as a new allele. Mutations are described according to the IMGT Scientific chart rules (http://imgt.cines.fr/textes/IMGTScientificChart/). Nucleotide positions, amino acid and codon positions are given in exons and in cDNA. For EX2, positions are according to the IMGT unique numbering for V-LIKE-DOMAIN [6].

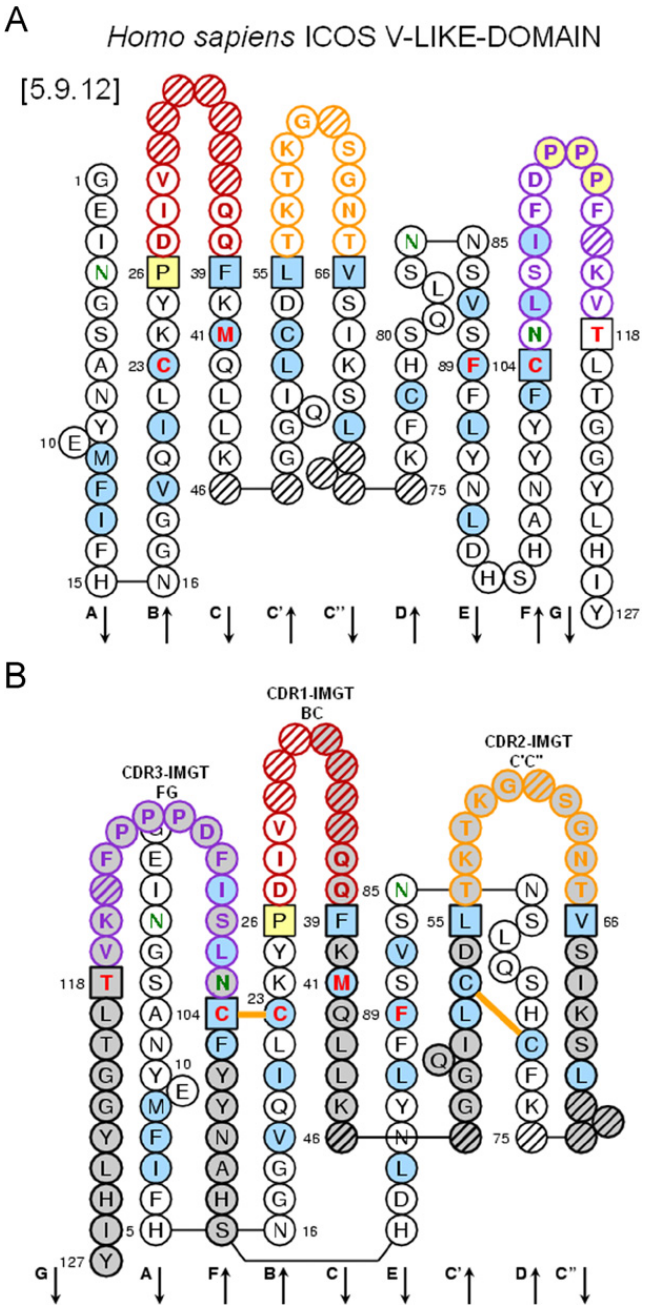

Fig. 8. IMGT Colliers de Perles of the *Homo sapiens* ICOS V-LIKE-DOMAIN. (A) IMGT Collier de Perles on one layer. (B) IMGT Collier de Perles on two layers. The ICOS V-LIKE-DOMAIN (112 aa) comprises nine antiparallel beta sheets [6]. Amino acid numeration is according to IMGT unique numbering for V-DOMAIN and V-LIKE-DOMAIN [6]. Hatched circles correspond to missing positions according to that numbering. Asparagines (N) that belong to potential N-glycosylation sites are in green (N4, N84 and N105). IMGT Collier de Perles was obtained from IMGT/3Dstructure-DB [38] (http://imgt.cines.fr).

lymphocyte hyperactivity characteristic of SLE. It has also been suggested that the PDCD1 polymorphism is a genetic modifier of the progression of multiple sclerosis (MP), possibly due to defect in PDCD1 mediated inhibition of T cell activation [75].

6. BTLA

6.1. BTLA inhibitory receptor

BTLA is a third inhibitory receptor expressed by T lymphocytes. BTLA is a 32 kDa monomer protein. BTLA is not expressed by naive T cells but is induced during activation and remains expressed on T helper type 1 (Th1) and resting B cells [24]. BTLA binds HVEM, a tumor necrosis factor receptor family member and this interaction exerts an inhibitory influence on the T cells [25]. BTLA differs from the CD28 family members (CD28, CTLA4, ICOS) and from PDCD1 by having an extracellular C-LIKE-DOMAIN, instead of a V-LIKE-DOMAIN [9]. As observed in PDCD1, the BTLA cytoplasmic region contains two ITIM motifs separated by 21 aa (in both human and mouse), the most distal one being part of an ITSM motif [76]. Upon phosphorylation of the ITIM and ITSM motifs which follows TR cross-linking on T cells, BTLA associates with the SHP1 and SHP2 phosphatases and attenuates the production of IL2 [76,77]. The pattern and position of the ITIM and ITSM are conserved in mammals and these motifs are also present in teleosts [9]. In addition to the ITIM (^{59}YASL^{62}) and ITSM (^{84}YASI^{87}) motifs, the human BTLA cytoplasmic region also contains two GRB2 binding sites ^{28}YDN^{30} and ^{45}YSN^{47} [76]. Only the proximal GRB2 motif is present in mouse. The mutational analysis of the tyrosines of either ITIM (Y59) or ITSM (Y84) disrupted or reduced BTLA ability to recruit SHP1 and SHP2 but the mutation of the Y28 of GRB2 motif had no effect [76,77].

6.2. BTLA gene exon–intron organization

The human BTLA gene is localized on chromosome 3 at 3q13.2 in REV orientation. The BTLA gene spans 33.1 kb and comprises five exons (EX1–EX5) which encodes 289 aa [24] (Table 3). EX1 (88 bp) encodes the L-REGION (23 aa) and 6 aa of the HI and is followed by a very long intron of 19,501 bp, EX2 (315 bp) encodes 4 aa of the HI and the V-LIKE-DOMAIN [D] (101 aa) and is followed by an intron of 8,099 bp. EX3 (144 bp) encodes the CO (18 aa), TM (26 aa) and 4 aa of the CY and is followed by an intron 1,407 bp. EX4 (47 bp) encodes

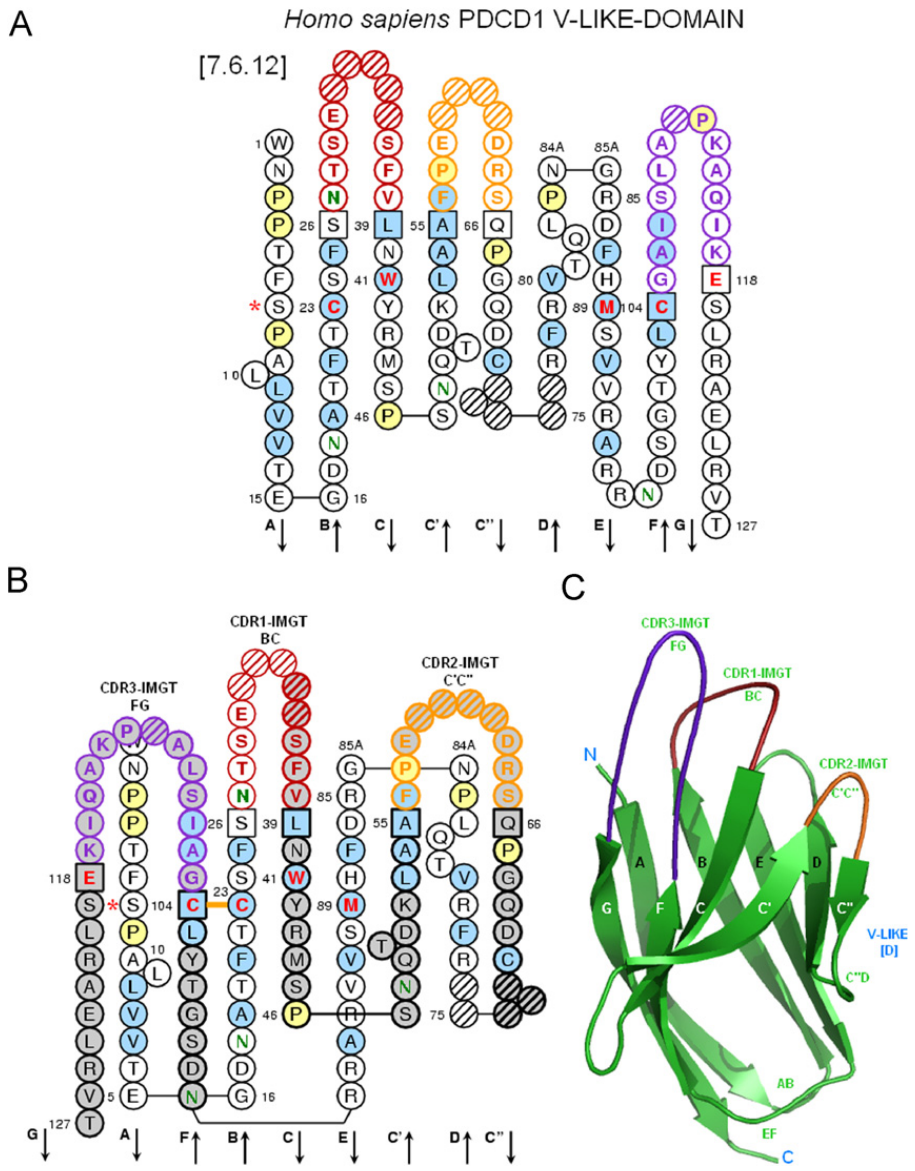

Fig. 9. IMGT Colliers de Perles of the *Homo sapiens* PDCD1 V-LIKE-DOMAIN and three-dimensional (3D) structure of the *Mus musculus* PDCD1 V-LIKE-DOMAIN. (A) IMGT Collier de Perles of the *Homo sapiens* PDCD1 V-LIKE-DOMAIN on one layer. (B) IMGT Collier de Perles of the *Homo sapiens* PDCD1 V-LIKE-DOMAIN on two layers (C) 3D structure of the *Mus musculus*. (A) ans (B) The *Homo sapiens* PDCD1 V-LIKE-DOMAIN (114 aa) comprises nine antiparallel beta sheets [6]. Amino acid numeration is according to IMGT unique numbering for V-DOMAIN and V-LIKE-DOMAIN [6]. Hatched circles correspond to missing positions according to that numbering. Asparagines (N) that belong to potential N-glycosylation sites are in green (N18, N27, N48 and N97). BC loop (CDR1-IMGT) is in red, C′C″ loop (CDR2-IMGT) in orange and FG loop (CDR3-IMGT) in purple. IMGT Collier de Perles was obtained from IMGT/3Dstructure-DB [38] (http://imgt.cines.fr). For (C) Ribbon representation was obtained with PyMOL (http://pymol.sourceforge.net/). PDB code and IMGT/3Dstructure-DB entry ID: 1npu.

16 aa of the CY and is followed by an intron of 3374 bp and EX5 (273 bp) encodes the remaining CY (91 aa).

The mouse BTLA gene is localized on chromosome 16 (B5, 29cM) and oriented in FWD orientation. It comprises six exons (EX1–EX6) and spans 28 kb. The mouse BTLA protein shares 49% aa identity with the human BTLA protein.

6.3. BTLA C-LIKE-DOMAIN IMGT Collier de Perles

The BTLA IMGT Collier de Perles shows the characteristics of a C-LIKE-DOMAIN (Table 8 and Fig. 10). There is an unusual extension of the C strand that forms a hairpin loop of 6 aa (positions 45A-45F) followed by 4 aa that forms a classical transversal CD

Table 8
Lengths of the strands and loops for the C-LIKE-DOMAIN of the BTLA protein

IMGT labels	C-LIKE-DOMAIN strands and loops		BTLA
	Numbering	Length	[D] [5.10.10]
A-STRAND	1–15	15	16 (+1.1)
AB-TURN	*15.1–15.3*		*+1*
B-STRAND	16–26	11	11
BC-LOOP	27–36	10	**5**
C-STRAND	39–45	7	7
CD-STRAND	*45A-45F*		***+10***
	45.1–45.4		
D-STRAND	77–84	8	8
DE-TURN	*84.1–84.2*		*+4*
	85.1–85.2		
E-STRAND	85–96	12	12
F-STRAND	97–104	8	8
FG-LOOP	105–117	13	**10**
G-STRAND	118–128	11	9
	Total length	95	101

Numbering is according to the IMGT unique numbering for C-DOMAIN and C-LIKE-DOMAIN [7]. Positions in italics correspond to the numbering of the additional positions in BTLA [D]. A complete numbering of the additional positions in C-LIKE-DOMAIN is described in [7]. BTLA has additional positions 45A-45F at the C-terminal end of the C strand that form a CD beta turn, followed by positions 45.1–45.4 that form classical transversal strand. Lengths of the BC-LOOP, CD-STRAND and FG-LOOP of the BTLA C-LIKE-DOMAIN [D] are shown between brackets (and in bold in the column). The BC-LOOP and FG-LOOP corresponds to the CDR1-IMGT and CDR3-IMGT of a V-LIKE-DOMAIN, whereas the CD-STRAND replaces the C′-STRAND, C′C″-LOOP (CDR2-IMGT) and C″-STRAND. Blank cells indicate no amino acids. A plus (+) sign indicates additional positions. The minus (-) sign indicates the absence of amino acids. IMGT Protein display amino acid sequence with IMGT numbering is shown in Fig. 2 and IMGT Colliers de Perles are shown in Fig. 10.

strand (positions 45.1–45.4). In addition to the classical intradomain disulfide bridge between 1st-CYS (position 23) and 2nd-CYS (position 104), there are two additional intradomain disulfide bonds between cysteine 1.1 and cysteine 28, and cysteine 44 and cysteine 45F, these last ones maintaining the structure of the hairpin between the C and CD strands. The number of disulfide bridges in the mouse BTLA protein is not constant between alleles. Indeed the BTLA*04 allele (GenBank/EMBL/DDBJ: AK041334) has the 44–45F disulfide bridge as in the human BTLA protein whereas the three other alleles BTLA*01 to BTLA*03 only have one disulfide bridge as there is a tryptophan instead of a cysteine at position 45F. The BTLA C-LIKE-DOMAIN has three N-glycosylation sites in human (N45B, N85.2, N99) and four N-glycosylation sites in mouse (N40, N45B, N84.1 and N99), with only two being at the same positions (N45B in the CD hairpin loop and N99 in the F-STRAND).

6.4. BTLA polymorphism

Four BTLA alleles have been identified in humans (Table 6). One alternative spliced transcript B lacks the TM and encodes a potentially soluble protein (Table 4, Fig. 5). Transcripts may be shorter of 6 codons in 5′ by the use of an ATG codon (methionine) located eighteen nucleotides downstream of the first ATG in EX1. Mouse BTLA seems to exhibit both structural and expression polymorphisms in the different strains. Four alleles have been identified. The BTLA*01 allele (129/SvEv) has been found expressed by B cells, T cells and dendritic cells, but not NK or macrophages whereas BTLA*04 allele (C57BL/6) is expressed by macrophages and NK cells [78]. Alleles BTLA*03 and BTLA*04 have been found expressed as alternatively spliced transcripts coding 305 aa and generated using an internal in-frame splice site at the 3′ end of EX3 (Table 4, Fig. 5).

7. Conclusion

The CD28 family member (CD28, CTLA4, ICOS), PDCD1 and BTLA proteins provide key second signals that can regulate the activation, inhibition and/or fine tuning of T cell response. CD28 predominates in regulating the activation of

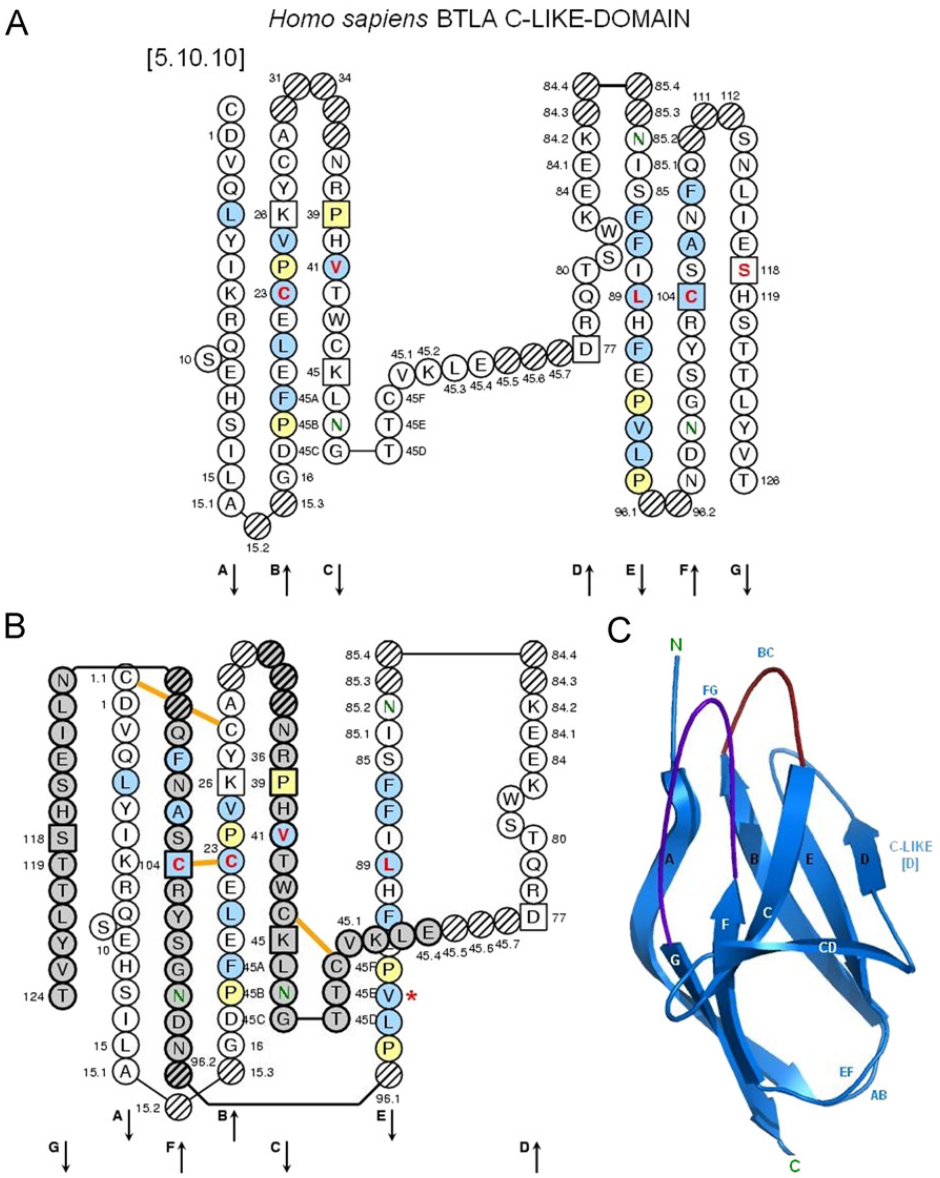

Fig. 10. IMGT Colliers de Perles and three-dimensional (3D) structure of the *Homo- sapiens* BTLA C-LIKE-DOMAIN. (A) IMGT Collier de Perles on one layer. (B) IMGT Collier de Perles on two layers. (C) 3D structure. (A) and (B) The BTLA C-LIKE-DOMAIN (101 aa) comprises seven antiparallel beta sheets [7]. Hatched circles correspond to missing positions according to that numbering. Asparagines (N) that belong to potential N-glycosylation sites are in green (N45B, N85.2 and N99). The C strand of the BTLA C-LIKE-DOMAIN has six additional amino acids (45A-45F) which extend into a hairpin loop like structure with a beta turn followed by a strand of 4 amino acids (45.1–45.4). [5.10.10] refer to the lengths of the BC loop (5 aa) , CD transversal strand (10 aa, 6 for the hairpin loop and 4 for the remaining CD) and FG loop (10 aa). In addition to the classical disulfide bridge 1st-CYS (23) and 2nd-CYS (104) there are two additional intradomain disulfide bonds between C1.1 and C28 and C44 and C45F. IMGT Collier de Perles from IMGT/3Dstructure-DB [38] (http://imgt.cines.fr). (C) Ribbon representation was obtained with PyMOL (http://pymol.sourceforge.net/). PDB code and IMGT/3Dstructure-DB entry ID: 2aw2.

naive T cells, whereas the other proteins seem to be particularly important in regulating previously primed T cells rather than antigen-inexperienced T cells. The aberrant regulation of CD28 family member, PDCD1 and BTLA proteins may lead to costimulatory disfunction and play a role in the initiation, progression and pathogenesis of autoimmune diseases. The understanding of these pathways in immune response will allow intervention in immune-mediated diseases through precise manipulation of these pathways. Homologs of these receptors are found in all vertebrates, including aves and fishes [9]. Phylogenetic and sequence analyses may provide insight into evolutionary relationship of the

CD28 family, PDCD1 and BTLA proteins. Precise IMGT definitions of the sequence alleles and of the alternative spliced transcripts are highly valuable to carry such analysis. The IMGT unique numbering for V-LIKE-DOMAIN and C-LIKE-DOMAIN provides a meaningful and easy comparison between domains that belong to different proteins (CD28, CTLA4, ICOS, PDCD1 and BTLA), and whatever the species. The IMGT Collier de Perles allow to bridge the gap between sequences and 3D structures. Thus rules based on IMGT-ONTOLOGY have proven to be crucial for the standardized description and analysis of the domains of the IgSF proteins.

Acknowledgments

We are grateful to Pierre Boudinot for discussing teleost data prior to publication. We thank Gérard Lefranc for fruitful discussion and Quentin Kaas for help in the IMGT Colliers de Perles. We are grateful to the IMGT® team for its constant motivation and expertize. IMGT® is a registered Centre National de la Recherche Scientifique (CNRS) mark. IMGT® is a National Bioinformatics RIO Platform since 2001 (CNRS, INSERM, CEA, INRA). IMGT® was funded in part by the BIOMED1 (BIOCT930038), Biotechnology BIOTECH2 (BIO4CT960037), 5th PCRDT Quality of Life and Management of Living Resources (QLG2-2000-01287) programmes of the European Union and received subventions from Association pour la Recherche sur le Cancer (ARC) and from the Réseau National des Génopoles (RNG), Génopole-Montpellier-Languedoc-Roussillon. IMGT® is currently supported by the CNRS, the Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche (MENESR) Université Montpellier II Plan Pluri-Formation, BIOSTIC-LR2004, ACI-IMPBIO IMP82-2004, GIS AGENAE, the Région Languedoc-Roussillon, Agence Nationale de la Recherche ANR (BIOSYS06_135457) and the ImmunoGrid project (IST-2004-028069) of the 6th framework programme of the European Union.

References

[1] Lefranc M-P, Lefranc G. The immunoglobulin Factsbook. London, UK: Academic Press; 2001 458pp.
[2] Lefranc M-P, Lefranc G. The T cell receptor FactsBook. London, UK: Academic Press; 2001 398pp.
[3] Frauwirth KA, Thompson CB. Activation and inhibition of lymphocytes by costimulation. J Clin Invest 2002;109:295–9.
[4] June CH, Ledbetter JA Linsley PS, Thompson CB. Role of the CD28 receptor in T-cell activation. Immunol Today 1990;11:211–6.
[5] Watts TH, DeBenedette MA. T cell co-stimulatory molecules other than CD28. Curr Opin Immunol 1999;11:286–93.
[6] Lefranc M-P, Pommié C, Ruiz M, Giudicelli V, Foulquier E, Truong L, et al. IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. Dev Comp Immunol 2003;27:55–77.
[7] Lefranc M-P, Pommié C, Kaas Q, Duprat E, Bosc N, Guiraudou D, et al. IMGT unique numbering for immunoglobulin and T cell receptor constant domains and Ig superfamily C-like domains. Dev Comp Immunol 2005;29:185–203.
[8] Duprat E, Kaas Q, Garelle V, Giudicelli V, Lefranc G, Lefranc M-P. IMGT standardization for alleles and mutations of the V-LIKE-DOMAINs and C-LIKE-DOMAINs of the immunoglobulin superfamily. Recent Res Devel Human Genet 2004;2:111–36.
[9] Bernard D, Hansen JD, Du Pasquier L, Lefranc M-P, Benmansour A, Boudinot P. Costimulatory receptors in jawed vertebrates: conserved CD28, odd CTLA4 and multiple BTLAs. Dev Comp Immunol 2007;31:255–71.
[10] Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nat Rev Immunol 2002;2:116–26.
[11] Collins M, Ling V, Carreno BM. The B7 family of immune-regulatory ligands. Genome Biol 2005;6:223.
[12] Aruffo A, Seed B. Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. Proc Natl Acad Sci USA 1987;84:8573–7.
[13] Dariavach P, Mattei MG, Golstein P, Lefranc M-P. Human Ig superfamily CTLA-4 gene:chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. Eur J Immunol 1988;18:1901–5.
[14] Harper K, Balzano C, Rouvier E, Mattei MG, Luciani MF, Golstein P. CTLA-4 and CD28 activated lymphocyte molecules are closely related in both mouse and human as to sequence, message expression, gene structure, and chromosomal location. J Immunol 1991;147:1037–44.
[15] Lee KM, Chuang E, Griffin M, Khattri R, Hong DK, Zhang W, et al. Molecular basis of T cell inactivation by CTLA-4. Science 1998;282:2263–6.
[16] Van der Merwe PA, Bodian DL, Daenke S, Linsley P, Davis SJ. CD80 (B7-1) binds both CD28 and CTLA-4 with a low affinity and very fast kinetics. J Exp Med 1997;185:393–403.
[17] Slavik JM, Hutchcroft JE, Bierer BE. CD80 and CD86 are not equivalent in their ability to induce the tyrosine phosphorylation of CD28. J Biol Chem 1999;274:3116–24.
[18] Hutloff A, Dittrich AM, Beier KC, Eljaschewitsch B, Kraft R, Anagnostopoulos I, et al. ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. Nature 1999;397:263–6.
[19] Tezuka K, Tsuji T, Hirano D, Tamatani T, Sakamaki K, Kobayashi Y, et al. Identification and characterization of rat AILIM/ICOS, a novel T-cell costimulatory molecule, related to the CD28/CTLA4 family. Biochem Biophys Res Commun 2000;276:335–45.
[20] Yoshinaga SK, Zhang M, Pistillo J, Horan T, Khare SD, Miner K, et al. Characterization of a new human B7-related

protein: B7RP-1 is the ligand to the co-stimulatory protein ICOS. Int Immunol 2000;12:1439–47.
[21] Mages HW, Hutloff A, Heuck C, Buchner K, Himmelbauer H, Oliveri F, et al. Molecular cloning and characterization of murine ICOS and identification of B7 h as ICOS ligand. Eur J Immunol 2000;30:1040–7.
[22] Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J 1992;11:3887–95.
[23] Youngnak P, Kozono Y, Kozono H, Iwai H, Otsuki N, Jin H, et al. Differential binding properties of B7-H1 and B7-DC to programmed death-1. Biochem Biophys Res Commun 2003;307:672–7.
[24] Watanabe N, Gavrieli M, Sedy JR, Yang J, Fallarino F, Loftin S-K, et al. BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. Nat Immunol 2003;4:670–9.
[25] Gonzalez LC, Loyet KM, Calemine-Fenaux J, Chauhan V, Wranik B, Ouyang W, et al. A coreceptor interaction between the CD28 and TNF receptor family members B and T lymphocyte attenuator and herpesvirus entry mediator. Proc Natl Acad Sci USA 2005;102:1116–21.
[26] Sedy JR, Gavrieli M, Potter KG, Hurchla MA, Lindsley RC, Hildner K, et al. B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. Nat Immunol 2005;6:90–8.
[27] Carreno BM, Collins M. BTLA: a new inhibitory receptor with a B7-like ligand. Trends Immunol 2003;24:524–7.
[28] Lafage-Pochitaloff M, Costello R, Couez D, Simonetti J, Mannoni P, Mawas C, et al. Human CD28 and CTLA-4 Ig superfamily genes are located on chromosome 2 at bands q33-q34. Immunogenetics 1990;31:198–201.
[29] Ling V, Wu PW, Finnerty HF, Agostino MJ, Graham JR, Chen S, et al. Assembly and annotation of human chromosome 2q33 Sequence containing the CD28, CTLA4, and ICOS gene cluster: analysis by computational, comparative, and microarray approaches. Genomics 2001;78: 155–68.
[30] Buonavista N, Balzano C, Pontarotti P, Le Paslier D, Golstein P. Molecular linkage of the human CTLA4 and CD28 Ig-superfamily genes in yeast artificial chromosomes. Genomics 1992;13:856–61.
[31] Kristiansen OP, Larsen ZM, Pociot F. CTLA-4 in autoimmune diseases a general susceptibility gene to autoimmunity? Genes Immun 2000;1:170–84.
[32] Shinohara T, Taniwaki M, Ishida Y, Kawaichi M, Honjo T. Structure and chromosomal localization of the human PD-1 gene (PDCD1). Genomics 1994;23:704–6.
[33] Giudicelli V, Lefranc M-P. Ontology for Immunogenetics: IMGT-ONTOLOGY. Bioinformatics 1999;15:1047–54.
[34] Lefranc M-P, Giudicelli V, Kaas Q, Duprat E, Jabado-Michaloud J, Scaviner D, et al. IMGT, the international ImMunoGeneTics information system®. Nucl Acids Res 2005;33:D593–7.
[35] Lefranc M-P, Clément O, Kaas Q, Duprat E, Chastellan P, Coelho I, et al. IMGT-Choreography for immunogenetics and immunoinformatics. In Silico Biol 5, 0006, 2004. Epub 〈http://www.bioinfo.de/isb/2004/05/006/〉. In silico Biol 2005;5: 45–60.
[36] Pommié C, Levadoux S, Sabatier R, Lefranc G, Lefranc M-P. IMGT standardized criteria for statistical analysis of immunoglobulin V-REGION amino acid properties. J Mol Recogn 2004;17:17–32.
[37] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The protein data bank. Nucl Acids Res 2000;28:235–42.
[38] Kaas Q, Ruiz M, Lefranc M-P. IMGT/3Dstructure-DB and IMGT/StructuralQuery, a database and a tool for immunoglobulin, T cell receptor and MHC structural data. Nucl Acids Res 2004;32:D208–10.
[39] Ledbetter JA, Imboden JB, Schieven GL, Grosmaire LS, Rabinovitch PS, Lindsten T, et al. CD28 ligation in T-cell activation: evidence for two signal transduction pathways. Blood 1990;75:1531–9.
[40] Turka LA, Ledbetter JA, Lee K, June CH, Thompson CB. CD28 is an inducible T cell surface antigen that transduces a proliferative signal in CD3+ mature thymocytes. J Immunol 1990;144:1646–53.
[41] Bromley SK, Iaboni A, Davis SJ, Whitty A, Green JM, Shaw AS, et al. The immunological synapse and CD28-CD80 interactions. Nat Immunol 2001;2:1159–66.
[42] Bour-Jordan H, Blueston JA. CD28 function: a balance of costimulatory and regulatory signals. J Clin Immunol 2002;22:1–7.
[43] Andres PG, Howland KC, Nirula A, Kane LP, Barron L, Dresnek D, et al. Distinct regions in the CD28 cytoplasmic domain are required for T helper type 2 differentiation. Nat Immunol 2004;5:435–42.
[44] Watanabe R, Harada Y, Takeda K, Takahashi J, Ohnuki K, Ogawa S, et al. Grb2 and Gads exhibit different interactions with CD28 and play distinct roles in CD28-mediated costimulation. J Immunol 2006;177:1085–91.
[45] Marengere LE, Okkenhaug K, Clavreul A, Couez D, Gibson S, Mills GB, et al. The SH3 domain of Itk/Emt binds to proline-rich sequences in the cytoplasmic domain of the T cell costimulatory receptor CD28. J Immunol 1997;159:3220–9.
[46] Holdorf AD, Green JM, Levin SD, Denny MF, Straus DB, Link V, et al. Proline residues in CD28 and the Src homology (SH)3 domain of Lck are required for T cell costimulation. J Exp Med 1999;190:375–84.
[47] Ward SG. CD28: a signalling perspective. Biochem J 1996;318:361–77.
[48] Lee KP, Taylor C, Petryniak B, Turka LA, June CH, Thompson CB. The genomic organization of the CD28 gene. Implications for the regulation of CD28 mRNA expression and heterogeneity. J Immunol 1990;145:344–52.
[49] Hanawa H, Ma Y, Mikolajczak SA, Charles ML, Yoshida T, Yoshida R, et al. A novel costimulatory signalling in human T lymphocytes by a splice variant of CD28. Blood 2002;99:2138–45.
[50] Gross JA, St John T, Allison JP. The murine homologue of the T lymphocyte antigen CD28. Molecular cloning and cell surface expression. J Immunol 1990;144:3201–10.
[51] Evans EJ, Esnouf RM, Manso-Sancho R, Gilbert RJ, James JR, Yu C, et al. Crystal structure of a soluble CD28-Fab complex. Nat Immunol 2005;6:271–9.
[52] Young JR, Davison TF, Tregaskes CA, Rennie MC, Vainio O. Monomeric homologue of mammalian CD28 is expressed on chicken T cells. J Immunol 1994;152:3848–51.
[53] Okkenhaug K, Wu L, Garza KM, La Rose J, Khoo W, Odermatt B, et al. A point mutation in CD28 distinguishes proliferative signals from survival signals. Nat Immunol 2001;2:325–32.

[54] Freeman GJ, Lombard DB, Gimmi CD, Brod SA, Lee K, Laning JC, et al. CTLA-4 and CD28 mRNA are coexpressed in most T cells after activation. Expression of CTLA-4 and CD28 mRNA does not correlate with the pattern of lymphokine production. J Immunol 1992;149:3795–801.

[55] Perkins D, Wang Z, Donovan C, He H, Mark D, Guan G, et al. Regulation of CTLA-4 expression during T cell activation. J Immunol 1996;156:4154–9.

[56] Eagar TN, Karandikar NJ, Bluestone JA, Miller SD. The role of CTLA-4 in induction and maintenance of peripheral T cell tolerance. Eur J Immunol 2002;32:972–81.

[57] Ling V, Wu PW, Finnerty HF, Sharpe AH, Gray GS, Collins M. Complete sequence determination of the mouse and human CTLA4 gene loci: cross-species DNA sequence similarity beyond exon borders. Genomics 1999;60:341–55.

[58] Schneider H, da Rocha Dias S, Hu H, Rudd CE. A regulatory role for cytoplasmic YVKM motif in CTLA-4 inhibition of TCR signalling. Eur J Immunol 2001;31:2042–50.

[59] Chuang E, Fisher TS, Morgan RW, Robbins MD, Duerr JM, Vander Heiden MG, et al. The CD28 and CTLA-4 receptors associate with the serine/threonine phosphatase PP2A. Immunity 2000;13:313–22.

[60] Stamper CC, Zhang Y, Tobin JF, Erbe DV, Ikemizu S, Davis SJ, et al. Crystal structure of the B7-1/CTLA-4 complex that inhibits human immune responses. Nature 2001;410:608–11.

[61] Djilali-Saiah I, Schmitz J, Harfouch-Hammoud E, Mougenot JF, Bach JF, Caillat-Zucman S. CTLA-4 gene polymorphism is associated with predisposition to coeliac disease. Gut 1998;43:187–9.

[62] Kouki T, Sawai Y, Gardine CA, Fisfalen ME, Alegre ML, DeGroot LJ. CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. J Immunol 2000;165:6606–11.

[63] Hudson LL, Rocca K, Song YW, Pandey JP. CTLA-4 gene polymorphisms in systemic lupus erythematosus: a highly significant association with a determinant in the promoter region. Hum Genet 2002;111:452–5.

[64] Hunt KA, McGovern DP, Kumar PJ, Ghosh S, Travis SP, Walters JR, et al. A common CTLA4 haplotype associated with coeliac disease. Eur J Hum Genet 2005;13:440–4.

[65] Wassink L, Vieira PL, Smits HH, Kingsbury GA, Coyle AJ, Kapsenberg ML, et al. ICOS expression by activated human Th cells is enhanced by IL-12 and IL-23: increased ICOS expression enhances the effector function of both Th1 and Th2 cells. J Immunol 2004;173:1779–86.

[66] Harada Y, Ohgai D, Watanabe R, Okano K, Koiwai O, Tanabe K, et al. A single amino acid alteration in cytoplasmic domain determines IL-2 promoter activation by ligation of CD28 but not inducible costimulator (ICOS). J Exp Med 2003;197:257–62.

[67] Grimbacher B, Hutloff A, Schlesier M, Glocker E, Warnatz K, Drager R, et al. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. Nat Immunol 2003;4:261–8.

[68] Haimila KE, Partanen JA, Holopainen PM. Genetic polymorphism of the human ICOS gene. Immunogenetics 2002;53:1028–32.

[69] Shilling RA, Pinto JM, Decker DC, Schneider DH, Bandukwala HS, Schneider JR, et al. Cutting edge: polymorphisms in the ICOS promoter region are associated with allergic sensitization and Th2 cytokine production. J Immunol 2005;175:2061–5.

[70] Carter LL, Carreno BM. Cytotoxic T-lymphocyte antigen-4 and programmed death-1 function as negative regulators of lymphocyte activation. Immunol Res 2003;28:49–59.

[71] Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I, Kobayashi SV, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol 2005;25:9543–53.

[72] Zhang X, Schwartz JC, Guo X, Bhatia S, Cao E, Lorenz M, et al. Structural and functional analysis of the costimulatory receptor programmed death-1. Immunity 2004;20:337–47.

[73] Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. J Immunol 2004;173:945–54.

[74] Prokunina L, Castillejo-Lopez C, Oberg F, Gunnarsson I, Berg L, Magnusson V, et al. A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. Nat Genet 2002;32:666–9.

[75] Kroner A, Mehling M, Hemmer B, Rieckmann P, Toyka KV, Maurer M, et al. A PD-1 polymorphism is associated with disease progression in multiple sclerosis. Ann Neurol 2005;58:50–7.

[76] Chemnitz JM, Lanfranco AR, Braunstein I, Riley JL. B and T lymphocyte attenuator-mediated signal transduction provides a potent inhibitory signal to primary human CD4 T cells that can be initiated by multiple phosphotyrosine motifs. J Immunol 2006;176:6603–14.

[77] Gavrieli M, Watanabe N, Loftin SK, Murphy TL, Murphy KM. Characterization of phosphotyrosine binding motifs in the cytoplasmic domain of B and T lymphocyte attenuator required for association with protein tyrosine phosphatases SHP-1 and SHP-2. Biochem Biophys Res Commun 2003;312:1236–43.

[78] Hurchla MA, Sedy JR, Gavrieli M, Drake CG, Murphy TL, Murphy KM. B and T lymphocyte attenuator exhibits structural and expression polymorphisms and is highly induced in anergic CD4+ T cells. J Immunol 2005;174:3377–85.