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T Cell Costimulation and Coinhibition: Genetics and Disease

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Abstract: T cell costimulatory and coinhibitory pathways are essential orchestrators and regulators of the adaptive immune response. In recent years, the costimulatory CD28 receptor and B7 ligand families have been expanded to include a total of four and seven members, respectively. Several polymorphisms, mutations, and deletions in both regulatory and protein-coding regions of these genes have subsequently been discovered and evaluated for genetic linkage to various human diseases. Here, we review this evidence as we discuss T cell costimulation and coinhibition in the context of genetic susceptibility to autoimmunity, cancer, and other diseases. As we gain further insight into the functional significance and mechanism of these immunoregulatory pathways by both genetic and immunological approaches, these receptors and ligands are poised to become key targets for immunotherapy. [*Discovery Medicine* 12(63):119-128, August 2011]

Introduction

The vertebrate immune system is regularly challenged to mount an immune response of appropriate specificity and magnitude to a vast array of foreign antigens, while restraining potentially deleterious responses to self and harmless environmental antigens. This requires a profound regulation of the immune response, especially at the level of the T cell, as these lymphocytes bear a central role in adaptive immunity and contribute to the cytokine milieu in which elements of innate immunity operate as well. A T cell recognizes a specific antigen presented by an antigen presenting cell (APC) in complex with major histocompatibility complex (MHC) I or II; this constitutes the first activation signal. In order to be fully activated, however, a second, APC-derived costimulatory signal delivered via the B7-1 or B7-2 ligand and transduced by the CD28 receptor on the surface of the T cell is also required. Upon activation, expression of the coinhibitory CTLA-4 receptor is upregulated on T cells and serves to limit T cell expansion. Thus this requirement for costimulation and coinhibition represents a key point in the control of T cell activation as well as clonal proliferation and deletion.

Recently, human and murine homologs of these ligands and receptors have been identified, and have been shown to have varied effects on T cell function (Collins *et al.*, 2005; Pentcheva-Hoang *et al.*, 2009). CD28 family receptors consist of a variable immunoglobulin domain (IgV), a transmembrane domain, and intracellular tyrosine-dependent signaling motifs (Collins *et al.*, 2005). To date the four members of this family are the costimulatory CD28 and ICOS and the coinhibitory CTLA-4 and PD-1; all but the monomeric PD-1 receptor exist on the cell surface as covalent homodimers (Chattopadhyay *et al.*, 2009). Ligands for these receptors belong to the B7 family of immunoglobulins, composed of extracellular IgV and constant immunoglobulin domains (IgC) linked by a transmembrane region to short, serine- and threonine-containing cytoplasmic domains (Collins *et al.*, 2005). In contrast to the classical B7-1/B7-2:CD28/CTLA-4 interactions which govern T cell responses at early stages in lymphoid organs, the five newly identified B7 family ligands ICOS-L (CD275) (Hutloff *et al.*, 1999; Swallow *et al.*, 1999; Yoshinaga *et al.*, 1999), PD-L1 (B7-H1, CD274), PD-L2 (B7-DC, CD273) (Dong *et al.*, 2002; 1999; Freeman *et al.*, 2000; Ishida *et al.*, 1992; Latchman *et al.*, 2001), B7-H3 (CD276) (Chapoval *et al.*, 2001), and B7x (B7-

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H4, B7S1) (Prasad *et al.*, 2003; Sica *et al.*, 2003; Zang *et al.*, 2003) are expressed on various non-lymphoid tissues as well, and serve to either co-inhibit or limit the extent of T cell function, or to alter the nature of the response. In some cases, receptors for these recently identified B7 family members are unknown. Thus these novel pathways represent modes of immunoregulation that impact the behavior of effector T cells in peripheral organs during an ongoing immune response. In this review, we discuss T cell costimulation and coinhibition in the context of genetic susceptibility to autoimmunity, cancer, and other diseases.

The Pathway of B7-1/B7-2 Ligands and CD28/CTLA-4 Receptors

Expression and function

CD28 is constitutively expressed on T cells. It binds two ligands on the surface of APCs, B7-1 (CD80) and B7-2 (CD86), and induces T cell proliferation by upregulating transcription of IL-2 (Sharpe and Freeman, 2002) and the anti-apoptotic Bcl-X_L (Boise *et al.*, 1995). The second principal member of costimulatory/coinhibitory family of receptors is cytotoxic T-lymphocyte antigen-4 (CTLA-4). CTLA-4 is expressed on both activated CD4 and CD8 T cells upon stimulation and negatively regulates activation by both inhibitory signaling within the T cell and by competing with CD28 for access to B7-1 and B7-2; in fact, the affinity of CTLA-4 for these ligands is much higher compared to that of CD28 (Grohmann *et al.*, 2002; Lin *et al.*, 1998; Prokunina *et al.*, 2002; Sharpe and Freeman, 2002). B7-1 and B7-2 are expressed on APCs, including B cells, macrophages, and dendritic cells (DCs), but also on activated T cells (Sharpe and Freeman, 2002). As *B7-1* and *B7-2* are located on chromosome 3 (Reeves *et al.*, 1997), adjacent to *CD28* and *CTLA-4*, they have been considered as candidates for genetic linkage with pathological conditions.

CTLA-4 polymorphisms and susceptibility to autoimmune diseases and cancer

Autoimmune disease emerges from a failure of the immune system to maintain non-responsiveness or tolerance to self antigens (Lesage and Goodnow, 2001). The *CTLA-4* gene, as well as CD28 and ICOS receptor genes, are located on human chromosome 2 (Chikuma *et al.*, 2003; Finger and Bluestone, 2002; Grohmann *et al.*, 2002; Lin *et al.*, 1998; Sharpe and Freeman, 2002). *CTLA-4* knockout mice developed a spontaneous multiorgan T cell lymphoproliferation resulting in death within three to four weeks (Chambers *et al.*, 1997; Tivol *et al.*, 1995; Waterhouse *et al.*, 1995). Additional work demonstrated that blockage of CTLA-4 signaling pathways by administration of an antagonistic CTLA-4-Ig fusion protein reduces the production of autoantibodies in a mouse model of systemic lupus erythematosus (SLE) (Finck *et al.*, 1994). These findings implicate CTLA-4 as a key molecule in the prevention of autoimmune disease, and, conversely, suggest that functional mutations in this gene may increase autoimmunity. The first indication of a genetic association of *CTLA-4* with autoimmune disease was in a study of Graves' disease (Yanagawa *et al.*, 1995). This study demonstrated a polymorphism in a non-coding 3′ region of *CTLA-4*. A later investigation identified microsatellites (Ueda *et al.*, 2003) associated with this gene that correlated with lower mRNA levels of the soluble (i.e., non-membrane bound), alternatively spliced form of *CTLA-4*. The *CTLA-4* gene has been found to be associated with several other diseases such as type 1 diabetes (Colucci *et al.*, 1997; Lamhamedi-Cherradi *et al.*, 2001). In addition, allelic variants located in the proximal 3′ flanking region of this gene were found to be associated with SLE incidence (Cunninghame Graham *et al.*, 2006). Similarly, a non-parametric linkage in celiac disease relied on a variation within the *CTLA-4/CD28* gene region that increased by 1.8-fold in affected individuals (Popat *et al.*, 2002). Association of the *CTLA-4* gene has also been demonstrated in type 1 diabetes (Douroudis *et al.*, 2009). To our knowledge, only one study failed to demonstrate linkage of this gene to an autoimmune disease, where it was reported that two SNPs were not associated with ulcerative colitis in Chinese Han patients (Hou *et al.*, 2005).

In contrast, studies of the relationship between *CTLA-4* polymorphisms and cancer bear further investigation. Whereas a genetic linkage has been reported in patients with well-differentiated cervical squamous cell carcinoma (Pawlak *et al.*, 2010), no correlation was observed between three polymorphisms of the *CTLA-4* gene and colorectal cancer in a Turkish cohort of patients (Dilmec *et al.*, 2008).

B7-1/B7-2 expression and genetic linkage to pathogenesis

In stark contrast to the *CTLA-4* gene which is strongly linked with the development of several pathological conditions, the *B7-1* and *B7-2* genes, located on chromosome 3, have not been reported to be associated with the development of any disease thus far examined. Through a mutation screening of the entire *B7-1* gene sequence in a population of patients with multiple sclerosis (MS), five genetic variants have been identified (Weinshenker *et al.*, 2000). However, none of those variants were associated with a differential course or

severity of MS or with altered protein structure or function. Similarly, a study of a cohort of Finnish individuals found no genetic linkage of the *B7-1* locus with celiac disease (Woolley *et al.*, 2002). Also, a lack of association in the *B7-1* and *B7-2* genes has been reported for other autoimmune diseases, e.g., rheumatoid arthritis and SLE (Matsushita *et al.*, 2000). Thus at the present time there is no evidence for the association of either *B7-1* or *B7-2* polymorphisms with altered disease risk.

The Pathway of ICOS-L/ICOS

Expression and function

The ICOS (CD278) receptor and its ligand ICOS-L (B7-RP, B7h, B7-H2, CD275) are costimulatory molecules of the expanded CD28/B7 family. On immune cells, ICOS is expressed on activated T cells and ICOS-L is expressed on B cells, DCs, and macrophages. On the other hand, ICOS-L tissue expression is broad and includes fibroblasts, endothelial cells, and epithelial cells (Sharpe and Freeman, 2002; Swallow *et al.*, 1999). In contrast to the constitutive expression of CD28, ICOS is induced on the T cell surface only upon activation, and engagement of this receptor by ICOS-L stimulates T cell differentiation and effector function (Greenwald *et al.*, 2005) including IL-4, IL-10, and IFN-γ production (Hutloff *et al.*, 1999; Yoshinaga *et al.*, 1999). ICOS has been reported to also play a role in the production of autoimmune autoantibodies by limiting access to ICOS-L on activated B cells (Liang *et al.*, 2002). This hypothesis has been validated by several studies in mouse models of rheumatoid arthritis and SLE where blocking these interactions resulted in reduced autoantibodies and disease progression (Hutloff *et al.*, 2004). Recent work demonstrates that ICOS is highly expressed on T follicular helper (T_{FH}) cells and that ICOS-L expression by B cells is required for the development of this recently described subset of lymphocytes (Choi *et al.*, 2011; Crotty, 2011; Nurieva *et al.*, 2008).

ICOS mutations and susceptibility to diseases

The *ICOS* gene is located on chromosome 2, adjacent to the genes for *CD28* and *CTLA-4* (Coyle *et al.,* 2000) and contains 5 exons and 4 introns spanning about 20 Kb (Ihara *et al.*, 2001). This gene region has been associated with numerous diseases such as MS, celiac disease, autoimmune thyroid disease, type 1 diabetes, and Crohn's disease (Barrett *et al.*, 2008). Studies demonstrated a high number of polymorphisms in this gene which are mostly silent mutations that do not change the amino acid sequence of the ICOS receptor (Haimila *et al.*, 2002). Further work genotyping 108 single-nucleotide polymorphisms (SNPs) in a group of Graves' disease patients found new susceptibility loci in a haplotype block containing the 5′ region of *ICOS* and *CTLA-4* (Ueda *et al.*, 2003). However, other studies uncovered no genetic association between the *ICOS* gene and disease incidence. For example, no allelic variants were detected in the coding region of the *ICOS* gene in a study of Japanese type 1 diabetes patients, except for two non-protein coding, microsatellite repeats in the fourth intron of the gene (Ihara *et al.*, 2001). Similarly, screening of German patients with cutaneous melanoma revealed 28 polymorphisms in the *ICOS*, *CTLA-4*, and *CD28* genes but none of these apparently contributed to the risk of melanoma development (Bouwhuis *et al.*, 2009). However, this study of course could not exclude a possible association of these polymorphisms with cutaneous melanoma in other segments of the population. Another investigation also reported the absence of association of the *ICOS* gene with susceptibility to type 1 diabetes (Douroudis *et al.*, 2009).

In contrast, it has been reported that ICOS deficiency is strongly associated with a human autosomal recessive disorder, the common variable immunodeficiency (CVID) (Grimbacher *et al.*, 2003). Specifically, a homozygous partial deletion of the *ICOS* gene in patients with CVID was shown to be associated with a deficiency in the expression of ICOS protein. As a consequence, the patients cannot generate or sustain normal numbers of memory B cells or serum immunoglobulin concentrations, leading to the clinical features of CVID (Grimbacher *et al.*, 2003). In addition, an association between SLE and the distal 3′ flanking region of *CTLA-4* gene on a haplotype that contains variants in the promoter of *ICOS* has been demonstrated (Cunninghame Graham *et al.*, 2006).

Taken together these studies provide clear evidence of genetic linkage between *ICOS* polymorphisms and CVID. However, further work is required to evaluate the genetic associations of ICOS with other diseases including type 1 diabetes, cutaneous melanoma, and SLE. To date, it seems that polymorphisms in other genes including *CTLA-4* and *CD28* may have a much stronger contribution to disease development.

ICOS-L and inflammatory bowel disease

ICOS-L, located on chromosome 21, is a costimulatory ligand predominantly expressed on B cells, activated monocytes, and dendritic cells. This gene has recently been implicated in the development of Crohn's disease, an inflammatory bowel disease (IBD) (Barrett *et al.*, 2008). A meta-analysis of a few thousand Crohn's dis-

ease cases and controls demonstrated an association between ICOS-L and Crohn's disease (Barrett *et al.*, 2008). Other meta-analyses corroborated these results through the identification of a variant in the 3′ region of *ICOS-L*, known as rs762421, which was associated with the risk of Crohn's disease (Duerr *et al.*, 2006; Haider *et al.*, 2007; Rioux *et al.*, 2007). The functional significance of this variant, located in a non-protein coding, intergenic region, is yet to be studied. Recent work indicates that ICOS-L also binds to CD28 and CTLA-4 (Yao *et al.*, 2011) and thus raises the question of which receptor-ligand interaction is responsible for the observed genetic linkage.

The Pathway of PD-L1/PD-L2 Ligands and PD-1 Receptor

Expression and function

PD-1 was initially identified as a gene highly expressed on cell lines undergoing programmed cell death (Ishida *et al.*, 1992). This receptor is expressed on activated T and B cells, regulatory T cells, and monocytes (Agata *et al.*, 1996; Freeman *et al.*, 2000) and binds to two ligands of the B7-family, PD-L1 and PD-L2. Whereas PD-L2 expression is mostly restricted to hematopoietic cells including DCs and macrophages (Greenwald *et al.*, 2005; Keir *et al.*, 2008; Latchman *et al.*, 2001; Liang *et al.*, 2003), PD-L1 is widely expressed on hematopoietic cells including monocytes, macrophages, mast cells, DCs, T and B cells, and on several parenchymal tissues including the vascular endothelium (Keir *et al.*, 2006) and epithelium of several organs such as the lung, liver, pancreas, and gut (Dong *et al.*, 2002; Grabie *et al.*, 2007; Greenwald *et al.*, 2005; Ishida *et al.*, 2002; Kanai *et al.*, 2003; Keir *et al.*, 2008; 2007; Liang *et al.*, 2003; Nakazawa *et al.*, 2004; Pinchuk *et al.*, 2008; Radtke and Clevers, 2005; Rodig *et al.*, 2003; Sharpe *et al.*, 2007). The interaction of these two ligands with the PD-1 receptor is well established as inhibiting CD4 and CD8 T cell proliferation by arresting the T cell cycle (Carter *et al.*, 2002; Greenwald *et al.*, 2005; Keir *et al.*, 2006). This functional evidence of the role of PD-L1 together with its widespread expression pattern on parenchymal tissue suggests that the PD-L1/PD-1 pathway is involved in the maintenance of peripheral tolerance, and that it influences the outcome of the immune response at local sites of inflammation. Several studies have already demonstrated that PD-L1 expression on tissue parenchyma inhibits the immune response in a diabetes model (Keir *et al.*, 2006) and impairs viral clearance during chronic infection (Mueller *et al.*, 2010). Other studies utilizing murine models of autoimmune encephalomyelitis (EAE) and diabetes showed an exacerbation of disease upon administration of blocking antibodies against PD-L1 (Ansari *et al.*, 2003; Salama *et al.*, 2003). These findings suggest that polymorphisms in *PD-L1* and *PD-1* genes may be crucial in the development of autoimmune diseases.

PD-1.3A and systemic lupus erythematosus

The *PD-1* gene is located on chromosome 2. So far, seven SNPs have been identified within this gene (Prokunina *et al.*, 2002) but only *PD-1.3A* has been shown to be strongly associated with SLE in studies of Northern Europeans (Lindqvist *et al.*, 2000) and Latin Americans (Prokunina *et al.*, 2002). *PD-1.3A* has a G-to-A SNP located in an enhancer-like domain in intron 4 of the *PD-1* gene, that alters a binding site of the runt-related transcription factor 1 (RUNX-1). This polymorphism modulates transcription of the gene, suggesting a mechanism by which it could contribute to the development of SLE in humans (Prokunina *et al.*, 2002).

Another study demonstrated differential PD-1 mRNA levels in individuals carrying the *PD-1.3A* allele (Kristjansdottir *et al.*, 2010). In addition, nuclear extracts from the human Jurkat T cell line showed specific binding to the product of this allele (Prokunina *et al.*, 2002). Finally, a Spanish study demonstrated a protective relationship between the *PD-1.3A* allele and SLE susceptibility (Ferreiros-Vidal *et al.*, 2007). These data support the function of PD-1 in maintaining T cell tolerance.

On the other hand, conflicting reports have emerged regarding the association of *PD-1.3A* allele with the risk of developing SLE, but it is possible that these discrepancies reflect background genetic variation among ethnic groups (Ferreiros-Vidal *et al.*, 2007; Sanghera *et al.*, 2004). For instance, meta-analyses demonstrated a significant linkage of the *PD-1.3A* allele with SLE in Latin American individuals but not in all European groups (Lee *et al.*, 2009). In addition, other studies indicated geographic variation in the frequency of the *PD-1.3A* allele across Europe, with the frequency decreasing from northern to southern Europe (Ferreiros-Vidal *et al.*, 2007). Another study (Nielsen *et al.*, 2004) reported an association between the *PD1 6867G* allele and increased risk of lupus nephropathy in Caucasian SLE patients. These analyses support the hypothesis that *PD-1.3A* might confer susceptibility to lupus nephritis in Europeans. Further investigations of *PD-1* polymorphisms in some European cohorts uncovered a strong association of another allele, *PD-1.5C*, with SLE (Lee *et al.*, 2009). Altogether these findings indicate a possible linkage of polymorphisms, especially *PD-1.3A*, to SLE, but discordance between individual stud-

ies suggests that further investigations should be undertaken.

PD-1 polymorphism and other diseases

PD-1 has been linked to several autoimmune diseases through genetic analyses of human patients. Many studies demonstrated that *PD-1* genetic polymorphisms were linked to rheumatoid arthritis (Prokunina *et al.*, 2002), type 1 diabetes mellitus (Nielsen *et al.*, 2003), MS (Kroner *et al.*, 2005), ankylosing spondylitis (Lee *et al.*, 2006), and Graves' disease (Newby *et al.*, 2007). In Japanese and Filipino populations, a higher frequency of a specific SNP was demonstrated in patients with subacute sclerosing panencephalitis (SSPE) in the *PD-1* allele (Ishizaki *et al.*, 2010).

In addition, recent findings demonstrated that PD-1 is markedly upregulated on the surface of exhausted virus-specific CD8 T cells in mice infected with lymphocytic choriomeningitis virus (Barber *et al.*, 2006) and in individuals with human immunodeficiency virus (Day *et al.*, 2006) and hepatitis C virus (Golden-Mason *et al.*, 2007; Penna *et al.*, 2007). Whether as yet undiscovered polymorphisms are associated with high PD-1 expression in infected patients remains to be investigated, but these results are consistent with the hypothesis that mutations or polymorphisms in genes encoding coinhibitory molecules may influence susceptibility to chronic viral infections.

PD-L1 polymorphism and diseases

The gene encoding PD-L1, situated on the short arm of chromosome 9, is highly conserved, particularly in higher mammals. The only other gene in close proximity is its sister ligand PD-L2, which lies 42 Kb from the *PD-L1* gene, in a distinct haplotype block (Mitchell *et al.*, 2009). One study showed that three of the eight SNPs in the *PD-L1* gene among a United Kingdom group and in two groups of Northern European patients were associated with Addison's and Graves' diseases, both of autoimmune etiology (Mitchell *et al.*, 2009). A recessive effect of the most strongly associated alleles was observed in both diseases. However, these results have not been recapitulated by investigations of SLE in human patients, where no link was uncovered between polymorphisms in the protein-coding regions of *PD-L1* and *PD-L2* and the development of disease (Wang *et al.*, 2007). Thus it seems that *PD-L1* polymorphisms may alter susceptibility to some but not necessarily all forms of autoimmune disease. Potentially complicating the interpretation is a recent finding that PD-L1 can bind to the B7-1 ligand and induce bidirectional coinhibitory signaling (Butte *et al.*, 2007).

The B7-H3 and B7x Pathways

B7-H3 (CD276), located on chromosome 15, is a coinhibitory ligand expressed by several kinds of hematopoietic cell, but transcription of this gene is not limited to immune organs and has been demonstrated in a variety of peripheral tissues. Of all the B7 family members, B7-H3 is the most conserved among a diverse assemblage of lineages including fish and terrestrial vertebrates, with more than 50% homology between species (Hansen *et al.*, 2009). In humans, the gene is expressed in two different forms, one with a domain organization typical of the B7 family, and the other with a duplication of the constant and variable domains that may have arisen prior to the evolutionary split between rodents and primates (Ling *et al.*, 2003; Sun *et al.*, 2002). The receptor for B7-H3 is unknown, and there are conflicting reports in the literature as to the function of the ligand, although most evidence to date points to a co-inhibitory rather than costimulatory function (Hofmeyer *et al.*, 2008). Overexpression of this molecule in the context of cancer generally correlates with poor prognosis, making it useful as a biomarker for severe or advanced stage of disease. This has been observed in lung, prostate, pancreatic, colorectal, and ovarian cancers, as well as neuroblastoma and clear cell renal cell carcinoma (Barach *et al.*, 2011). As B7-H3 is one of the newest members of the B7 family, the relationship between polymorphisms in this gene and disease susceptibility has not been thoroughly explored. One study examined the link between three coding and one non-coding (i.e., in the promoter region) SNPs to the autoimmune disorder myasthenia gravis (Sakthivel *et al.*, 2006) but found no evidence of association.

B7x (B7-H4, B7S1), located on chromosome 1, is the most recently discovered member of the B7 family. This protein exerts a coinhibitory effect on activated T cells, although the receptor mediating this effect is currently unknown. The *B7x* gene is transcribed in various lymphoid and peripheral tissues, but protein expression is scarce in healthy human tissue (Choi *et al.*, 2003; Tringler *et al.*, 2005). In contrast, overexpression of this gene in the setting of human breast, lung, prostate, ovarian, kidney, brain, and pancreatic cancers has been demonstrated by several groups (Barach *et al.*, 2011). In some malignancies, the tumor or tumor vasculature expresses B7x, and elevated serum-soluble B7x has also been observed. In general, high expression levels of this gene correlated with disease severity or poor prognosis, and may represent a means of immune evasion by the tumor.

As the newest member of the B7 family of ligands,

information regarding genetic polymorphisms of *B7x* is only beginning to emerge. An interesting study (Zhang *et al.*, 2009) identified three SNPs in untranslated or intronic (i.e., non-protein coding) regions of the *B7x* gene which were associated with rates of sporadic breast cancer in a Chinese Han population. It was surmised that these SNPs affect the efficiency of cellular production of B7x rather than the function of the protein itself. Two SNPs were associated with a decreased risk of cancer, while one SNP was linked to a greater incidence of cancer, increased lymph node metastasis, and altered steroid hormone receptor status. These attributes are of prognostic value in patient management, and receptor status in particular is often utilized to predict responsiveness to hormone therapy. In addition, a "hypothesis-free," genome-wide association study identified ten SNPs in non-coding regions of the *B7x* gene which were significantly associated with juvenile idiopathic arthritis (JIA) (Hinks *et al.*, 2009). This discovery is of special interest, as JIA is a relatively common rheumatic disease of complex, poorly understood etiology whose genetic component, for the most part, remains to be elucidated.

Concluding Remarks

Costimulatory and coinhibitory pathways are pivotal mechanisms in the determination of T cell function. Given the varied roles and temporospatial expression patterns of the B7 family ligands and CD28 family receptors, it seems logical that functional polymorphisms in either the protein-coding or regulatory regions of these genes may alter the initiation or subsequent course of the immune response. Indeed, recent genetic linkage studies examining such mutations in both human patients and animal models of cancer, autoimmune, and other diseases confirm that these costimulatory and coinhibitory pathways profoundly affect the nature of the immune response in both lymphoid and peripheral tissues. While in many instances there is an apparent discordance among conclusions reached by different investigations, particularly in studies of human populations, it is important to realize that the etiology of most cancers and autoimmune diseases is multifactorial and that background genetic variation among and within ethnic groups naturally confounds interpretation. Nonetheless, identification of these disease-linked allelic variants and dissection of the affected ligand-receptor interactions and downstream intracellular signaling pathways hold great promise for approaches in rational drug design. In 2005, the FDA approved Orencia (Bristol-Myers Squibb), a CTLA-4 Ig fusion protein that competes with CD28 for binding to B7-1 and B7-2, for the treatment of adult rheumatoid arthritis; a few years later, its use was extended to JIA (Felix *et al.*, 2010). Early 2011 has already witnessed the approval of another biologic, Yervoy (Bristol-Myers Squibb), a monoclonal antibody which blocks the interaction of CTLA-4 with B7-1 and B7-2, for the treatment of metastatic melanoma (Sondak *et al.*, 2011). Looking ahead as the function and expression patterns of more recently discovered B7 and CD28 family members are elucidated in various disease contexts via genetic linkage and immunological studies, these novel costimulatory and coinhibitory pathways will likely be considered as drug targets in the footsteps of CTLA-4. In light of their distinct temporospatial expression patterns relative to the founding members of these families, it will be interesting to see whether the manipulation of these more recently identified receptor-ligand interactions will result in more targeted therapies and fewer side effects.

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Disclosure

The authors report no conflicts of interest.

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