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Review

Alternate costimulatory molecules in T cell activation: differential mechanisms for directing the immune response

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Summary. T cells are required for an effective immune response against a wide range of pathogens and for the generation of immunological memory. T cell activation can be divided into two phases: an antigen-specific signal delivered through the T cell antigen receptor, and a costimulatory signal delivered through accessory molecules on the T cell surface. Following activation, T cells differentiate to acquire distinct effector functions depending on the costimulatory signal, cytokine environment, and the pathogen itself. Although CD28 has been identified as the dominant costimulatory molecule, several other molecules have been described as having a costimulatory function. This review will focus on recent evidence for the existence of alternate costimulatory molecules, and the differential roles they might play in the activation, development, and survival of T cells.

Key words: T cells, Costimulation, Memory, Effector, Activation

Introduction

Initiation of the cellular adaptive immune response involves the interaction of T lymphocytes with their specific antigen in the context of major histocompatibility molecules expressed on antigen-presenting cells (APCs). Effective T cell activation results in proliferation, differentiation, and the acquisition of effector functions. This activation is a tightly regulated process that requires 2 distinct signals: an antigen-specific signal delivered through the T cell antigen receptor (TCR) and a second, or costimulatory signal, delivered through the interaction of accessory molecules on the T cell with their ligands on the antigen-presenting cell. T cells receiving a signal only through the TCR will become anergic or undergo apoptosis (Chambers and Allison, 1999), and entry into these states can be prevented by costimulation.

Although there is no definitive list of requirements for the classification of T cell costimulatory molecules, it has become accepted that costimulatory signaling should function to increase IL-2 production, promote cell division and clonal expansion, protect T cells from anergy/apoptosis, and generate long-lived memory cells (Frauwirth and Thompson, 2002). Costimulatory signaling is generally thought to synergize with signaling through the TCR, lowering the antigen threshold for activation. Recent work has supported this hypothesis. Using an analysis of global gene expression during T cell activation, it was demonstrated that costimulation through CD28 serves primarily to further induce levels of gene expression already induced by TCR signaling alone (Diehl et al., 2002; Riley et al., 2002). However, costimulation through either of the CD28 family members ICOS or CTLA4, resulted in modulation of different cohorts of genes compared with CD28.

A considerable body of evidence has established CD28 as the dominant costimulatory molecule on the surface of T cells (Lenschow et al., 1996). Costimulation through CD28 synergizes with TCR signaling to activate events not generally observed in response to either stimulus used alone. These events include activation of PI 3-kinase, sphingomyelinase, tyrosine kinases, JNK, and NF-AT (Ward et al., 1993; Hutchcroft and Bierer, 1994; Su et al., 1994, Boucher et al., 1995; Raab et al., 2001). Particularly pertinent to the present discussion of alternate second signals is that costimulation through CD28 leads to the production of large amounts of IL-2 and also generates both Th1 and Th2 responses. Costimulation through CD28 protects T cells from apoptosis through the upregulation of the anti-apoptotic gene Bcl-xL, leading to substantial clonal expansion (Boise et al., 1995). CD28 also is capable of generating long-lived memory cells, which can respond rapidly and vigorously to a secondary challenge. The present

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discussion will focus on alternate T cell costimulatory molecules, and the emerging picture of the role they might play in T cell activation and differentiation during modulation of the immune response. We will be guided by the hypothesis that T cell phenotype, and thus cell signaling mechanisms and gene expression patterns, will differ according to the costimulatory ligands encountered by the cell.

T cell activation in the absence of CD28

Despite its importance as a costimulatory molecule, studies in CD28-deficient mice demonstrate that not all T cell-dependent immune responses are dependent on CD28. Initial studies showed that CD28-deficient mice had impaired responses to lectins, particularly in T cell-dependent B cell responses. However, the cytotoxic capacity of T cells in response to lymphocytic choriomeningitis virus (LCMV) infection and skin allograft rejection was largely similar between CD28-deficient and wild type mice, indicating that alternate costimulatory molecules might be providing a level of signaling redundancy or possibly inducing alternate signaling pathways (Shahinian et al., 1993; Kawai et al., 1996). Additional studies employing antigen-specific CD4+ T cells showed that initial proliferation and activation marker expression were the same between CD28-deficient and wild-type cells during the first 24 to 48 hours after antigen stimulation. However, CD28-deficient T cells produced significantly less IL-2, were less responsive to lower antigen concentrations, and failed to maintain their proliferative capacity (Lucas et al., 1995). The importance of lessons learned from CD28-deficient mice is underscored by the observation that a significant percentage of T cells in humans are CD28(-). The peripheral blood CD8+ T cell pool may consist of up to 50% CD28(-) T cells in healthy individuals, and a similar, albeit much lower frequency, CD4+ CD28(-) population has been identified (Morishita et al., 1986; Azuma et al., 1993).

Modulation of CD28 expression levels following activation appears to be controlled by a variety of factors, including cell division (Fiorentini et al., 1999), IL-2-family member cytokines (Posnett et al., 1999), and the binding of specific motifs within the CD28 regulatory region (Vallejo et al., 1999). However, the possible contribution of alternate costimulatory molecules to this process has not yet been examined (Arosa, 2002). Although the phenotype of CD28(-) T cells closely resembles that of memory cells, it is not clear that all CD28(-) T cells arise from a previous antigen encounter. Therefore, the role that alternate costimulatory signals may play in the generation, maintenance, and activation of CD28(-) T cells remains largely unknown.

Alternate costimulatory molecules

The number of molecules other than CD28 that have been identified as providing a costimulatory function during T cell activation continues to grow. Assignment of a surface protein as a costimulatory receptor is often based on its ability to augment proliferation or effector functions in conjunction with signaling through the TCR. The list includes, but is not limited to, CD2 (Bierer et al., 1988), CD5 (Ledbetter et al., 1985), CD9 (Tai et al., 1996), CD27 (van Lier et al., 1987), CD44 (Huet et al., 1989), CD46 (Astier et al., 2000), CD81 (Todd et al., 1996), LFA-1 (van Seventer et al., 1992), ICAM-1 (Chirathaworn et al., 2002), VLA-4 (Davis et al., 1990), OX40 (Gramaglia et al., 1998), 4-1BB (Shuford et al., 1997), LIGHT (Wan et al., 2002), SLAM (Aversa et al., 1997), ICOS (Hutloff et al., 1999), CTLA-4 (Walunas et al., 1996), and PD-1 (Freeman et al., 2000). While none of these molecules appear to be completely redundant with CD28, there is evidence that different molecules can act to promote, to inhibit, or in some instances maintain T cell activation. In addition, some of these molecules may influence T cell activation and differentiation by directing the acquisition of distinct effector functions. Although a thorough discussion of all the molecules mentioned above is beyond the scope of

Table 1. Effect of different costimulatory molecules on aspects of T cell activation.

COSTIMULATORY MOLECULE	PROLIFERATION EARLY/LATE[1]	INCREASED IL-2	PROTECTION FROM APOPTOSIS	CYTOKINE POLARIZATION
CD28	+ / +	+	+	Th1 and Th2
ICOS	- / +	-	N.D.	Th2
CTLA-4	- / -	-	-	-
PD-1	- / -	-	-	-
LFA-1	+ / -	-	-	Th1
ICAM-1[2]	+ / +	+	+	Th1
4-1BB	- / +	+	+	Primarily Th1
OX40	- / +	+	N.D.	Th2
LIGHT	- / +	+	N.D.	Th1

N.D.: No direct evidence. [1]: Early refers to costimulatory events that contribute immediately to onset of the proliferative response. Late refers to events that are thought to contribute to sustaining proliferation because of markedly later expression of the signaling molecule by the T cell or because of continued use. [2]: Early proliferation is delayed compared to LFA-1 or CD28.

this review, we will briefly highlight the evidence for several of these molecules where recent studies have given insight into their role during T cell activation and differentiation (summarized in Table 1).

CD28 family members (ICOS , CTLA-4, and PD-1)

ICOS

ICOS (Inducible COStimulator) is expressed only on T cells that have undergone activation, and like CD28, ICOS signaling potentiates cell proliferation, and enhances production of cytokines. ICOS appears to modulate expression of a subset of those cytokines regulated by CD28, and these include IL-4, IL-5, IL-10, IFN-γ, and TNF-α, but not IL-2 (Hutloff et al., 1999). The nature of the cytokines that are modulated suggests a role for ICOS in regulating B cell differentiation, and this is supported by the observation that ICOS expression seems to be limited to germinal center T cells *in vivo* (Hutloff et al., 1999). ICOS is the only CD28 family member that binds the B7 family member B7-h, expressed on B cells and macrophages (Yoshinaga et al., 1999). The importance of ICOS in regulating Th2-driven B cell responses was confirmed in $ICOS^{-/-}$ mice, which have an absence of serum IgE, smaller germinal centers, impaired antibody responses and impaired Ig class switching (Dong et al., 2001; McAdam et al., 2001; Tafuri et al., 2001). In addition, Th1 responses also are suppressed in $ICOS^{-/-}$ mice and this is presumed to be a result of decreased cytokine responses during the primary immune response (Bertram et al., 2002).

CTLA-4

Cytotoxic T lymphocyte antigen-4 (CTLA-4) is a member of the CD28 family and is upregulated following T cell activation. Although some initial reports showed that it had a positive effect on T cell responses (Zheng et al., 1998), the bulk of the data support its role as a negative regulator of T cell activation (e.g. Chambers et al., 2001). Some controversy remains as to whether the primary mode of action of CTLA-4 is the result of direct signaling through CTLA-4, or whether CTLA-4 sequesters the CD28 ligands B7-1 and B7-2 (Carreno et al., 2000) rendering them inaccessible to CD28. It seems most likely that both possibilities contribute to the function of CTLA-4. The importance of CTLA-4 in attenuating T cell responses was demonstrated in *CTLA-4*-deficient mice, which developed a massive lymphoproliferative disorder (Waterhouse et al., 1995).

PD-1

Program death 1 (PD-1) is an activation-induced CD28 family member that contains an immunoreceptor tyrosine-based inhibitory motif (ITIM), and is expressed on T cells, B cells, and monocytes (Agata et al., 1996). The ligands for PD-1 are induced on monocytes (PD-L1/B7-H1) and dendritic cells (PD-L2/B7-DC) following exposure to IFN-γ or IFN-γ plus LPS (Freeman et al., 2000), and also on T cells in response to stimulation through the TCR and CD28 (Bennett et al., 2003). Engagement of PD-1 inhibits TCR- and TCR plus ICOS-induced proliferation, largely through the inhibition of IL-2 production (Bennett et al., 2003). In contrast to stimulation through CTLA-4, costimulation through CD28 or the addition of exogenous IL-2 can overcome PD-1-mediated inhibition. This suggests that the level of IL-2 induced by costimulation, or present in the local milieu, might determine the outcome of PD-1 engagement (Carter et al., 2002). In support of its role as an immune attenuator, PD-1-deficient mice develop autoimmune pathologies (Nishimura et al., 1999). PD-1 also presents a potential for therapeutic intervention of immune-mediated diseases, and indeed, in one study, PD-1 targeting promoted allograft survival (Ozkaynak et al., 2002).

Thus, the CD28 family includes surface molecules capable of contributing positive or negative effects on T cell activation and proliferation, and in no case do the effects of these family members mimic exactly the effects of CD28.

Leukocyte function-associated Antigen-1 (LFA-1)

The importance of the role of the ß-2 integrin, LFA-1, in T cell : APC interaction has been highlighted by its localization to the peripheral supra-molecular activation cluster (pSMAC) within the immunological synapse (Monks et al., 1998; Grakoui et al., 1999). Traditionally, LFA-1 was known for its participation in intercellular adhesion and leukocyte extravasation. However, in addition to these adhesion effects, stimulation through LFA-1 is capable of transmitting molecular signals into the cell. This results in the cytoskeletal reorganization necessary for synapse formation (Wulfing et al., 1998). Initial work studying the ability of LFA-1 to deliver a costimulatory signal showed that LFA-1 could induce naïve T cell proliferation in conjunction with an antigen signal, but induced only low levels of IL-2 (Zuckerman et al., 1998). Additional work in our laboratory and others has further shown that costimulation through LFA-1 fails to induce clonal expansion after the initial burst of proliferation, and the cells exhibit a tendency to become apoptotic (Zuckerman et al., 1998; Ragazzo et al., 2001; Kohlmeier et al, 2003). Compared with CD28, costimulation through LFA-1 leads to differential processing of procaspase-3 and -9 (Palmer et al., 2001), suggesting that the ability of costimulatory signals to affect caspase processing may determine their ability to protect activated T cells from apoptosis. Thus, despite the observation that costimulation through LFA-1 can induce T cell proliferation leading to several rounds of cell division, the inability of LFA-1 to promote clonal expansion or significant IL-2 secretion, and to protect from apoptosis does not support its function as a “stand-

alone" costimulatory molecule. There is evidence, however, that LFA-1 signaling polarizes talin to the T cell : APC interface independent of signaling through the TCR or CD28 (Sedwick et al., 1999). This suggests that a level of signaling cooperativity involving the TCR and LFA-1, plus other surface molecules, might be required for optimal T cell : APC interaction and activation.

It is interesting to note that TCR and LFA-1 stimulation synergize to activate the same early signaling events attributed to costimulatory signaling through CD28, including PI 3-kinase, sphingomyelinase, and JNK (Ni et al., 1999). Because the outcomes of these two costimulatory events (CD28 or LFA-1) are markedly different, this suggests that, whereas engagement of these pathways is necessary for initial T cell activation, they are not, by themselves, sufficient to maintain a fully effective response. However, blocking the interaction of LFA-1 with its ligands, the ICAMs, does enhance Th2 cytokine production (Salomon and Bluestone, 1998). Also, in contrast to costimulation through CD28, nonpolarized T cell clones costimulated through LFA-1 produced Th1 cytokines exclusively, suggesting that some role does exist for LFA-1 in the polarization of T cell responses toward Th1 (Jenks and Miller, 2000, Chirathaworn et al., 2002). Thus, in spite of its inability to fully activate T cells, costimulation through LFA-1 may serve to guide some level of T cell differentiation. Additional models, incorporating an increasing number of costimulatory molecules and cytokines, will help to elucidate the contribution of LFA-1 to costimulatory signaling and T cell differentiation.

Intercellular adhesion molecule-1 (ICAM-1)

The previously accepted roles for ICAM-1 in T cell activation were to serve as a migration guide on the surface of endothelial cells during the extravasation of leukocytes and to serve as a ligand on an APC for LFA-1 on a T cell (Marlin and Springer, 1987). ICAM-1 is expressed at low but detectable levels on resting and naïve T cells, and is upregulated by inflammatory cytokines or after stimulation through the TCR (Roebuck and Finnegan, 1999). This low level of ICAM-1 is active as a signaling molecule, however. We demonstrated some years ago (Chirathaworn et al., 1995) that stimulation through ICAM-1 on resting T cells was able to transmit signals into the cell. Recently, we described the ability of ICAM-1, resident on a T cell, to deliver a costimulatory signal that is similar in many ways to that delivered through CD28. Costimulation through ICAM-1 resulted in activation of PI 3-kinase by 5 minutes, expression of CD69 by 6 hours, transcription and secretion of Th1 cytokines by 8 hours, extensive T cell proliferation, and clonal expansion of naïve CD4+ T cells (Chirathaworn et al., 2002). Unpublished data from our laboratory demonstrate that costimulation of naïve cells through ICAM-1 or CD28 generates equivalent amounts of IL-2 mRNA, whereas costimulation through LFA-1 generates considerably less. However, we find that in contrast to CD28, ICAM-1 costimulation is unable to stabilize the IL-2 message longer than its normal half-life of 30 minutes (Fig. 1).

Although costimulation through ICAM-1 is similar to LFA-1 in that both elicit primarily Th1 cytokines, the outcome of activation varies greatly between the two. Initially, cell division induced by ICAM-1 costimulation is delayed compared to that caused by LFA-1 or CD28. Although we have demonstrated very rapid molecular responses to signaling through ICAM-1 (Chirathaworn, et al., 1995), we have hypothesized that the delay in proliferation represents a necessity that the level of ICAM-1 be increased above a certain threshold to enable full activation. This is supported by elevation of ICAM-1 expression on naïve CD4+ T cells between 12 and 24 hours after TCR signaling (Kohlmeier et al., 2003). One implication of these observations is a biphasic response of T cells to stimulation through ICAM-1. Following the initial proliferative lag, costimulation through ICAM-1 promotes marked clonal expansion, and protects from apoptosis to a level similar to CD28 (Kohlmeier et al., 2003). Surprisingly, costimulation of a mixed population of T cells through ICAM-1 produced an equivalent number of T cells with a memory phenotype when

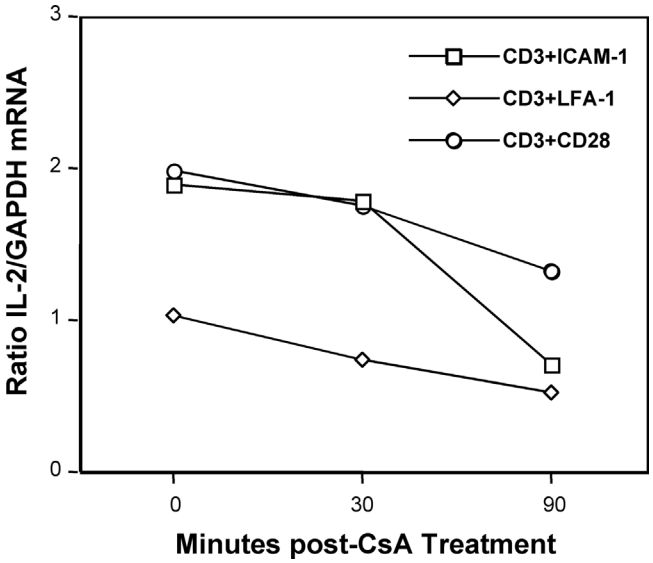

Fig. 1. Costimulation through ICAM-1 did not stabilize IL-2 mRNA to the same extent as did CD28. Human peripheral blood T cells were stimulated in vitro with anti-CD3 plus either anti-ICAM-1, anti-LFA-1, or anti-CD28 for 4 days. Cultures were then expanded in the presence of 100 U/ml of recombinant IL-2 for an additional 2 days. Dead cells were removed by passage over a ficoll gradient, and cultures were re-stimulated for an additional 6 hours prior to the addition of cyclosporin A (CsA) to inhibit further transcription of IL-2. Total RNA was collected at 0, 30, and 90 minutes following CsA treatment and mRNA was amplified by PCR using specific primers for IL-2 and GAPDH. Densitometry was performed using BitImage software and the data represent the intensity of the IL-2 message relative to the GAPDH message for that individual sample.

compared to CD28, and this was in stark contrast to the effects of costimulation through LFA-1. It will be interesting in future studies to test the efficacy of costimulation through ICAM-1 in an antigen-specific system, and to investigate any ability of ICAM-1 stimulation to modulate T cell differentiation.

TNF Receptor Family Members (4-1BB, OX40, and LIGHT)

Stimulation through any of several members of the TNF receptor (TNFR) family is known to augment T cell proliferation. However, although these proteins are related, the phenotypic outcomes they induce are different. This supports our basic contention that the nature of the array of costimulatory signals delivered to a given T cell can differentially modulate the phenotypic outcome of the cell when it also is stimulated through the TCR.

4-1BB

4-1BB (CD137) is expressed on both activated CD4+ and CD8+ T cells, but supports a higher level of proliferation by CD8+ T cells compared to CD4+ T cells (Shuford et al., 1997). Also, 4-1BB is one of the few costimulatory molecules capable of inducing high levels of IL-2 production (DeBennedette et al., 1997). The temporal expression patterns of 4-1BB suggest that it may function primarily to sustain T cell activation following CD28 down-regulation (Vinay and Kwon, 1998), and this is supported by the observation that T cells from chronic disease states show an increased sensitivity to 4-1BB costimulation (Michel et al., 1998). In addition, the administration of agonistic antibodies against 4-1BB potentiates anti-tumor responses in mice bearing syngeneic tumors (Melero et al., 1997). Finally, mice targeted for 4-1BB or 4-1BBL, exhibit diminished antiviral CTL responses, skin allograft rejection and graft-versus-host disease (DeBenedette et al., 1999; Tan et al., 1999; Tan et al., 2000) but no defects in antibody production against viruses. Thus, 4-1BB seems to provide an augmentation function for the cellular immune response, and the immune response is prolonged in cells receiving a signal through 4-1BB. It is of considerable interest to ask how 4-1BB is regulated, and whether it is restricted to certain T cell subsets.

OX 40

OX40 expression also is limited to activated T cells, and OX40 ligand (OX40L) is expressed on dendritic cells, activated B cells, and activated T cells (Chen et al., 1999). The function of OX40 appears to differ markedly from that of 4-1BB. OX40 augments the proliferative response to sub-optimal antigen stimulation, and OX40 costimulation increases production of the Th2 cytokines IL-4 and IL-5, but not IFN-γ (Flynn et al., 1998; Gramaglia et al., 1998). Its relevance to disease states has been demonstrated by the ability of an OX40-Ig fusion protein to reduce the severity of experimental autoimmune encephalitis (EAE) in mice (Weinberg et al., 1999). Finally, $OX40^{-/-}$ mice develop fewer antigen-specific CD4+ T cells than wild type littermates, suggesting a role for OX40 in the generation of T cell memory (Gramaglia et al., 1999).

LIGHT

A recent report has described a role for the TNF receptor family member LIGHT in T cell activation. LIGHT stands for: 'homologous to Lymphotoxins, exhibits Inducible expression, and competes with HSV Glycoprotein D for Herpesvirus entry mediator, a receptor expressed on T lymphocytes'. The secreted TNFR protein DcR3/TR6 is a ligand for LIGHT, as well as for Fas ligand and TL1A (TNF-like factor-1A) (Yu et al., 1999). Similar to the case with OX40, costimulation through LIGHT shows enhanced proliferation to sub-optimal TCR stimulation in both Th1- and Th2-polarized T cells, but unlike OX-40, LIGHT augments Th1 and not Th2 cytokine production (Wan et al., 2002). Interestingly, there also is evidence to support the role of LIGHT as a costimulatory ligand upon its interaction with TR2 on a T cell. TR2 (TNFR-related 2, herpesvirus entry mediator) is expressed on both resting and activated CD4+ and CD8+ T cells. LIGHT-TR2 interaction can augment T cell proliferation and cytokine production (Kwon et al., 1997). It is especially intriguing that such closely related costimulatory molecules elicit such distinct T cell phenotypes. Elucidation of the pathways involved in transduction of costimulatory signals from these molecules and others undoubtedly will help to explain some of the distinct phenotypic outcomes.

Roles of alternate costimulatory molecules

As the number of identified potential costimulatory molecules continues to increase, and their effects on T cell differentiation are further defined, it is interesting to consider the possible role(s) of such a complex system in governing T cell activation. One obvious role that is supported by existing data is the manner in which specific costimulatory signals, or a lack thereof, influence the cytokine polarity of T cells. LFA-1, ICAM-1, 4-1BB, LIGHT, and SLAM all promote a Th1 phenotype, either by directly upregulating Th1 cytokines or by inhibiting Th2 cytokines. Alternatively, ICOS and OX40 promote Th2 responses, and CD28 seems to promote the appearance of both Th1 and Th2 cells. In at least one case, polarization to a Th1 phenotype by costimulation through LFA-1 was not reversible by additional costimulation through CD28 (Jenks and Miller, 2000). Future work will undoubtedly examine the ability of other costimulatory molecules to terminally differentiate cells to a Th1 or Th2 phenotype, and will determine the participation of exogenous cytokines on

these types of polarizations.

Another important consideration is the role that different costimulatory signals may play in directing T cell homing through the regulation of adhesion molecules and chemokine receptors. Although there currently is very little published information, a substantial amount of indirect evidence supports this notion. It is well established that Th1 and Th2 cells express different chemokine receptors. Specifically, the chemokine receptors CCR5 and CXCR3 are preferentially expressed on Th1 cells, whereas CCR4 and CCR8 are expressed on Th2 cells (Bonecchi et al., 1998; Colantonio et al., 2002). It is therefore possible that the polarizing effect of different costimulatory molecules plays a role in determining chemokine receptor expression, and thus the ability of the cells to travel to specific sites. We have found that costimulation through CD28, but not LFA-1 or ICAM-1, upregulates expression of the B cell follicle homing receptor CXCR5 (unpublished data). This is logical in that Th2 (but not Th1) polarized T cells capable of providing B cell help would preferentially migrate to B cell areas, and LFA-1 and ICAM-1 seem to induce only cells with a Th1 phenotype.

CCR7 is a chemokine receptor of particular interest in that it targets cells to lymphoid tissues. Thus, memory T cells have been grouped into CCR7(-) effector memory cells which are thought not to home to lymph nodes, and CCR7(+) central memory cells which recirculate between the blood and nodes in a manner that is similar to naïve T cells (Sallusto et al., 1999). Two models have been proposed to account for the appearance of these two memory cell populations. In one case, both populations would arise independently and would show only marginal overlap in repertoire (Sallusto and Lansavecchia, 2001; Baron et al., 2003). In the other, more linear model, effector memory cells are proposed to arise from central memory cells and in this scenario, overlap of repertoire would occur (Wherry et al., 2003). The participation of alternate or multiple costimulatory signals on the generation of these two distinct populations under either of these models has not yet been investigated.

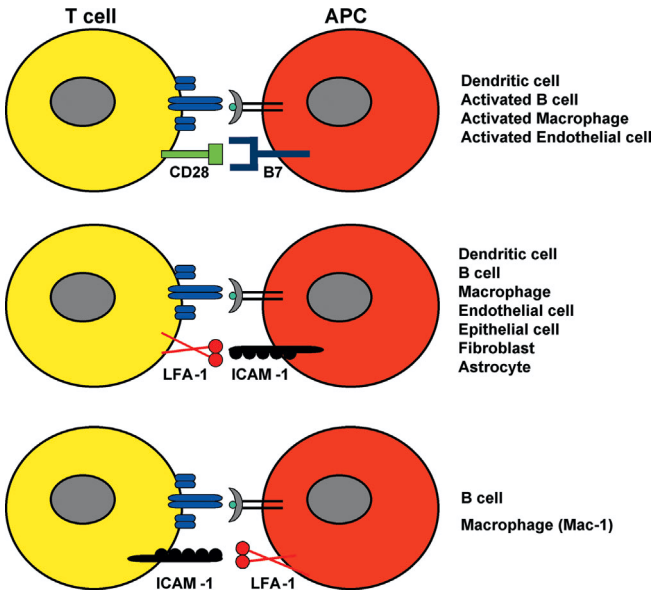

Fig. 2. Schematic diagram of the manner in which specific costimulatory molecules may allow T cells to 'perceive' the type of cell with which they are in contact.

An alternate purpose for such a wide array of costimulatory molecules might be to serve as a sensory array, allowing the T cell to 'perceive' the cell type with which it is interacting based on the surface molecules that are engaged, as we have proposed earlier (Chirathaworn et al., 2002). For example (Fig. 2), a T cell receiving costimulation through CD28 could be interacting with dendritic cells, activated B cells or macrophages or endothelial cells in certain states of activation. Similarly, costimulation through LFA-1 could occur through interaction with any cell expressing ICAM-1, such as endothelial cells, epithelial cells, fibroblasts, astrocytes, and leukocytes as well as conventional APCs (Hayflick et al., 1998). Costimulation through ICAM-1 could come only from leukocytes and in the case of class II antigen presentation, from B cells, macrophages or certain dendritic cells (e.g. Hart and McKenzie, 1998). Therefore, a T cell may perceive that it is interacting with a nonlymphoid cell if it receives a signal through LFA-1 in conjunction with stimulation through the TCR. However, that T cell could sense that it is interacting in a lymphoid environment by receiving signals through the TCR plus ICAM-1, or the TCR plus ICAM-1 plus LFA-1. Because only leukocytes are capable of stimulating ICAM-1 on the T cell, this interaction could provide a means by which a T cell can distinguish between leukocytes and nonlymphoid cells and respond appropriately.

Into the future

A growing body of evidence dealing with the role of alternate or multiple costimulatory molecules has begun to demonstrate the complexity of the cell-cell interactions that govern lymphocyte activation. The implications for understanding the mechanisms controlling T cell activation and the ability to modulate T cell development are staggering and are especially pertinent with regard to adoptive immunotherapy. Because costimulation can influence T cell activation, homing, effector functions, and memory cell generation, costimulation-dependent therapies could allow for the creation of specialized T cell subsets capable of migrating to and responding vigorously against specific antigens. Such approaches are already being investigated for the generation of tumor-specific T cells both *in vivo*

and *ex vivo* (e.g. Diehl et al., 1999; Weinberg et al., 2000; Marshall and Marks, 2001).

Future analyses of mice lacking one or more costimulatory molecules or their ligands should assist in sorting out the unique or overlapping roles of these signals in regulating the immune response. Building mice to address these questions will occupy a great deal of time and a major allocation of resources. The use of artificial APCs expressing various costimulatory ligands alone or in combination also will prove to be beneficial in dissecting the contribution of the array of costimulatory molecules to T cell activation and differentiation (Deeths and Mescher, 1999; Jenks and Miller, 2000; Ragazzo et al., 2001). This type of study frequently can be accomplished in a more expeditious manner than creating genetically altered mice.

Although there are several limitations, the careful application of specific monoclonal antibodies in selected combinations can provide rapid information that can be verified using mice or engineered APCs. Antibodies must be characterized according to their ability to be agonist, antagonist, or blocking. Some receptors respond equally well regardless of whether an antibody is immobilized or free in solution. The classic example of this is costimulation through CD28. Other signals must be delivered by immobilized antibody, examples of which include LFA-1 (Geppert and Lipsky, 1988) and ICAM-1 (Chirathaworn and Benedict, unpublished data). Finally, some antibodies are agonist under one circumstance and antagonist under different circumstances. One recent example is the PD-1 system, where immobilized antibody delivered signals through PD-1, whereas antibody free in solution blocked signals delivered through cell:cell contact (Bennett et al., 2003). Despite these and other limitations, the use of antibody to provide costimulatory signals remains useful for examining cell signaling in a specific manner.

Concluding remarks

T cell costimulatory signals synergize with signaling through the TCR to induce T cell activation and effector functions. Although the number of molecules described as having a costimulatory function continues to grow, their unique contributions to proliferation, clonal expansion, cytokine production and polarization, protection from apoptosis, cell differentiation and the generation of long-lived memory cells are only now beginning to be elucidated. The primary costimulus is delivered through CD28. Interestingly, none of the alternate costimulatory molecules described thus far appear to be completely redundant, suggesting that all potentially play an important role in eliciting optimal immune function. Further investigation of known, and as yet unidentified costimulatory molecules will clarify this issue, and studies of antigen-specific T cells and disease models using alternate costimulatory molecules alone or in combination will allow determination of the precise role of these molecules in directing the T cell immune response.

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