Minireview

Contrasting Alloreactive CD4+ and CD8+ T Cells: There's More to It Than MHC Restriction

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Surface expression of CD4 or CD8 is commonly used to identify T-cell subsets that recognize antigen presented by class II MHC or class I MHC, respectively. This holds true for T cells that respond to allogeneic MHC molecules that are directly recognized as foreign, as well as peptides from allogeneic MHC molecules that are indirectly presented by self MHC molecules. CD4 or CD8 expression was initially believed to define cytokine secreting helper T cells or cytotoxic cells, respectively. However, this association of phenotype and function is not absolute, in that CD4+ cells may possess lytic activity and CD8+ cells secrete cytokines, notably IFNγ. Recently, additional fundamental differences in the immunobiology of these T-cell subsets have been identified. These include differences in costimulatory requirements, cytokine responsiveness, cytokine production, cell survival, and the maintenance of memory. This review will survey these differences, emphasizing alloreactive T-cell responses as well as relevant observations that have been made in other systems.

Functions of CD4 and CD8

The concept that subpopulations of T cells could be delineated by specific cell surface markers was first established in 1975 by Cantor and Boyse (1), who defined murine T-cell subsets based on Lyt1 and Lyt2 antigens. Their work, and subsequent independent studies with both mouse and human cell lines established that this phenotypic difference between T cells had a functional importance as well, since helper CD4+ T cells (L3T4) responded exclusively to antigens (Ag) presented by class II MHC, while CD8 (Lyt2) cytotoxic T cells recognized Ag in the context of class I MHC presentation (2–5). Indeed, monoclonal antibodies (mAb) specific for CD8 and CD4 blocked T-cell interactions with class I and class II MHC–Ag complexes, indicating an essential role for CD4 and CD8 as coreceptors for T-cell receptor (TCR) recognition of Ag in the context of MHC [reviewed in (6)]. Both coreceptors bind to their respective MHC molecule at sites distal from the polymorphic antigen binding domains: CD4 binds to the β_2 domain of MHC class II, while the binding site of CD8 has been mapped to the α_3 domain of MHC class I. Coreceptor involvement with TCR engagement increases the affinity of the TCR for Ag-MHC complexes, thereby enhancing the activation of T cells 100-fold or more (7).

Though CD4 and CD8 both mediate coreceptor function and are members of the immunoglobulin superfamily, their structures differ greatly from one another. CD4 is a 55–60-kDa monomeric glycoprotein consisting of 4 immunoglobulin-like domains with a flexible hinge between the second and third domains (7,8). In contrast, CD8 is expressed as a disulfide-linked homodimer of two α chains (38 kDa) or as a heterodimer of α and β chains (28–30 kDa), with each chain containing one immunoglobulin-like domain (8). The CD8 isoforms are expressed in specific contexts, with thymocytes and peripheral T cells expressing CD8$\alpha\beta$, while intraepithelial lymphocytes in gut express either CD8$\alpha\alpha$ or CD8$\alpha\beta$ (9,10). CD4 and CD8 exert their coreceptor function through their association with p56^{lck} (Lck), a SRC family tyrosine kinase that phosphorylates several intracellular substrates, thereby initiating the signaling cascade of T-cell activation. Lck itself is positively regulated by the common leukocyte antigen, CD45. Signaling through CD45 activates a tyrosine phosphatase, which then dephosphorylates a COOH-terminal tyrosine that negatively regulates Lck function. Engagement of the TCR activates Lck, which in turn phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAMS) located at the cytoplasmic tail of the CD3 chains. This allows for binding of the Syk family protein kinase ZAP 70 to the ζ chains of the TCR, thus providing protein kinase function for the TCR itself, which results in a series of second messenger cascades (11). However, activation of Lck through CD4 and CD8 is highly regulated and can be affected by the isoform of CD45 expressed by the T cell, the length of contact between Ag and TCR, and the

presence of costimulatory molecules such as CD28 (6,7,12). Fundamental differences in CD4 and CD8 structure also play a role, as signaling initiated through CD4 or CD8αβ results in a greater activation of Lck than signaling through CD8αα (6,7). In addition, CD8 association with Lck appears to play an important role in the rapid activation of effector and memory T lymphocytes (13).

Recent studies have demonstrated a role for CD4 and CD8 coreceptors in lipid raft formation. These rafts, which are rich in cholesterol and glycosphingolipids, seclude specific proteins while excluding others, and serve as platforms on the plasma membrane to facilitate signaling (14). The sequestering of Lck within lipid rafts in particular appears to regulate activation of the cell. For instance, formation of lipid rafts stabilizes the association of CD8 and Lck (15). Importantly, CD45 is excluded from rafts, and its tyrosine phosphatase activity may activate only coreceptor-associated Lck sequestered at the edge of lipid rafts (14). In fact, visualization of immune synapse formation showed that active Lck is only detected at the periphery of synapse formation (16). The role of Lck and coreceptors in the TCR mediated signaling appears to be brief, since activated Lck and CD4 are no longer visualized in the mature immune synapse (16,17).

CD8+ T Cells Have Survival Advantages Over CD4+ T Cells

In both mouse (18) and man (19), cardiac allograft rejection is characterized by the dominant presence of CD8+ cells over CD4+ cells among graft infiltrating cell (GIC) populations. This may reflect preferential expansion of donor-specific CD8+ cells in secondary lymphoid tissues (18) as well as preferential apoptosis of CD4+ cells among the GIC (19). Whether preferential recruitment and/or retention (20,21) of CD8+ cells contributes to this dominance has not been established. The CD8+ GIC enrichment may also be due to the fact that CD8+ cells have a selective survival advantage over CD4+ cells. In infectious disease models, CD8+ cells have been shown to have a greater proliferative capacity than CD4+ cells (22) and may continue to proliferate once the antigenic stimulus has been removed (23). Further, CD8+, but not CD4+ cells, may undergo 'bystander' activation in response to bacterial pathogens (24). Indeed, CD4+ cells appear to have an intrinsically lower capacity for survival in general, which is reflected by their gradual disappearance in thymectomized animals and an increased sensitivity to apoptosis relative to CD8+ cells (25). This is further emphasized by the finding that virus-specific memory CD8+ cells persist in stable numbers, whereas memory CD4+ cells decline with time (26). The persistence of memory CD8+ cells is likely due to high-level expression of the anti-apoptotic protein Bcl-2 (27). Collectively, these observations indicate that CD8+ cells are generally 'heartier' than their CD4+ counterparts.

CD4+ and CD8+ T-Cell Interactions

Following the historic association of T-cell phenotype and function (1–5), the concept that CD4+ cells provided the necessary 'help' for CD8+ CTL received support from a number of experimental systems (28–37). The nature of the help provided by CD4+ cells for CD8+ CTL expansion and development has been attributed to IL-2 production (28) and CD40 ligand (CD40L) expression (35,36) by CD4+ cells. CD40L expression by CD4+ cells is believed to activate CD40 expressing APC, thereby enhancing their stimulatory capacity for CD8+ CTL (35,36). This notion of CD4+ and CD8+ cell interactions was applied to allograft rejection, where it became widely accepted that graft-reactive CD8+ CTL served as the terminal effector cell in the rejection response, while CD4+ cells provided the signals required for CTL development and expansion [reviewed in (38)]. This paradigm was supported by studies where *in vivo* treatment with anti-CD4 mAb markedly prolonged allograft survival (18,39–42). Indeed, transient depletion of CD4+ cells in cardiac allograft recipients eliminates IL-2 producing helper cells, prevents CTL activation, and eliminates the development of intragraft inflammatory endothelia, which is required for mononuclear cell infiltration into the graft (18). However, it should be noted that CD8+ effector cells may develop independently of CD4+ help, and that this process may be influenced by the route of Ag delivery (43), the frequency of the CD8+ effector cells (44,45), and the avidity of the TCR for Ag (46). CD4-independent CD8 responses have been reported in models of contact hypersensitivity (47), autoimmune diabetes (48), tumor rejection (49), and islet xenograft rejection (50), indicating that this phenomenon is widespread.

We reported that IFNγ-deficient (IFNγ–/–) cardiac allograft recipients develop CD4-independent CD8+ effector cells that are insensitive to treatment with anti-CD40L mAb (51). This contrasts with cardiac allograft rejection in wild-type (WT) recipients, which is prevented by treatment with either anti-CD4 or anti-CD40L mAb. Treatment of WT allograft recipients with anti-CD4 or anti-CD40L mAb prevents CD8+ cell activation, yet allows these cells to be maintained in a quiescent precursor state (18,52). It is of interest that CD8+ cells represent a major source of IFNγ in WT cardiac allograft recipients (53), yet the removal of this Th1 cytokine markedly influences the behavior of CD8+ effector cells, making them much more difficult to suppress. Unlike their CD8+ counterparts, CD4+ effector cells in IFNγ–/– mice are readily suppressed by anti-CD40L therapy (51). Similar observations were made by Newell et al. (54), who identified costimulation blockade-resistant CD8+, but not CD4+ cells in an intestinal transplant model using IFNγ sufficient CD4–/– vs. CD8–/– mice as recipients. In this system, membrane lymphotoxin (LT) serves as a critical effector molecule, in that blocking membrane LT with a LT receptor fusion protein inhibits rejection (55). Hence, under certain

circumstances CD4-independent, costimulation blockade-resistant CD8⁺ cells emerge that may be less susceptible to immunosuppressive therapies than are CD4⁺ cells. Whether these cells represent a distinct or differentiated subset of CD8⁺ cells is not known; however, costimulation blockade-resistant CD8⁺ cells have been reported to express the surface marker, asialo GM1 (56). It should also be noted that the appearance of costimulatory blockade-resistant CD8⁺ cells may be influenced by the mouse strain employed as the transplant recipient. Indeed, Williams et al. (57) demonstrated that C57BL/6, but not C3H/HeJ mice develop costimulation blockade-resistant CTL and IFNγ-producing cells following skin grafting.

The idea that the CD8⁺ CTL represents 'the' terminal effector cell in allograft rejection (38) was initially called into question by several reports that documented that CD4⁺ cells could mediate rejection independently of CD8⁺ cells (58–62). The mechanism(s) by which CD4⁺ T cells mediate rejection have not been completely defined, but polarized CD4⁺ cells that secrete either IFNγ (Th1) or IL-4 (Th2) are equally effective at inducing cardiac allograft rejection (63). CD4⁺ Th1 likely mediate tissue damage through a delayed type hypersensitivity (DTH) response (64), as well as by promoting graft infiltration and up-regulating the graft's MHC for immune recognition by graft reactive T cells (65). However, the mechanisms by which CD4⁺ Th2 mediate rejection are less clear. We have reported that depletion of CD8⁺ cells induces Th2 cytokine production by CD4⁺ cells within cardiac allografts, which is associated with the accumulation of eosinophils in the transplant (59). Eosinophils and Th2 cytokines are not readily detectable in unmodified cardiac allograft rejection, where CD8⁺ cells and Th1 cytokines dominate the response (18,53,59). This observation was further explored by Braun et al. (66), who reported that IFNγ production by CD8⁺ cells inhibited IL-5 production by CD4⁺ cells, which was responsible for the eosinophilia within rejecting cardiac transplants. Hence, CD8⁺ cells may negatively regulate cytokine production by CD4⁺ cells. CD8⁺ cells that have been polarized to produce Th2 cytokines also mediate cardiac allograft rejection, which is characterized by an eosinophil influx (67). Further, the CD4-independent, anti-CD40L-resistant CD8⁺ cells that mediate cardiac allograft rejection in IFNγ –/– mice recruit numerous eosinophils and neutrophils into the graft (51). However, eosinophils are not necessary for rejection in the IFNγ –/– mouse, since neutralizing IL-4 abrogates eosinophil accumulation but does not prevent rejection (51). Mechanisms by which eosinophils may contribute to acute allograft rejection have been recently reviewed (68), and eosinophils have been implicated in chronic skin allograft rejection as well (69). Hence, it appears that Th2 cytokine production by either CD4⁺ or CD8⁺ cells results in 'nontraditional' mechanisms of graft rejection, thereby detracting from the once popular idea that Th2 may be beneficial in the context of transplantation (70).

Collectively, these observations raise important points regarding CD4⁺/CD8⁺ T-cell interactions in transplantation: First, transplant immunologists are accustomed to the processes by which CD4⁺ cells regulate CD8⁺ T-cell behavior and the rejection response [reviewed in (71)]. However, we are just beginning to understand CD8⁺ T-cell regulation of CD4⁺ cell behavior and how this may influence the composition of GIC. Second, under certain conditions, CD8⁺ T cells have a mind of their own and often choose not to play by what we view as the immunologic rules.

Cytokine Regulation of CD4⁺ and CD8⁺ T Cells

Since the initial description of mouse CD4⁺ Th1 and Th2 clones (72), it has been well established that polarized IFNγ-producing Th1 and IL-4-producing Th2 may be induced from heterogeneous populations of cells in both mouse and man [reviewed in (73–75)]. While this was originally found with CD4⁺ cells, it became apparent that CD8⁺ T cells could also assume these polarized phenotypes (76). Several factors are involved in Th1 vs. Th2 differentiation, and the local cytokine milieu markedly influences which phenotype a T cell will adopt: IL-12 and IFNγ favor Th1 and IL4 favors Th2 development (74,75). The down-stream regulators or 'master switches' for Th1 and Th2 development are the transcription factors T-bet and GATA-3, respectively [reviewed in (75)]. GATA-3 is strongly associated with Th2 differentiation, IL-4 production and Stat 6 activation, and is not expressed in Th1 cells (77–79). T-bet is expressed in Th1, but not Th2, and leads to strong transactivation of the IFNγ gene (80). Indeed, transduction of T-bet into polarized Th2 converts these cells into IFNγ-producing Th1 and represses IL-4 and IL-5 production (80).

We have reported that alloreactive CD8⁺ cells do not require biologically active IL-12p70 to differentiate into IFNγ-producing Th1 (53), suggesting that the Th1 phenotype represents the default pathway for CD8⁺ cells. Indeed, CD8⁺ cells do not require signaling through Stat 4 for IFNγ production when stimulated through the TCR, whereas CD4⁺ cells do (81). Several factors may be involved in the predisposition of CD8⁺ cells to acquire the Th1 phenotype. For example, the IFNγ promoter has been shown to remain demethylated for prolonged periods of time in CD8⁺ cells, even in the absence of repeated TCR stimulation, favoring transcription of the IFNγ gene (82). Further, the IL-18 receptor (IL-18R) has been reported to be expressed at higher levels on CD8⁺ cells than on CD4⁺ cells (83). Since IL-18 shares Th1-inducing activity with IL-12 [reviewed in (84)], preferential expression of IL-18R by CD8⁺ cells over CD4⁺ cells may explain the differential responsiveness of these T-cell subsets to this cytokine (85). Specifically, adding IL-18, but not IL-12 to primary mixed lymphocyte cultures (MLC) results in

preferential expansion of CD8$^+$ cells that produce 20- to 30-fold more IFNγ upon secondary stimulation (85). Finally, while the p40 subunit of IL-12 is antagonistic for biologically active IL-12p70 on cells that have been stimulated with mitogens or exogenous Ag (86), several reports indicate that IL-12p40 may be stimulatory (53,87–89). Indeed, we have found that alloreactive CD8$^+$ cells respond to IL-12p40 with increased IFNγ production both *in vitro* (87) and *in vivo* (53). Using p35 –/– and p40 –/– mice as cardiac allograft recipients, we found that IL-12p40 may substitute for IL-12p70 in promoting IFNγ-producing CD8$^+$ cells (53). While not yet tested, it is interesting to speculate that these *in vivo* effects of IL-12p40 may result from the ability of p40 to complex with p19, yielding the composite cytokine IL-23 (90). Similarly to IL-12, IL-23 stimulates IFNγ production. If IL-23 mediates the stimulatory effects of p40 on IFNγ production *in vivo*, our observations (53) would predict that IL-23 has preferential activity on alloreactive CD8$^+$ T cells over CD4$^+$ cells.

Glimcher's group recently reported that the Th1-inducing transcription factor T-bet is required for IFNγ production by CD4$^+$ and NK cells, but not by CD8$^+$ cells (91). This very interesting observation sheds further light on why CD8$^+$ cells acquire such a recalcitrant Th1 phenotype that is not dependent on IL-12p70 (53) or Stat 4 activation (81). Further piecing the puzzle together is a recent report from Flavell's group (92), which demonstrates that the Th1-inhibiting activity of TGFβ (93) is likely due to the ability of TGFβ to inhibit T-bet expression. Since CD4$^+$, but not CD8$^+$ cells are dependent on T-bet for IFNγ production, it now makes biologic sense that CD4$^+$ and CD8$^+$ cells exhibit differential sensitivity to TGFβ. Lotz et al. (94) reported that human CD4$^+$ clones are more sensitive than their CD8$^+$ counterparts to the antiproliferative effects of TGFβ. Further, we reported (95) that cardiac allograft rejection by CD4$^+$ cells is prevented by TGFβ gene transfer, whereas CD8$^+$ cells are resistant to this therapy. Interestingly, the protective effects of TGFβ gene therapy are associated with muted Th1 responses, and the protective effects on graft survival can be overridden by recipient treatment with exogenous IL-12 (95).

Finally, it appears that CD8$^+$ cells are more dependent on IL-15 as a growth and maintenance factor than their CD4$^+$ counterparts [reviewed in (96)]. IL-15 is structurally related to IL-2 and signals through the IL-2R β and γ chains complexed with an IL-15 specific α chain [reviewed in (97)]. While IL-15 shares the T-cell growth factor (TCGF) activity of IL-2, IL-15 is biologically distinct from IL-2 in several ways (96,97). Unlike IL-2, IL-15 is produced by a variety of cells types, but not by activated T cells. In addition, IL-15, rather than IL-2, is required for the generation of primary CD8$^+$ effector cells during viral infections and the maintenance of CD8, but not CD4$^+$ memory cells (98–101). Unlike IL-2, IL-15 plays a role in homeostatic lymphocyte recirculation (102) and may promote the survival of activated lymphocytes, as opposed to promoting activation-induced cell death (AICD) (103). In the context of transplantation, IL-15, rather than IL-2, is the TCGF most frequently associated with rejection when human renal biopsies are assessed for these cytokine transcripts (104). Further, an antagonistic IL-15 fusion protein prevents costimulation blockade-resistant rejection of allogeneic islets by CD8$^+$ cells (105), and an antagonistic soluble fragment of the IL-15Rα chain markedly prolongs survival of minor Ag mismatched cardiac allografts (106).

In summary, the cytokine requirements for the growth, maintenance, and function of CD4$^+$ and CD8$^+$ T cells are quite dissimilar. Given this, it comes as no surprise that selective cytokine manipulation aimed at preventing allograft rejection by CD4$^+$ and CD8$^+$ T cells has met with limited success. Indeed, manipulating cytokines combined with the depletion of either CD4$^+$ (106) or CD8$^+$ (95) T cells has proven necessary in experimental cardiac transplantation.

Costimulatory Requirements for CD4$^+$ and CD8$^+$ T Cells

The importance of T-cell costimulation in allograft rejection has been studied extensively [reviewed in (107–111)]. Hence, we will briefly highlight differences in costimulatory requirements for CD4$^+$ and CD8$^+$ cells here. While costimulation blockade resistance is a recurring phenomenon for alloreactive CD8$^+$ cells, this does not appear to be the case for CD4$^+$ cells (51,54–56,112). These studies have examined the relative resistance of CD8$^+$ cells to blockade of the CD28/B7 and/or the CD40/CD40L pathways, and similar observations have been made in TCR transgenic systems (113), in models of bacterial (114,115) and viral (116,117) infection, and in TNFα-mediated diabetes (118). Yet conflicting reports exist, which demonstrate a strict dependency on costimulation in CD8$^+$ effector cell development (35,36,119–121). The explanation for costimulation dependence or independence of CD8$^+$ cells may lie in the strength and persistence of the stimulating Ag. Indeed, Andreasen et al. (122) compared the costimulation dependency of CD8$^+$ cells during infection with lymphocytic choriomeningitis virus (LCMV), which replicates widely and extensively, and vesicular stomatitis virus (VSV), which spreads poorly in mice. This study demonstrated that the primary CD8$^+$ effector cell response to LCMV did not require CD40L or CD28, whereas the CD8 (and CD4) response to VSV was markedly impaired.

While the CD28/B7 and CD40/CD40L pathways have received the most attention in transplantation, other costimulatory molecules may contribute to effector cell development and graft rejection [reviewed in (123)]. Of these, 4–1BB (CD137) and 4–1BBL have been implicated in the development of CD8$^+$ T cells [reviewed in (124)]. 4–1BB and 4–1BBL are members of the TNFR and TNF superfamilies, respectively. 4–1BB is primarily expressed on activated T cells and 4–1BBL is expressed on mature

dendritic cells (DC), activated B cells, and activated macrophages (123,124). Since 4–1BB and 4–1BBL expression on resting cells is low or absent, it is believed that the 4–1BB/4–1BBL pathway plays a minor role in early activation events *in vivo*. Indeed, stimulatory, agonistic mAb to 4–1BB have a greater effect on activated T cells than on resting T cells (125), indicating that 4–1BB may play a role in costimulation of T cells once CD28 has been down regulated (126). Shuford et al. (127) reported that costimulation though 4–1BB stimulates CD8⁺ cells to a greater extent than CD4⁺ cells, while the converse holds true for CD28 costimulation. Further, *in vivo* treatment with stimulatory anti-4–1BB mAb amplifies H-2ᵈ-specific CTL responses in a graft vs. host disease (GVHD) model, and accelerates cardiac and skin allograft rejection (127). Subsequent reports documented that 4–1BB ligation favors the survival of CD8⁺ over CD4⁺ cells following superantigen stimulation (128), and that 4–1BBL –/– mice mount poor CD8⁺ but normal CD4⁺ T-cell responses to LCMV infection (129,130). While both CD4⁺ and CD8⁺ cells express 4–1BB following allogeneic stimulation in MLC, 4–1BB ligation augments proliferation and IFNγ production by CD8⁺ cells to a greater extent than CD4⁺ cells (131). Collectively, these studies suggest that 4–1BB is involved in costimulation of CD8⁺ cells and plays only a minor role in CD4⁺ cell activation. However, contrasting reports indicate that 4–1BB ligation serves to costimulate both CD4⁺ and CD8⁺ T cells (132–134), inducing IL-4 production from CD4⁺ cells and IFNγ production from CD8⁺ cells (133). Hence, differential utilization of the 4–1BB/4–1BBL costimulatory pathway by CD4⁺ vs. CD8⁺ cells is controversial, and the involvement of this pathway in transplant rejection remains to be resolved.

Concluding Remarks

In summary, it appears that CD4⁺ and CD8⁺ T cells have more dissimilarities than similarities. These differences are summarized in Table 1, along with relevant references that support conflicting results. Hence, it seems that the initial reports by Cantor and Boyse (1,2) that T-cell phenotype correlates with function were correct. However, this association between phenotype and function is much more complex than we had originally envisioned. Rather than simply defining cells with lytic (CD8⁺) or helper (CD4⁺) function, it is now apparent that these T-cell subsets have differential costimulatory and cytokine requirements for their maturation into effector cells.

Acknowledgments

Table 1: Summary of differences in CD4⁺ and CD8⁺ T-cell biology. Conflicting observations are noted by italics

	CD4⁺ T cells	CD8⁺ T cells	Selected References
Cytokine requirements			
IL-12p70 required for Th1 polarization	Yes	No	(53)
Signaling through Stat-4 required	Yes	No	(81)
IL-12p40 responsive	No	Yes	(53,87–89)
	Yes		*(88)*
IL-18R expressed	Low	High	(83)
T-bet required for IFNγ production	Yes	No	(91)
TGFβ suppression	Yes	No	(94,95)
Require IL-15 for growth	No	Yes	(96–102)
Costimulatory requirements			
CD28/B7 dependent	Yes	No	(54,56,112–114,116)
		Yes	*(122)*
CD40/CD40L dependent	Yes	No	(51,56,115,117,118)
		Yes	*(35,36,119–122)*
4–1BB/4–1BBL responsive	No	Yes	(127–131)
	Yes		*(132–134)*
Roles in transplant rejection			
Mediate CTL function	No	Yes	(18,38,58,61)
Regulate CTL development	Yes	No	(18,38)
Regulate CTL entry into the graft	Yes	No	(18)
DTH response	Yes	Yes	(38,61,63)
Promote eosinophil infiltration	Yes (Th2)	No	(59)
		Yes, if Th2	*(51,67)*

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