Signal transduction in T lymphocytes

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ABSTRACT

T lymphocytes utilize a variety of surface receptors to transmit environmental signals across the plasma membrane and initiate biochemical events leading to responses such as proliferation, anergy, cytokine secretion, and death. The T cell receptor complex, CD28, IL-2 receptor, and CD95 each couple to distinct sets of cytoplasmic signaling events to modulate the biological responses of T cells. Deficiency or defective function of proteins involved in signaling through these receptors are associated with murine and human disease.

Introduction

The recent revolution in molecular biology has produced significant advances in our understanding of the biochemical basis for the responsiveness of numerous vertebrate tissues, including immune cells. The T lymphocyte serves as a prime example of how techniques of isolation and functional characterization of individual cellular proteins have led to improved knowledge of the mechanisms behind such diverse responses as activation, cytokine secretion, proliferation, induction of anergy, and programmed cell death. Remarkable progress has also been made in the understanding of how specific interactions of external stimuli (cytokines, cell surface proteins, or antigens) with T cell surface receptor complexes cause biochemical alterations which can modulate processes such as gene transcription and cell cycle progression.

Here we present an overview of current thinking about the structure and function of several types of T cell surface receptors: the T cell antigen receptor/CD3 complex, and examples of co-stimulatory molocules, cytokine receptors, and "death" receptors. Receptor links with cytoplasmic signaling machinery and examples of disrupted signaling resulting in disease will also be considered.

The T cell receptor

The T cell antigen receptor (TCR) is a multimeric protein complex which plays a critical role in the activation of numerous cellular responses ranging from proliferation to death. It is distinguished from other surface receptors on T cells by its vast clonal diversity, a property which permits specific responses to a universe of foreign peptide antigens. Functionally, the TCR is composed of both antigen-recognition and signaling subunits (1). TCR α and β (or in a small minority of T cells, γ and δ) proteins comprise the antigen recognition module. Members of the immunoglobulin superfamily of proteins, TCR α and β chains contain extracellular domains which confer the ability to recognize peptide antigen bound to self Major Histocompatibility Complex (MHC) molecules. The α and β chain cytoplasmic domains are short and devoid of known function.

TCR-mediated signaling across the plasma membrane therefore depends upon the proteins of the associated CD3 complex (CD3-ε, -γ, -δ and -ξ) (2). The cytoplasmic domains of these invariant CD3 proteins possess one or more short sequences of central importance for TCR signaling called immune receptor tyrosine-based activation motifs (ITAMs) (3) [reviewed in (4)].

Activation of several families of protein tyrosine kinases (PTKs) is the earliest detectable biochemical event following TCR ligation (5). With PTK activation, tyrosine residues within the CD3 chain ITAMs become phosphorylated upon TCR engagement (6) (Fig. 1). Lck, a membrane-associated PTK of the src family, constitutively associates with the co-receptor molecules CD4 and CD8 (7) and is required for antigen-induced CD3-ITAM phosphorylation (8, 9). The structurally-related PTK, fyn, also associates with the TCR complex (10), and appears critical for the optimal TCR-mediated proliferation of thymocytes (11). Phos-

Table I. Disease associations with deficient T cell signaling molecules.

Signaling event affected	Deficient protein	Disease features
TCR signaling		
Coupling of TCR to PTK activation	ZAP-70	Severe immune deficiency; impaired T cell development in mice and humans.
Down-regulation of PTK activity	SHP-1	"Moth-eaten" mice show autoimmunity and inflammatory lung disease.
IL-2R signaling		
Coupling IL-2 binding to JAK activation	Common gamma chain	Severe immune deficiency, impaired lymphocyte development in mice and humans
Phosphorylation of STAT proteins	JAK3	Severe immune deficiency in humans.
CD95 signaling		
Membrane recruitment and activation of protease activity	CD95	lpr and gld mice and patients with ALPS show glomerulonephritis, anti-DNA antibodies, hemolytic anemia.

pho-tyrosine residues within ITAMs display high binding affinity for specialized regions termed SH2 (src homology-2) domains (12, 13). Cytoplasmic proteins containing SH2 domains are inducibly recruited into complexes with ITAM-containing molecules. For example, following TCR ligation, ZAP-70 (Zeta-Associated Phosphoprotein of 70 kilodaltons), a cytoplasmic PTK containing two SH2 domains, associates with the phosphorylated ITAMs of the CD3-ξ or -ε chains at the plasma membrane (14, 15).

Several lines of evidence suggest that ZAP-70 and the src family PTKs are necessary for effective TCR signaling. In mice made deficient in these molecules by homologous recombination ("knock-outs"), T cell development is impaired, and thymocytes or T cells display marked abnormalities in signaling after TCR ligation (16, 17). ZAP-70- and lck-deficient transformed cell lines also show severe blocks in TCR-mediated signaling (8, 18). Finally, a rare human immunodeficiency state has been linked to the functional absence of ZAP-70 (Table I). These patients lack CD8+ cells and show profound CD4+ cell unresponsiveness, again suggesting that ZAP-70 is required both for development of T cell subsets and for mature T cell signal transduction (19-21).

Phosphatases also regulate TCR-induced PTK activity. CD45 is an abundant transmembrane protein containing protein tyrosine phosphatase (PTP) activity in its cytoplasmic region (22). Given the above observations that early PTK activity correlates with T cell activation, some investigators predicted that PTPs, like CD45, would inhibit TCR signaling. Surprisingly, studies in T cell clones, tumor lines, and CD45 deficient mice revealed the opposite: TCR signaling is abolished in the absence of CD45 (23-25). An explanation for this paradox is suggested by work showing that CD45 can dephosphorylate a negative regulatory tyrosine residue on src family kinases such as lck, thereby maintaining the kinases in a state poised for activation (26-28) (Fig. 1). In contrast to CD45, the cytoplasmic PTP SHP-1 behaves as a negative regulator of TCR signaling (29-31). SHP-1 deficiency causes lymphoproliferative and autoimmune disease in an inbred mouse strain known as "moth-eaten" (32-34). Recent work suggests that SHP- 1 associates with and inhibits the activity of ZAP-70 after TCR ligation, supplying a possible mechanistic link between SHP-1 deficiency and autoimmunity (29).

In addition to CD3 ITAMs, TCR-stimulated PTKs phosphorylate a number of cytoplasmic proteins. Among the TCR-dependent PTK substrates implicated in signal transduction are the Linker protein of Activated T cells (LAT) (35), SH2-containing Leukocyte Protein of 76 kD (SLP-76) (36), and Cbl (37). LAT appears important for coupling TCR stimulation to activation of phospholipase C-γl(PLC-γ1) (35). SLP-76 is absolutely required for thymocyte development;

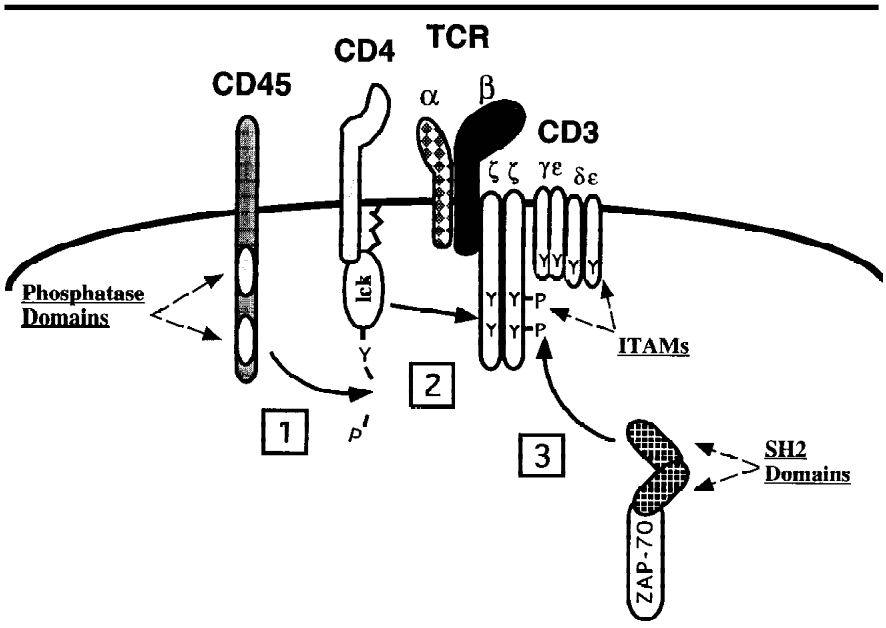

Fig. 1. Membrane-proximal events in TCR signaling. The tyrosine phosphatase CD25 dephosphorylates the negative regulatory tyrosine residue on the co-receptor-associcated PTK lck, maintaining lck in the active form (1). Engagment of the TCR α and β brings activated lck into proximity with ITAM-bearing CD3 chains. Lck phosphorylates the ξ chain (2). The phosphorylated ξ chain interacts with the tandem SH2 domains of the cytoplasmic PTK ZAP-70 (3), allowing activation of ZAP-70 and the phosphorylation of downstream substrates.

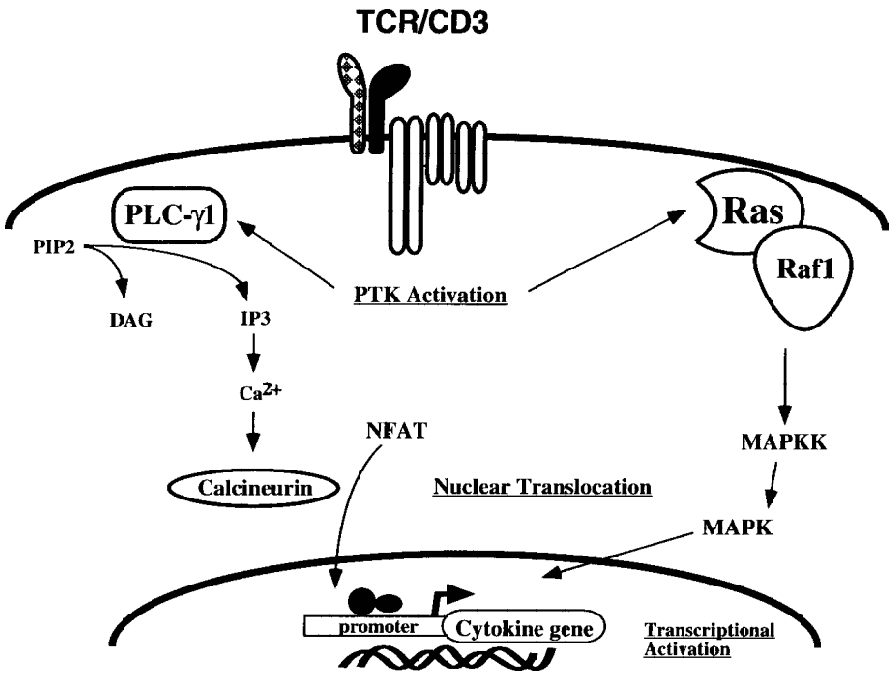

Fig. 2. Signaling pathways activated by TCR engagement. TCR ligation results in activation of PTKs such as ZAP-70 (see Fig. 1). The lipid modulator PLC-γ1 becomes phosphorylated and activated by membrane-proximal PTKs. Hydrolysis of phosphatidyl-inositol bis-phosphate (PIP2) by PLC-γl releases diacylglycerol (DAG) and inositol tris-phosphate (IP3). IP3 stimulates an increase in the intracellular calcium concentration, which activates the phosphatase calcineurin. Calcineurin dephosphorylates NFAT, thereby signaling NFAT translocation to the nucleus.
TCR-stimulated PTK activity also enhances Ras activity. Active Ras binds and stimulates the kinase Raf1, which phosphorylates and activates a cascade of serine/threonine kinases. Upon phosphorylation and activation, the most membrane-distal component in the cascade, MAPK, translocates to the nucleus. Transcription factors activated by MAPK cooperate with NFAT proteins to upregulate the transcription of IL-2 and other cytokine genes.

SLP-76-deficient mice have no peripheral T cells due to a block in thymocyte ontogeny at an early stage (38, 38b). Moreover, a SLP-76-deficient variant of a human leukemic cell line displays a profound block in TCR-induced calcium signaling (39). Cbl likely plays an inhibitory role in TCR signaling (40). Recent work suggests that Cbl recruits a protein complex implicated in the induction of T cell anergy to the plasma membrane (41).

Downstream of PTK activation, TCR stimulation results in the activation of several well-defined signaling cascades which ultimately modulate gene transcription and cellular responses (Fig. 2). PLC-γl, a prominent substrate of the TCR-stimulated PTKs (42-44), is an early component in one such signaling cascade. When activated, PLC-γ1 hydrolyzes phosphatidyl inositol 4,5-bisphosphate (PIP2). The "second messengers" diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) are products of PIP2 hydrolysis. IP3 stimulates a rapid increase in intracellular free calcium, and DAG causes protein kinase C activation, both of which lead to transcriptional activation and cellular responses. The cytoplasmic serine phosphatase calcineurin is activated by the intracellular calcium increase (45). Calcineurin acts to enhance the translocation of members of the nuclear factor of activated T cells (NFAT) family of transcription factors (46). Optimal transactivation of the IL-2 gene following TCR stimulation requires NFAT binding to response elements in the IL-2 promoter (47). The dependency of IL-2 upregulation upon calcineurin function has been exploited clinically in organ transplantation. Current immunosuppressive regimens often include FK506 or cyclosporin, two drugs which selectively inhibit calcineurin phosphatase activity (48).

In addition to the phosphotidylinositol-derived "second messengers", TCR ligation initiates signaling events along a second major pathway - the Ras/Mitogen Activated Protein Kinase (MAPK) cascade. Ras is a membrane-associated protein which inducibly associates with and activates Raf-1, a serine/threonine kinase (49). Raf-1 in turn phosphorylates and activates a series of kinases, culminating with the activation of MAPK (50, 51). MAPK translocates to the nucleus upon phosphorylation, and there activates transcription factors implicated in IL-2 and other cytokine gene expression (52).

CD28 mediated co-stimulation

Optimal T cell activation requires a "second signal" in addition to TCR engagement. Receipt of an isolated TCR stimulus by a resting T cell induces long-term unresponsiveness to antigen known as anergy (53). CD28 serves to block anergy induction and mediate the co-stimulation of T cells (54). CD28 is constitutively expressed as a homodimer on the majority of T cells. Its physiologic ligands include CD80 and CD86 (also known as B7-1 and B7-2) which are expressed on B cells and other antigen presenting cells (55, 56). In addition to preventing anergy in TCR-activated cells, CD28 mediates a number of effects, including cooperation with TCR signals to induce IL-2 gene transcription (57, 58), the prevention of apoptosis (59), and the promotion of Th2 responses (60).

CD28 may couple to a number of signaling pathways to mediate these effects. Like the TCR, CD28 possesses no intrinsic enzymatic activity, but a tyrosine-based motif within its cytoplasmic tail becomes phosphorylated upon CD28 ligation (61). Inducible phospho-tyrosine-mediated associations of CD28 with the adapter protein Grb2 (62), the PTK Itk (63), and PI3-kinase (61) have been reported. In a T cell hybridoma transfected with mutant CD28 molecules, the inability to bind Grb2 correlates with marked impairment in the ability of CD28 to co-stimulate IL-2 production (64). CD28 may require src family PTK (e.g. fyn) to induce thymocyte proliferation, but not to induce cytokine gene transcription (65).

CD28 has also been implicated in the activation of the spingomyelinase/Jun kinase (JNK) signaling cascade. Mice deficient in SEK1, a kinase immediately upstream of JNK, exhibit impaired IL-2

production and proliferation mediated by CD28 ligation. This suggests that CD28 may utilize JNK activation for optimal co-stimulation of cytokine upregulation (66).

CD28 may modulate cytokine gene transcription through the removal of inhibitory influences on TCR-stimulated signaling cascades. TCR-mediated activation of Rap 1, a Ras family member with GTPase function, has been correlated with inhibition of the MAPK cascade and with the induction of anergy in T cells (41). TCR stimulation of T lymphocyte blasts causes robust Rap1 activation. However, combining anti-CD28 stimulus with TCR ligation completely abrogates the Rap1 activation, while simultaneously enhancing TCR-mediated MAPK activity (67). These data suggest a model for Rap1 acting as a molocular switch capable of potentiating or inhibiting activating signals emanating from the TCR. CD28 regulation of Rap1 activity may determine whether a TCR stimulus will lead to anergy or to productive stimulation.

CD28 also augments T cell proliferation, through both the upregulation of growth factors and the induction of anti-apoptotic proteins. Early work suggested that CD28 ligation causes stabilization of IL-2 messenger RNA (68). Recent work revealed that CD28 co-stimulation renders cells much less susceptible to CD95-mediated apoptosis (see below). This effect correlates with the upregulation of Bcl-XL, an anti-apoptotic protein (59).

The CD28 cognate ligands CD80 and CD86 also bind with high affinity to CTLA-4 (69), a T cell surface protein homologous to CD28 which acts to down-regulate proliferation (70). The cross-reactivity of CTLA-4 with CD28 ligands has been exploited therapeutically using a fusion protein containing the extracellular domain of CTLA-4. Blockade of CD28 function with a CTLA-4 fusion protein prolongs the acceptance of pancreatic xenografts (71) and ameliorates the disease activity in lupus-prone NZB mice (72). T cell inhibitory signaling via CTLA-4 may proceed through recruitment of the tyrosine phosphatase SHP-2 to the receptor (73).

IL-2 receptor

The capacity to become activated and proliferate in response to IL-2 is a defining feature of T lymphocytes. The effects of IL-2 upon T lymphocytes are mediated by at least three surface-expressed gene products: the α, β, and γ subunits of the IL-2 receptor (IL-2R). The β and γ subunits can each bind IL-2 when expressed alone, but the IL-2R with the highest affinity for IL-2 contains all three proteins (74).

Like the TCR, IL-2R complexes contain no intrinsic enzymatic activity. In contrast to the TCR α and β chains, however, the IL-2R-β and -γ chains each contain both ligand recognition and signal transduction domains. The large cytoplasmic region of IL-2R-β confers the ability to associate with a number of enzymes: the src family PTKs lck (75) and fyn (76), the active subunit of the lipid modulator PI-3 kinase (77), and the Janus family kinases JAK1 and JAK3 (78). The adaptor protein Shc, which has been implicated in the activation of the Ras/MAPK cascade in other receptor signaling systems, also binds IL-2R-β (79). The IL-2R-γ chain, also known as the "common γ chain" because it participates in receptor complexes for at least four other cytokines beside IL-2, is structurally related to IL-2R-β and constitutively associates with JAK3 (80).

Heterodimerization of the IL-2R-γ and IL-2R-β chains occurs upon IL-2 binding, and several signaling pathways are activated. First, the IL-2R-associated JAK3 becomes activated (81, 82), an event critical for the rapid tyrosine phosphorylation of several members of a family of transcription factors known as STATs (Signal Transducers and Activators of Transcription) (Fig. 3). IL-2 induced phosphorylation permits homo- or heterodimerization of STAT3 and STAT5. Heterodimerized STAT proteins translocate to the nucleus and bind consensus response elements within the promoters of a number of genes involved in cell cycle progression and differentiation (83). A recent study also suggested that PI-3 kinase-dependent activation of the Ras/MAPK cascade occurs after IL-2R

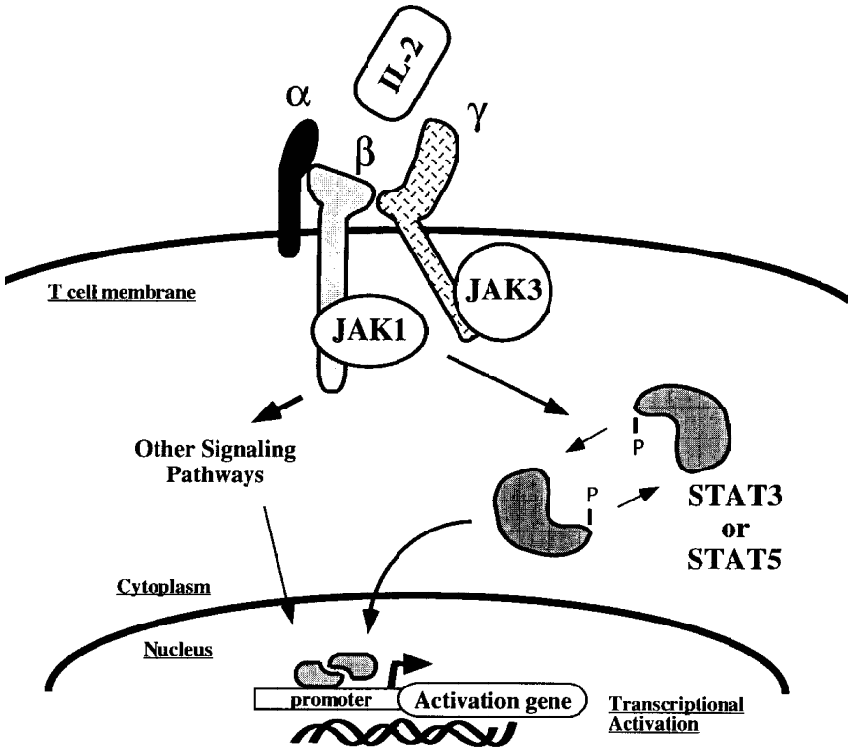

Fig. 3. Prominent intermediates in IL-2 signaling. IL-2 binding to the T cell results in oligomerization of combinations of α, β, and γ surface receptor components. The β- and γ-associated PTKs JAK1 and JAK3 phosphorylate and activate each other. The JAKs phosphorylate STAT family members, which then dimerize, translocate to the nucleus, and bind response elements in the promoters of target activation genes. Growing evidence suggests that additional signaling pathways triggered by the activation of enzymes such as Phosphatidyl-inositol-3 kinase and lck also contribute to IL-2 effects on gene transcription.

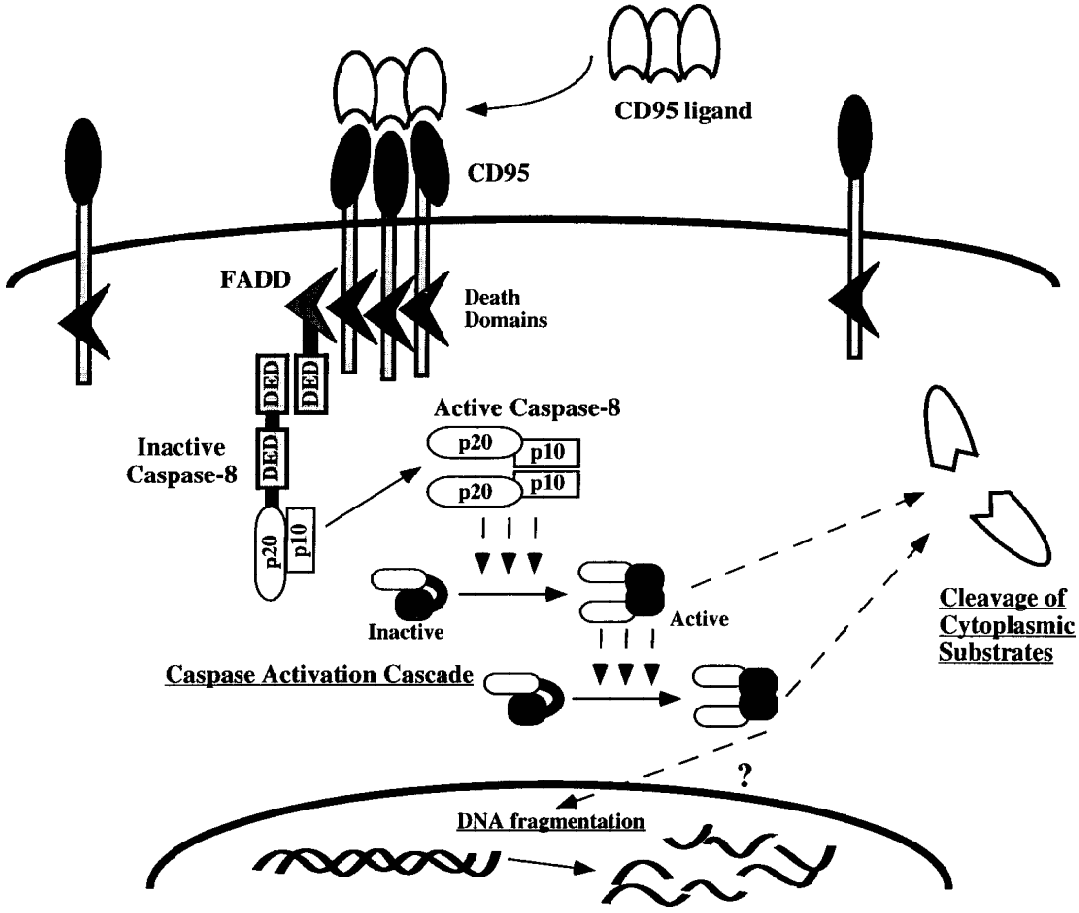

Fig. 4. CD95 signals result in cysteine protease activation and apoptosis. Trimerized CD95 ligand induces oligomerization of CD95 at the T cell surface. Cytoplasmic Death Domains (DD) borne by CD95 proteins aggregate and stimulate the homotypic association of a DD within FADD. FADD also contains a Death Effector Domain (DED) which mediates the recruitment of another DED-containing protein, Caspase-8. Upon relocalization to the CD95-FADD complex, Caspase-8 undergoes auto-catalysis, cleaving the protease-containing p20/p10 regions away from the DEDs. Enzymatically-active Caspase-8 tetramers derived from the p20 and p10 regions can then activate additional Caspase family members. Numerous cytoplasmic proteins serve as Caspase substrates in a prologue to apoptosis. Caspases may also play a role in the activation of endonucleases which cleave DNA.

engagement (77).

Murine and human disease states have been attributed to defects in several IL-2R signaling molecules (Table I). Deficiency of functional common γ chain causes human X-linked severe combined immunodeficiency (XSCID), characterized by the complete absence of T cells and defective humoral and cellular immune responses (84). γ chain defects account for an estimated 50% of primary human SCID. However, the loss of γ chain participation in IL-2 signaling is likely not responsible for absent T cell development in XSCID, as other patients deficient in IL-2 itself develop normal T cell populations (85). Interleukin-7 and its receptor are unique among the cytokine receptor complexes containing the common γ chain in that their function is absolutely required for T cell development (86). Thus, γ chain defects likely block T cell development due to depressed IL-7 receptor function (87). Another subgroup of SCID patients exhibit absent or defective JAK3, the primary kinase associated with the common γ chain (88).

Paradoxically, mice lacking the IL-2R-α (89) or -β (90) chains or IL-2 (91) display autoimmunity. For example, IL-2R-β knockout mice display inappropriate T cell activation, massive infiltration of tissues by myeloid cells, and early death due to autoimmune hemolytic anemia (90). One model proposed to explain these findings holds that IL-2R signaling results not only in the well-characterized endpoint of cellular proliferation, but simultaneously activates the genes responsible for programmed cell death (74).

CD95 signaling

Prevention of host tissue damage caused by activated, proliferating T lymphocytes responding to antigen challenge requires that such cells be rapidly removed after the antigenic stimulus has been eradicated. In the past decade, major advances in our knowledge of the molecular underpinnings of cellular disposal processes have occurred. One major route whereby activated peripheral T cells may be deleted is through the specific ligation of one of a family of "death receptors" on the cell surface and the induction of programmed cell death, or apoptosis (92).

At least five surface receptors capable of engaging a cytoplasmic death-inducing signaling machine have been cloned from human cells. All are members of the tumor necrosis factor receptor (TNFR) family, and share homologous cytoplasmic domains responsble for coupling to downstream signaling events (92). CD95 (fas/APO-1) is one such TNFR family member which has been implicated in both murine and human

autoimmune disease and in activation-induced cell death (AICD) (93).

The cognate ligand for CD95, CD95L exists as a trimer, and probably induces CD95 oligomerization upon binding (94) (Fig. 4). A 70-amino acid region of the CD95 cytoplasmic domain is required for apoptotic signaling and is thus termed the "death domain" (95-97). The CD95 death domain mediates association with an homologous region within the cytoplasmic protein FADD (Fas-associated death domain-containing protein) (98). FADD contains an additional region termed the "death effector domain" (DED) which mediates inducible association between FADD and Caspase-8 (FLICE) (99, 100). Caspase-8 belongs to a superfamily of cytoplasmic cysteine proteases which cleave other proteins after aspartate residues (101). FADD association likely induces the homo-oligomerization of Caspase-8, leading to autocatalysis and conversion to the active form of the protease (102). Active Caspase-8 can in turn cleave and activate additional caspases, each capable of cleaving numerous cellular substrates (103). The resulting widespread cleavage of cytoplasmic proteins by caspases represents a prologue to apoptotic cell death.

CD95 signaling defects cause autoimmune disease (Table I). The MRL *lpr/lpr* and *gld* strains of inbred mice have long served as models for human systemic lupus erythematosus (SLE). Recently, the autoimmune syndromes of massive lymphadenopathy, splenomegaly, glomerulonephritis, and production of anti-DNA antibodies observed in these mice were explained by inactivating mutations in CD95 (lpr) or CD95 ligand (gld) (104, 105). Patients suffering from Autoimmune Lympho-Proliferative Syndrome (also known as Canale-Smith disease) exhibit clinical features similar to those seen in mice; they also bear inactivating mutations in the CD95 gene (106, 107). CD95/CD9SL function has been studied in human SLE. One SLE patient with a mutation in the CD95 ligand gene has been reported (108); however, the vast majority of SLE patients have intact CD95/CD95 ligand function (109).

Recent work has implicated CD95 as a critical mediator of the phenomenon of "immune privilege" (110, 111). Immune privileged sites such as the testes and the posterior chamber of the eye are notable for the absence of inflammatory cellular infiltrates after infection or trauma. The eyes of *lpr/lpr* mice, however, appear to have lost the ability to inhibit infiltration of activated T lymphocytes after viral challenge. Interestingly, *lpr* mice which have been lethally irradiated and reconstituted with immune progenitors from syngeneic, but non-*lpr* mice show reconstitution of ophthalmic immune privilege (110). This finding suggested a model for CD95 mediation of immune privilege: in the normal, immune-privileged eye, CD95 ligand is expressed constitutively. When CD95-expressing, activated T lymphocytes enter the eye they undergo apoptosis.

Conclusion

Optimal antigen-specific vertebrate immune responses require tightly regalated T cell function. T cells depend upon receptors such as TCR, CD28, IL-2R, and CD95 to transduce environmental signals across the plasma membrane. The integration of signals emanating from these and other receptors further depends upon a complex network of cytoplasmic enzymes, adaptor proteins, and transcription factors. These signaling molecules ensure that the T cell can respond with a biologic outcome - proliferation, activation, cytokine secretion, or apoptosis - appropriate to the environmental stimulus. Functional defects in components of surface-receptor coupled signaling cascades may cause either immune deficiency or autoimmunity. Pharmacologic targeting of receptor-coupled signaling events has allowed the recent development of increasingly specific and effective therapies for autoimmune conditions and for transplant rejection. Further basic investigations into the mechanisms of T cell signal transduction will probably contribute more to the understanding of T cell biology and ultimately, improved diagnosis and treatment of T cell-related disease.

Acknowledgements

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References

1. WEISS A, LITTMAN DR: Signal transduction by lymphocyte antigen receptors. *Cell* 1994; 76: 263-74.
2. WEISS A: T cell antigen receptor signal transduction: A tale of tails and cytoplasmic proteintyrosine kinases. *Cell* 1993; 73: 209-12.
3. RETH M: Antigen receptor tail clue. *Nature* 1989; 338: 383-4.
4. CHAN AC, SHAW AS: Regulation of antigen receptor signal transduction by protein tyrosine kinases. *Curr Opin Immunogy* 1996; 8: 394-401.
5. JUNE CH, FLETCHER MC, LEDBETTER JA, SAMELSON LE:Increases in tyrosine phosphorylation are detectable before phospholipase C activation after T cell receptor stimulation. *J Immunol* 1990; 144: 1591-9.
6. LETOURNEUR F, KLAUSNER RD: Activation of T cells by a tyrosine kinase activation domain in the cytoplasmic tail of CD3ε. *Science* 1992; 255: 79-82.
7. SHAW AS, AMREIN KE, HAMMOND C, STERN DF, SEFTON BM, ROSE JK: The lck tyrosine protein kinase interacts with the cytoplasmic tail of the CD4 glycoprotein through its unique amino-terminal domain. *Cell* 1989; 59: 627-36.
8. STRAUS DB, WEISS A: Genetic evidence for the involvement of the lck tyrosine kinase in signal transduction through the T cell antigen receptor. *Cell* 1992; 70: 585-93.
9. VEILLETTE A, ABRAHAM N, CARON L, DAVIDSON D: The lymphocyte-specific tyrosine protein kinase pS61ck. *Semin Immunol* 1991; 3: 143-52.
10. SAMELSON LE, PHILLIPS AF, LUONG ET, KLAUSNER RD: Association of the fyn protein-tyrosine kinase with the T-cell antigen receptor. *Proc Natl Acad Sci USA* 1990; 87: 4358-62.
11. STEIN PL, LEE HM, RICH S, SORIANO P: ppS9fyn mutant mice display differential signaling in thymocytes and peripheral T cells. *Cell* 1992; 70: 741-50.
12. SCHLESSINGER J: SH2/SH3 signaling proteins. *Curr Opin Genetics & Dev* 1994; 4: 25-30.
13. IRVING BA, CHAN AC, WEISS A: Functional characterization of a signal transducing motif present in the T cell antigen receptor zeta chain. *J Exp Med* 1993; 177: 1093- 103.
14. CHAN AC, IWASHIMA M, TURCK CW, WEISS A: ZAP-70: A 70 kd protein-tyrosine kinase that associates with the TCR zeta chain. *Cell* 1992; 71: 649-62.
15. WANGE RL, MALEK SN, DESIDERIO S, SAMELSON LE: Tandem SH2 domains of ZAP70 bind to T cell antigen receptor zeta and CD3 epsilon from activated Jurkat T cells. *J Biol Chem* 1993; 268: 19797-801.
16. MOLINA T, KISHIHARA K, SIDEROVSKI D, *et al.*: Profound block in thymocyte development in mice lacking pS61ck. *Nature* 1992; 357: 161-4.
17. NEGISHI I, MOTOYAMA N, NAKAYAMA K, *et al.*: Essential role for ZAP-70 in both positive and negative selection of thymocytes. *Nature* 1995; 376: 4358.
18. WILLIAMS BL, SCHREIBER KL, ZHANG W, *et al.*: Genetic evidence for differential coupling

of Syk family kinases to the T-cell receptor: Reconstitution studies in a ZAP-70-deficient Jurkat T-cell line. *Mol Cell Biol* 1998; 18: 1388-99.

19. ELDER ME, LIN D, CLEVER J, *et al*.: Human severe combined immunodeficiency due to a defect in ZAP-70, a T cell tyrosine kinase. *Science* 1994; 264: 1596-9.
20. CHAN AC, KADLECEK TA, ELDER ME, *et al*.: ZAP-70 deficiency in an autosomal recessive form of severe combined immunodeficiency. *Science* 1994; 264: 1599-601.
21. ARPAIA E, SHAHAR M, DADI H, COHEN A, ROLFMAN CM: Defective T cell receptor signaling and CD8+ thymic selection in humans lacking zap-70 kinase. *Cell* 1994; 76: 947-58.
22. KORETZKY GA: Role of the CD45 tyrosine phosphatase in signal transduction in the immune system. *FASEB J* 1993; 7: 420.
23. KORETZKY GA, PICUS J, THOMAS ML, WEISS A: Tyrosine phosphetase CD45 is essential for coupling T-cell antigen receptor to the phosphatidyl inositol pathway. *Nature* 1990; 346: 66-8.
24. KISHIHARA K, PENNINGER J, WALLACE VA, *et al*.: Normal B lymphocyte development but impaired T cell maturation in CD45-exon6 protein tyrosine phosphatase-deficient mice. *Cell* 1993; 74: 143-56.
25. PINGEL JT, THOMAS ML: Evidence that the leukocyte-common antigen is required for antigen-induced T lymphocyte proliferation. *Cell* 1989; 58: 1055-65.
26. BURNS CM, SAKAGUCHI K, APPELLA E, ASHWELL JD: CD45 regulation of tyrosine phosphorylation and enzyme activity of src family kinases. *J Biol Chem* 1994; 269: 13594-600.
27. OSTERGAARD HL, SHACKELFORD DA, HURLEY TR, *et al*.: Expression of CD45 alters phosphorylation of the lck-encoded tyrosine protein kinase in murine lymphoma T-cell lines. *Proc Natl Acad Sci USA* 1989; 86: 8959-63.
28. MCFARLAND ED, HURLEY TR, PINGEL JT, SEFTON BM, SHAW A, THOMAS ML: Correlation between Src family member regulation by the protein-tyrosine-phosphatase CD45 and transmembrane signaling through the T-cell receptor. *Proc Natl Acad Sci USA* 1993; 90: 1402-6.
29. PLAS DR, JOHNSON R, PINGEL JT, *et al*.: Direct regulation of ZAP-70 by SHP-1 in T cell antigen receptor signaling. *Science* 1996; 272: 1173- 6.
30. MUSCI MA, BEAVES SL, ROSS SE, YI T, KORETZKY GA: Surface expression of hemopoietic cell phosphatase fails to complement CD45 deficiency and inhibits TCR-mediated signal transduction in a Jurkat T cell clone. *J Immunol* 1997; 158: 1565-71.
31. PANI G, FISCHER KD, MLINARIC-RASCAN I, SIMINOVITCH KA: Signaling capacity of the T cell antigen receptor is negatively regulated by the PTP1C tyrosine phosphatase. *J Exp Med* 1996; 184: 839-52.
32. SHALTZ LD, SCHWEITZER PA, RAJAN TV, *et al*.: Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. *Cell* 1993; 73: 1445-54.
33. TSUI HW, SIMINOVITCH KA, DE SOUZA L, TSUI FW: Moth-eaten and viable moth-eaten mice have mutations in the haematopoietic cell phosphatase gene. *Nat Genet* 1993; 4: 124-9.
34. LORENZ U, RAVICHANDRAN KS, BURAKOFF SJ, NEEL BG: Lack of SHPTP1 results in src-family kinase hyperactivation and thymocyte hyper-responsiveness. *Proc Natl Acad Sci USA* 1996; 93: 9624-9.
35. ZHANG W, SLOAN-LANCASTER J, KITCHEN J, TRIBLE RP, SAMELSON LE: LAT: The ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. *Cell* 1997; 92: 83-92.
36. MOTTO DG, ROSS SE, WU J, HENDRICKS-TAYLOR LR, KORETZKY GA: Implication of the GRB2-associated phosphoprotein SLP-76 in T cell receptor-mediated interleukin 2 production. *J Exp Med* 1996; 183: 1937-43.
37. DONOVAN JA, WANGE RL, LANGDON WY, SAMELSON LE: The protein product of the c-cbl protooncogene is the 120-kDa tyrosine-phosphorylated protein in Jurkat cells activated via the T cell antigen receptor. J Biol Chem 1994; 269: 22921-4.
38. CLEMENTS JL, YANG B, ROSS-BARTA SE, *et al*.: Requirement for the leukocyte-specific adapter protein SLP-76 for normal T cell development. *Science* 1998; 281 (5375): 416-9.

38b. PIVNIOUK V, TSITSIKOV E, SWINTON P, RATHBUN G, ALT FW, GEHA RS: Impaired viability and profound block in thymocyte development in mice lacking the adaptor protein SLP-76. *Cell* 1998; 94: 229-38.

39. YABLONSKI D, KUHNE MR, KADLECEK T, WEISS A: Uncoupling of non-receptor tyrosine kinases from PLC-gamma-1 in an SLP-76-deficient T cell. *Science* 1998; 281 (5375): 413-6.
40. OTA Y, SAMELSON LE: The product of the proto-oncogene c-cbl: A negative regulator of the Syk tyrosine kinase. *Science* 1997; 276: 418-20.
41. BOUSSIOTIS VA, FREOMAN GJ, BEREZOVSKAYA A, BARBER DL, NADLER LM: Maintenance of human T cell anergy: Blocking of IL-2 gene transcription by activated Rapl. *Science* 1997; 278: 124-8.
42. SECRIST JP, KARNITZ L, ABRAHAM RT: T-cell antigen receptor ligation induces tyrosine phosphorylation of phospholipase C-gamma 1. *J Biol Chem* 1991; 266: 12135-9.
43. WEISS A, KORETZKY G, SCHATZMAN RC, KADLECEK T: Functional activation of the T-cell antigen receptor induces tyrosine phosphorylation of phospholipase C-gamma 1. *Proc Natl Acad Sci USA* 1991; 88: 5484-8.
44. PARK DJ, RHO HW, RHEE SG: CD3 stimulation causes phosphorylation of phospholipase C-gamma 1 on serine and tyrosine residues in a human T-cell line. *Proc Natl Acad Sci USA* 1991; 88: 5453-6.
45. LOH C, SHAW KT, CAREW J, *et al*.: Calcineurin binds the transcription factor NFAT1 and reversibly regulates its activity. *J Biol Chem* 1996; 271: 10884-91.
46. LUO C, SHAW KT, RAGHAVAN A, *et al*.: Interaction of calcineurin with a domain of the transcription factor NFAT1 that controls nuclear import. *Proc Natl Acad Sci USA* 1996; 93: 8907-12.
47. NORTHROP JP, HO SN, CHEN L, *et al*.: NF-AT components define a family of transcription factors targeted in T-cell activation. *Nature* 1994; 369: 497-502.
48. LIU J, FARMER JD, JR., LANE WS, FRIEDMAN J, WEISSMAN I, SCHREIBER SL: Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* 1991; 66: 807-15.
49. DOWNWARD J, GRAVES J, CANTRELL D: The regulation and function of p21ras in T cells. *Immunol Today* 1992; 13: 89-92.
50. GUPTA S, WEISS A, KUMAR G, WANG S, NEL A: The T-cell antigen receptor utilizes Lck, Raf-1, and MEK-1 for activating mitogen-activated protein kinase. Evidence for the existence of a second protein kinase C-dependent pathway in an Lck-negative Jurkat cell mutant. *J Biol Chem* 1994; 269: 17349-57.
51. SIEGEL JN, KLAUSNER RD, RAPP UR, SAMELSON LE: T cell antigen receptor engagement stimulates c-raf phosphorylation and induces c-raf-associated kinase activity via a protein kinase C-dependent pathway. *J Biol Chem* 1990; 265: 18472-80.
52. SETH A, GONZALEZ FA, GUPTA S, RADEN DL, DAVIS RJ: Signal transduction within the nucleus by mitogen-activated protein kinase. *J Biol Chem* 1992; 267: 24796-804.
53. SCHWARTZ RH: Models of T cell anergy: Is there a common molecular mechanism? *J Exp Med* 1996; 184:1-8.
54. HARDING FA, MCARTHUR JG, GROSS JA, RAOLET DH, ALLISON JP: CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature* 1992; 356: 607-9.
55. LINSLEY PS, CLARK EA, LEDBETTER JA: T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB- 1. *Proc Natl Acad Sci USA* 1990; 87: 5031-5.
56. INABA K, WITMER-PACK M, INABA M, *et al*.: The tissue distribution of the B7-2 costimulator in mice: Abundant expression on dendritic cells *in situ* and during maturation *in vitro*. *J Exp Med* 1994; 180: 1849-60.
57. LINSLEY PS, BRADY W, GROSMAIRE L, ARUFFO A, DAMLE NK, LEDBETTER JA: Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 rnRNA accumulation. *J Exp Med* 1991; 173: 721-30.
58. THOMPSON CB, LINDSTEN T, LEDBETTER JA, *et al*.: CD28 activation pathway regulates the production of multiple T-cell-derived lymphokines/cytokines. *Proc Natl Acad Sci USA* 1989; 86: 1333-7.
59. NOEL PJ, BOISE LH, GREEN JM, THOMPSON CB: CD28 costimulation prevents cell death during primary T cell activation. *J Immunol* 1996; 157: 636-42.
60. TAO X, CONSTANT S, JORRITSMA P, BOTTOMLY K: Strength of TCR signal determines the costimulatory requirements for Th1 and Th2 CD4+ T cell differentiation. *J Immunol* 1997; 159: 5956-63.
61. AUGUST A, DUPONT B: CD28 of T lymphocytes associates with phosphatidylinositol 3-kinase. *Int Immunol* 1994; 6: 769-74.
62. SCHNEIDER H, CAI YC, PRASAD KV, SHOELSON SE, RUDD CE: T cell antigen CD28 binds to the GRB-2/SOS complex, regulators of p21ras. *European J Immunol* 1995; 25:

1044-50.
63. PRASED KV, CAI YC, RAAB M, *et al.*: T-cell antigen CD28 interacts with the lipid kinase phosphatidylinositol 3-kinase by a cytoplasmic Tyr(P)-Met-Xaa-Met motif. *Proc Natl Acad Sci USA* 1994; 91: 2834-8.
64. KIM HH, THARAYIL M, RUDD CE: Growth factor receptor-bound protein 2 SH2/SH3 domain binding to CD28 and its role in co-signaling. *J Biol Chem* 1998; 273: 296-301.
65. ONG CJ, LIM AS, TEH HS: CD28-induced cytokine production and proliferation by thymocytes are differentially regulated by the p59fyn tyrosine kinase. *J Immunol* 1997; 159: 2169-76.
66. NISHINA H, BACHMANN M, OLIVEIRA DOS-SANTOS AJ, *et al.*: Impaired CD28-mediated interleukin 2 production and proliferation in stress kinase SAPK/ERK1 kinase (SEK1)/ mitogen-activated protein kinase kinase 4 (MKK4)-deficient T lymphocytes. *J Exp Med* 1997; 186: 941-53.
67. REEDQUIST KA, BOS JL: Co-stimulation through CD28 suppresses T cell receptor-dependent activation of the Ras-like small GTPase Rapl in human T lymphocytes. *J Biol Chem* 1998; 273: 4944-9.
68. LINDSTEIN T, JUNE CH, LEDBETTER JA, STELLA G, THOMPSON CB: Regulation of lymphokine messenger RNA stability by a surface-mediated T cell activation pathway. *Science* 1989; 244: 339-43.
69. LINSLEY PS, BRADY W, URNES M, GROSMAIRE LS, DAMLE NK, LEDBETTER JA: CTLA-4 is a second receptor for the B cell activation antigen B7. *J Exp Med* 1991; 174: 561-9.
70. WALUNAS TL, LENSCHOW DJ, BAKKER CY, *et al*.: CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1994; 1: 405-13.
71. LENSCHOW DJ, ZENG Y, THISTLETHWAITE JR, *et al*.: Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA41g. *Science* 1992; 257: 789-92.
72. FINCK BK, LINSLEY PS, WOISY D: Treatment of murine lupus with CTLA4Ig. *Science* 1994; 265: 1225-7.
73. MARENGERE LE, WATERHOUSE P, DUNCAN GS, MITTRUCKER HW, FENG GS, MAK TW: Regulation of T cell receptor signaling by tyrosine phosphatase SYP association with CTLA-4. *Science* 1996; 272: 1170-3.
74. THEZE J, ALZARI PM, BERTOGLIO J: Interleukin 2 and its receptors: Recent advances and new immunological functions. *Immunol Today* 1996; 17: 481-6.
75. MINAMI Y, KONO T, YAMADA K, *et al*.: Association of p561ck with IL-2 receptor beta chain is critical for the IL-2-induced activation of pS61ck. *Embo J* 1993; 12: 759-68.
76. KOBAYASHI N, KONO T, HATAKEYAMA M, *et al*.: Functional coupling of the src-family protein tyrosine kinases pS9fyn and pS3/561yn with the interleukin 2 receptor: Implications for redundancy and pleiotropism in cytokine signal transduction. *Proc Natl Acad Sci USA* 1993; 90: 4201-5.
77. KARNITZ LM, BURUS LA, SUTOR SL, BLENIS J, ABRAHAM RT: Interleukin-2 triggers a novel phosphatidylinositol 3-kinase-dependent MEK activation pathway. *Mol Cell Biol* 1995; 15: 3049-57.
78. ZHU MH, BERRY JA, RUSSELL SM, LEONARD WJ: Delineation of the regions of interleukin-2 (IL-2) receptor beta chain important for association of Jak1 and Jak3. Jak1-independent functional recruitment of Jak3 to Il-2Rbeta. *J Biol Chem* 1998; 273: 10719-25.
79. RAVICHANDRAN KS, BURAKOFF SJ: The adapter protein Shc interacts with the interleukin2 (IL- 2) receptor upon IL-2 stimulation. *J Biol Chem* 1994; 269: 1599-1602.
80. MIYAZAKI T, KAWAHARA A, FUJII H, *et al*.: Functional activation of Jak1 and Jak3 by selective association with IL-2 receptor subunits. *Science* 1994; 266: 1045-7.
81. WITTHUHN BA, SILVENNOINEN O, MIURA O, *et al.*: Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells. *Nature* 1994; 370: 153-7.
82. JOHNSTON JA, KAWAMURA M, KIRKEN RA, *et al*.: Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. *Nature* 1994; 370: 151-3.
83. DARNELL JE Jr: STATs and gene regulation. *Science* 1997; 277: 1630-5.
84. ISHII N, ASAO H, KIMURA Y, *et al*.: Impairment of ligand binding and growth signaling of mutant IL-2 receptor gamma-chains in patients with X-linked severe combined immunodeficiency. *J Immunol* 1994; 153: 1310- 17.
85. WEINBERG K, PARKMAN R: Severe combined immunodeficiency due to a specific defect in the production of interleukin-2. *N Engl J Med* 1990; 322: 1718-23.
86. VON FREEDEN-JEFFRY U, VIEIRA P, LUCIAN LA, MCNEIL T, BURDACH SE, MURRAY R: Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J Exp Med* 1995; 181: 1519-26.
87. PESCHON JJ, MORRISSEY PJ, GRABSTEIN KH, *et al.*: Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. *J Exp Med* 1994; 180: 1955-60.
88. MACCHI P, VILLA A, GILLANI S, *et al.*: Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). *Nature* 1995; 377: 65-8.
89. WILLERFORD DM, CHEN J, FERRY JA, DAVIDSON L, MA A, ALT FW: Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* 1995; 3: 521-30.
90. SUZUKI H, KUNDIG TM, FURLONGER C, *et al.*: Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. *Science* 1995; 268: 1472-6.
91. SCHERLE H, HOLTSCHKE T, HUNIG T, SCHIMPL A, HORAK I: Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. *Nature* 1991; 352: 621-4.
92. NAGATA S: Apoptosis by death factor. *Cell* 1997; 88: 355-65.
93. NAGATA S, GOLSTEIN P: The Fas death factor. *Science* 1995; 267: 1449-56.
94. TANAKA M, SUDA T, TAKAHASHI T, NAGATA S: Expression of the functional soluble form of human fas ligand in activated lymphocytes. *Embo J* 1995; 14: 1129-35.
95. ITOH N, NAGATA S: A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen. *J Biol Chem* 1993; 268: 10932-7.
96. CASCINO I, PAPOFF G, DE MARIA R, TESTI R, RUBERTI G: Fas/Apo-1 (CD95) receptor lacking the intracytoplasmic signaling domain protects tumor cells from fas-mediated apoptosis. *J Immunol* 1996; 156: 13- 17.
97. PETERSON E, LATINIS K, KORETZKY G: Molecular characterization of a CD95 signaling mutant. *Arthritis Rheum* 1998; 41: 1047-53.
98. CHINNAIYAN AM, O'ROURKE K, TEWARI M, DIXIT VM: FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 1995; 81: 505-12.
99. BOLDIN MP, GONCHAROV TM, GOLTSEV YV, WALLACH D: Involvement of MACH a novel MORTl/FADD-interacting protease, in Fas/ APO-1- and TNF receptor-induced cell death. *Cell* 1996; 85: 803-15.
100. MUZIO M, CHINNAIYAN AM, KISCHKEL FC, *et al*.: FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO- 1) death-inducing signaling complex. *Cell* 1996; 85: 817-27.
101. ALNEMRI ES: Mammalian cell death proteases: A family of highly conserved aspartate specific cysteine proteases. *J Cell Biochem* 1997; 64: 33-42.
102. MUZIO M, STOCKWELL BR, STENNICKE HR, SALVESEN GS, DIZIT VM: An induced proximity model for caspase-8 activation. *J Biol Chem* 1998; 273: 2926-30.
103. FERNANDES-ALNEMRI T, ARMSTRONG RC, KREBS J, *et al*.: *In vitro* activation of CPP32 and Mch3 and Mch4, a novel human apoptotic cysteine protease containing two FADD-like domains. *Proc Natl Acad Sci* 1996; 93: 7464-9.
104. NAGATA S, SUDA T: Fas and Fas ligand: lpr and gld mutations. *Immunol Today* 1995; 16: 39-43.
105. WATANABE-FUKONAGA R, BRANNAN CI, COPELAND NG, JENKINS NA, NAGATA S: Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* 1992; 356: 314-7.
106. FISHER GH, ROSENBERG FJ, STRAUS SE, *et al*.: Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* 1995; 81: 935-46.
107. DRAPPA J, VAISHNAW AK, SULLIVAN KE, CHU JL, ELKON KB: Fas gene mutations in the Canale-Smith syndrome, an inherited lymphoproliferative disorder associated with autoimmunity. *N Engl J Med* 1996; 335: 1643-9.
108. WU J, WILSON J, HE J, XIANG L, SCHUR PH, MOUNTZ JD: Fas ligand mutation in a patient with systemic lupus erythematosus and lymphoproliferative disease. *J Clin Invest* 1996; 98: 1107-13.
109. MYSLER E, BINI P, DRAPPA J, *et al*.: The apoptosis-l/Fas protein in human systemic lupus erythematosus. *J Clin Invest* 1994; 93: 1029-34.
110. GRIFFITH TS, BRUNNER T, FLETCHER SM, GREEN DR, FERGUSON TA: Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* 1995; 270: 118992.
111. BELLGRAU D, GOLD D, SELAWRY H, MOORE J, FRANZUSOFF A, DUKE RC: A role for CD95 ligand in preventing graft rejection. *Nature* 1995; 377: 630-2.