Published in final edited form as:

Curr Opin Organ Transplant. 2019 February ; 24(1): 12–19. doi:10.1097/MOT.0000000000000599.

TNF-driven cell death in donor organ as a barrier to immunological tolerance

Rosalind L. Ang^{a,#} and Adrian T. Ting^{a,#}

^aPrecision Immunology Institute, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, New York, NY 10029, USA.

Abstract

Purpose of review: Regulated cell death (RCD) is likely to play a role in organ rejection but it is unclear how it may be invoked. A well-known trigger of RCD is tumor necrosis factor- α (TNF), which activates both caspase-dependent apoptosis or caspase-independent necroptosis. TNF is best known as a pro-inflammatory cytokine because it activates NF κ B and MAPK signaling to induce expression of pro-inflammatory genes.

Recent findings: Emerging data from animal models now suggest that TNF-induced cell death can also be inflammatory. Therefore, the role of cellular demise in regulating immunity should be considered. In transplantation, TNF could have a role in cellular injury or death from ischemia reperfusion (IR) injury and this may dictate organ survival. The default response to TNF in most cells is survival, rather than death, due to the presence of cell death checkpoints. However, cells succumb to TNF-driven death when these checkpoints are disrupted and sensitivity to death likely reflects a reduction in molecules that fortify these checkpoints. We propose that a cell's propensity to die in response to TNF may underlie allograft rejection.

Summary: Genetic, epigenetic and post-translational control of death checkpoint regulators in donor tissues may determine graft survival. Therapeutically, drugs that prevent donor cell demise could be useful in preventing organ rejection.

Keywords

TNF; Cell Death; Apoptosis; Necroptosis; Ubiquitin; E3 ligase; Deubiquitinase; Checkpoint

Role of TNF in transplant rejection

Transplantation of solid organs from allogeneic donors has been the treatment of choice for end stage organ disease and advances in our understanding of transplantation immunology have resulted in clinical strategies that have greatly enhanced graft survival (1, 2). Not surprisingly, due to the central role of MHC allo-antigens in graft rejection, transplant immunology has focused on how adaptive immune mechanisms such as the priming and

[#]Corresponding Authors: Precision Immunology Institute, Box 1630, Icahn School of Medicine at Mount Sinai, 1 Gustave L Levy Place, New York, NY 10029, USA., rosaling.ang@mssm.edu, Phone: 212-659-9416, adrian.ting@mssm.edu, Phone: 212-659-9410.

Conflicts of interest

We declare that there are no financial conflicts of interest.

activation of lymphocytes are involved in the process of allograft rejections (3, 4). These insights have led to the development of a number of immunosuppressive agents, which by and large, inhibit activation of T cells (e.g., steroids, calcineurin and mTOR inhibitors). These targeted strategies, predicated on modulating host immune responses, have significantly reduced the rates of acute rejection and increased short-term graft survival (2). However, long-term allograft survival remains suboptimal (2) and new intervention strategies to prolong graft survival are likely to come from a greater understanding of cellular processes that are occurring within the donor cells and tissues.

The transplanted organ brings with it cells of both hematopoietic origin (e.g., resident macrophages) and non-hematopoietic origin (e.g., vasculature endothelial cells, stromal fibroblasts, cardiomyocytes and renal tubular cells). Quite surprisingly, how cells in the donor tissue are involved in organ injury following IR during surgical implantation and in the subsequent encounter with recipient immune cells are not well understood. Organ failure is ultimately a result of the death of cells in the transplanted tissue and understanding the decision-making process that determines life versus death may reveal therapeutic targets. In this review, we will expound on the hypothesis that the response of donor cells to TNF, depending on whether this cytokine is perceived by donor cells as a pro-survival or a pro-death signal, will determine graft tolerance or rejection.

The thesis that TNF has a role in allograft rejection was suggested by early observations of systemic TNF elevation in patients experiencing allograft rejection (5, 6) and in animal transplant models (7, 8). Subsequent studies demonstrated that blocking TNF prolonged allograft survival in rodent models implicating a pathogenic role for TNF in graft rejection (9–12). This is now being tested in a clinical trial examining whether TNF blockade by infliximab will extend allograft survival and function in renal transplants (NCT02495077).

Exactly how TNF is involved in allograft rejection is unclear. TNF is an inflammatory cytokine and blocking TNF has proven to be highly effective in a number of inflammatory disorders (13–15). This pro-inflammatory role of TNF is thought to be due to the induction of NF κ B and MAPK signaling, and subsequent transcription of downstream pro-inflammatory genes including other cytokines, chemokines, receptors and adhesion molecules (16–19). The expression of these genes would lead to the recruitment of innate and adaptive cells to the graft, similar to what occurs at the site of an infection. Because this paradigm of TNF's pro-inflammatory mechanism is well established in other inflammatory disorders (e.g., inflammatory bowel disease and rheumatoid arthritis), the conventional wisdom is that a similar mechanism may underlie TNF's role in graft rejection. This pro-inflammatory role of TNF is often synonymous with a pro-survival effect because NF κ B transcription factors also upregulate a number of pro-survival genes that inhibit cell death. However, TNF also possesses cytotoxic potential as evidenced by its initial isolation as a factor that kills tumor cells (20). It is currently unclear whether this cytotoxic response to TNF has any role in graft rejection and under what circumstances this response could come into play. With the increasing appreciation that cellular fate can shape the immunological responses (21), the survival or death (apoptosis or necroptosis) of cells in the transplanted organ may be pertinent to graft survival outcome and to induction of immunotolerance. We

will discuss the current understanding of susceptibility to TNF-induced cellular cytotoxicity and how that may link to graft survival in solid organ transplantation.

Molecular regulation of the cellular responses to TNF

To study and understand the biological function of TNF-driven cytotoxicity, an appreciation of its intricate signaling pathways is warranted (22). TNF can trigger two opposing cellular fates – survival or death, and most cells have the machinery to induce both responses. However, the default response to TNF in a majority of cells is survival rather than cell death and the molecular basis for this behavior has become better understood. There are two receptors for TNF: the 55 kD TNFR1 and the 75 kD TNFR2 (18). Due to its more widespread expression, TNFR1 is the dominant receptor that transduces TNF signaling. Interaction of TNF with its TNFR1 leads to trimerization of the receptor that results in the formation of membrane-bound TNFR1 signaling complex I (i.e., TNFR1-SC). This TNFR1-SC is comprised of signaling molecules including TRADD, TRAF2, cIAP1/2, LUBAC, RIPK1, TAB2/3-TAK1 and NEMO-IKK α/β . The complex is highly regulated by post-translational modifications including ubiquitination and phosphorylation that dictate cellular fate. We had proposed that there are two sequential cell death checkpoints in the TNFR1 pathway (22–24) (Figure 1A). Cell death occurs when either of these cell death checkpoints is disrupted (Figure 1B). The early checkpoint is a transcription-independent checkpoint that depends on the ubiquitination of RIPK1 by both the TRAF2/cIAP1/2 and LUBAC E3 ubiquitin ligases that catalyzes the formation of lysine 63 (K63)-linked and linear (M1)-linked poly-ubiquitin chains, respectively. The addition of these non-degradative ubiquitin chains on RIPK1 serves to protect against cell death in the following ways. First, ubiquitinated RIPK1 serves as a docking platform for NEMO/IKK α/β and TAB2/3/TAK1 kinase complexes, and this prevents RIPK1 from associating with death-signaling molecules. Second, TAK1 can now phosphorylate and activate IKK. In turn, IKK phosphorylates and further suppresses the death-signaling function of RIPK1 (25), as well as phosphorylates and inhibits CYLD, a deubiquitinase that dismantles K63-linked poly-ubiquitin chains (26). These phosphorylation events serve as additional reinforcement of the early cell death checkpoint. The IKK complex also phosphorylates I κ B α leading to its degradation. This event results in the release and translocation of NF κ B to the nucleus to induce transcription of pro-survival genes such as cFLIP and BCL2, providing long term protection against death. The induction of NF κ B-mediated gene transcription to protect against death is referred to as the late checkpoint. Failure in either checkpoint leads to death upon TNF stimulation.

The NF κ B-dependent late checkpoint was the first checkpoint to be discovered when it was shown that cells with a genetic deficiency or a blockade in NF κ B-dependent gene transcription were more sensitive to cell death when treated with TNF (27–29). In the absence of NF κ B-dependent pro-survival protein synthesis, a cytoplasmic signaling complex termed Complex IIa, comprising of TRADD, FADD and CASPASE 8 is formed (30). One way by which NF κ B deficiency leads to death sensitivity is because cFLIP, an NF κ B-regulated gene, is not replenished when NF κ B is defective (30, 31). cFLIP normally forms a complex with PROCASPASE 8 to limit its catalytic activity and so a reduction in cFLIP level results in auto-cleavage of PROCASPASE 8 to generate a p18/p10 tetrameric

holoenzyme that initiates the apoptotic cascade. Active CASPASE 8 can cleave PROCASPASE3 directly to generate active CASPASE 3, or it can do this indirectly by cleaving BID to generate tBID, which then causes the release of cytochrome C from the mitochondria leading to CASPASE3 activation.

The ubiquitin-dependent early checkpoint was discovered a decade later when it was shown that blocking the non-degradative ubiquitination of RIPK1 by mutating its ubiquitin acceptor site caused RIPK1 to associate with CASPASE 8 to initiate apoptosis (32). Similarly, treating cells with SMAC mimetics, which are compounds that inhibit the cIAP1/2 E3 ligases from ubiquitinating RIPK1, had the same effect (33, 34). This particular complex of RIPK1 that forms with CASPASE 8 and FADD has been termed Complex IIb. Thus, ubiquitination of RIPK1 blocks death by preventing RIPK1 from interacting with CASPASE 8. One unique feature of death conferred by the failure of the early checkpoint and the involvement of RIPK1 is the ability of the apoptotic response to be shunted to necroptosis, an alternative form of cell death, when caspases are inhibited (35). In the presence of caspase inhibitors, CASPASE 8 is prevented from cleaving CYLD and RIPK1 (36, 37). Removal of ubiquitin chains from RIPK1 by CYLD then promotes the interaction of RIPK1 with RIPK3. The latter then phosphorylates MLKL resulting in MLKL oligomerization to trigger necroptosis. For both RIPK1-dependent apoptosis and necroptosis, its kinase activity is essential. Thus, ubiquitination of RIPK1 or the lack thereof can be considered as a toggle between survival (when RIPK1 is ubiquitinated) or death (when it is not ubiquitinated).

Immunological consequences of TNF-induced cell death

Molecularly, the mechanism by which TNF induces cell death when the late checkpoint fails is different from when the early checkpoint fails. The death that occurs when the late NF κ B-dependent checkpoint fails could be considered 'death by starvation' as it is due to a loss of an essential sustenance (i.e., NF κ B-dependent pro-survival molecules). In contrast, the death that occurs when the early ubiquitin-dependent checkpoint fails could be considered 'death by execution.' In this case, death is manifested as a result of RIPK1 gaining a death-inducing capability following the removal of the restraint imposed by ubiquitination. The biological effects and the immunological sequelae of TNF-induced cell death conferred by the failure of either checkpoints are likely to be different. The use of mouse knockout models, which necessarily involve the deletion of pro-survival genes in the checkpoints to reveal the death response, have begun to shed light on this. Deletion in intestinal epithelial cells (IEC) of *Nemo* (thereby leading to the failure of both checkpoints with RIPK1 initiating the cell death) led to severe intestinal inflammation (38). In contrast, combined deletion of *Rel* members of the NF κ B family in the same tissue (thereby leading to failure of only the late checkpoint with no RIPK1 involvement) did not lead to colitis (38). Hence, RIPK1-dependent death appears to be highly inflammatory. Another insightful mouse model is that of the *cpdm* strain, which has a spontaneous loss-of-function mutation in the *Sharpin* gene. This mouse strain exhibits immunodeficiency together with multi-organ inflammation including pronounced dermatitis (39). SHARPIN together with HOIP/RNF31 and HOIL1/RBCK1 forms the Linear Ubiquitination Assembly Complex (LUBAC), an E3 ligase that catalyzes the addition of linear ubiquitin chains on molecules such as RIPK 1 and NEMO as part of the early checkpoint. SHARPIN deficiency sensitizes cells to RIPK1-dependent

death when treated with TNF (40–42). *In vivo*, the inflammatory phenotype of the *Sharpin*-deficient *cpdm* strain can be reversed by a compound deletion in *Tnf* (42) or by a K45A knock-in mutation of *Ripk1* that disables its kinase activity and thus RIPK1-dependent death (43). Removal of death-signaling molecules FADD or CASPASE 8 in combination with RIPK3 also had the same effect of reversing the inflammation in the *cpdm* strain (44, 45). These findings indicate that TNF-driven cell death, conferred by the failure of the early checkpoint and the resulting RIPK1-dependent death, is inflammatory.

In both the IEC-specific knockout of *Nemo*, deletion of *Ripk3*, which disables necroptosis but leaves apoptosis intact, still resulted in colitis in a proportion of mice (38). Similarly, deletion of *Ripk3* only partially ameliorated the dermatitis in the *Sharpin*-deficient *cpdm* mice (45). These results strongly suggest that TNF-induced RIPK1-dependent apoptosis is inflammatory. A well-accepted concept in cell death is that apoptosis is a non-immunogenic form of death whereas necrotic death such as necroptosis is inflammatory and immunogenic. This concept was formulated based in part on observations that apoptosis often occurs during normal developmental processes without any signs of inflammation. An example of this occurs in the thymus where the bulk of thymocytes die by apoptosis because they failed to be selected during thymic education. Despite the high level of thymocyte death, there is no inflammation because apoptotic debris are rapidly ingested and cleared away by phagocytes. However, it is possible that RIPK1-dependent apoptosis induced by TNF is qualitatively different and can be inflammatory. One possible reason is that RIPK1-dependent death may be accompanied by gene expression of cytokines and chemokines that are inflammatory (46). Alternatively, the presence of TNF when the cells are dying by apoptosis may cause the cellular debris taken up by innate phagocytic cells to be processed and presented differently. Necroptotic death is widely accepted to be inflammatory due to the release of damage-associated molecular patterns (DAMPs), cellular contents that are ligands for pattern-recognition receptors. This form of death would be triggered by TNF during an infection with a microbial agent that encodes a CASPASE 8 inhibitor or if the responding cells lack CASPASE 8 expression due to epigenetic or transcriptional silencing.

Mechanisms by which TNF-dependent death leads to graft rejection

At present, there is little evidence to indicate that TNF-mediated cell death has a role in human transplants. More compelling evidence has come from mouse models in which various death-signaling components have been genetically altered (47). It has been reported that *Ripk3*^{−/−} renal and cardiac allografts survived longer than their wild type counterparts (48, 49) suggesting that necroptosis may be involved. Administration of necrostatin-1, a compound that inhibits the kinase activity of RIPK1, reduced organ damage and failure in a renal ischemia reperfusion injury model (50). However, both RIPK1 and RIPK3 can also activate apoptosis and have inflammatory functions beyond cell death (51). The defect in *Mkl1* knockout is currently believed to be restricted to necroptosis and has been proposed to be more definitive for necroptosis loss-of-function (51). A role for RIPK1-dependent apoptosis in allograft rejection cannot be ruled out at this point.

RIPK1-dependent death can only occur when there is a disruption in the early cell death checkpoint, but it is not clear when and how this could occur during organ transplantation.

The best described manner to disrupt this early checkpoint (without resorting to pharmacological or genetic manipulation) is through simultaneous ligation of both TNFR1 and TNFR2 by TNF. *In vitro* experiments have shown that in cells that are normally resistant to TNF-induced death and which do not express TNFR2, the introduction of TNFR2 into these cells will render them sensitive to cell death in a RIPK1-dependent manner (52). Ligation of TNFR2 leads to the degradation of TRAF2 (53) and the loss of this K63-linked ubiquitin E3 ligase component toggles the TNFR1 output towards RIPK1-dependent cell death. Since TNFR2 expression is more limited whereas TNFR1 expression is more widespread, sensitivity to RIPK1-dependent cell death could be a function of TNFR2 expression level. In a similar manner, reduced expression of RIPK1's E3 ligases (*e.g.*, TRAF2, cIAP1/2, LUBAC components) and/or elevated expression of RIPK1's deubiquitinases (*e.g.*, CYLD) could also shift the response to cell death. The epigenetic and transcriptional regulation of these ubiquitin-modifying molecules are not well understood. Furthermore, these molecules are also subjected to post-translational regulation. TRAF2 and cIAP1/2 stability can be regulated by proteosomal degradation and CYLD can be cleaved by proteases. Both transcriptional and post-translational regulation of their expression will be influenced by other signals the cells are also receiving. We propose that the balance in expression of molecules that fortify the early checkpoint versus molecules that drive cell death within an individual donor organ could determine their susceptibility to TNF-induced cell death and, in turn, the degree of IR injury (Figure 2A). Therefore, survival of the allograft may reflect the contention between two opposing responses intrinsic to cells of each donor. Deceased donor transplants involve extended cold storage prior to transplant surgery. It is not known whether extended cold exposure or the subsequent reperfusion affects the expression level of the checkpoint regulators. It is also unclear as to the source of TNF during IR. In a mouse model of transplant that mimics human deceased donor transplants, cardiac allografts that were subjected to prolonged cold ischemia caused an elevation in TNF level 48 hours after the transplant compared to those that did not experience cold ischemia (54). Although the mechanisms responsible for the elevation in TNF induced by ischemia are still unclear, TNF increase could, at least in part, explain the negative impact of prolonged ischemia on graft outcomes. Therefore, understanding the molecular determinants of how TNF-driven cell death within the allograft tissue is brought about could be a fruitful area for future studies.

Since allograft rejection requires an adaptive response, the initial injury resulting from TNF-induced cell death has to be translated into activation of allo-reactive effector T cells. A simple model to account for this is that DAMPs released by donor cells undergoing necroptosis activate dendritic cells presenting allo-antigen to T cells (Figure 2B). DAMPs could also activate monocytes/macrophages to produce inflammatory cytokines. This response is sometimes referred to as necroinflammation. It is unclear why necroptosis rather than apoptosis would be activated in an allograft setting. As discussed earlier, RIPK1-dependent apoptosis could be inflammatory and a role for this death in graft rejection cannot be ruled out. It is also unclear whether the type of death program activated may be different in different tissues/organs or in organs obtained from different donors. Outstanding questions remained regarding the circumstances under which RIPK1-dependent apoptosis or

necroptosis occurs, and the immunological consequence of either death response during rejection.

One final area where sensitivity to TNF-driven cell death could affect graft survival is at the effector phase of the allo-response. Primed allo-reactive CD8 T cells typically kill their target via the perforin pathway (Figure 2B). Effector T cells also produce TNF, which can be cytotoxic to donor cells if those cells have a defective early checkpoint. In addition to T cells, other immune cells including NK cells, macrophages and dendritic cells also produce TNF, and they could potentially kill donor cells via TNF if there is a checkpoint failure in donor cells (Figure 2C). This broadens the universe of cytotoxic cells if the donor tissue possesses the appropriate sensitivity. We propose that just as in the initial IR injury, the balance between opposing regulators of the early checkpoint could render donor cells sensitive to TNF-mediated destruction during the effector phase.

Conclusion

Our objective in this review is to provide an in-depth discussion of how TNF regulates two opposing cell fates and to apply newfound molecular understanding of these mechanisms towards understanding allograft survival/rejection. Future studies to address the questions we discussed could provide targets for therapeutic strategies that modulate the cellular responses of donor cells/tissues. This, in combination with existing strategies that modulate host immune cell function, could lead to long lasting allograft survival.

Summary

- TNF elevation is associated with allograft rejection in transplant patients and blocking TNF prolonged graft survival in animal models.
- TNF is a pro-inflammatory cytokine, and this has been largely attributed to its transcription-dependent regulation of downstream inflammatory genes.
- TNF can also trigger cell death and emerging experimental evidence suggests that this cellular response is also inflammatory.
- The default response to TNF in most cells is survival, rather than death, and this is due to the presence of two sequential cell death checkpoints.
- The early checkpoint is dependent on non-degradative ubiquitination of RIPK1 and is transcription-independent whereas the late checkpoint is dependent on NF κ B-mediated gene transcription.
- When the early checkpoint fails, RIPK1 converts to a death-signaling mode and the cell succumbs to death via either CASPASE 8-dependent apoptosis or RIPK3/MLKL-dependent necroptosis.
- IR injury may be mediated in part by TNF-induced death of donor cells if there is a dearth of molecules that fortify the early checkpoint.
- DAMPs and cellular debris from dying cells may enhance priming of allo-reactive T cells by antigen-presenting cells.

- Deficiency in checkpoint-fortifying molecules will also render donor cells more vulnerable to death caused by TNF produced by both innate and adaptive immune cells.
- Blocking TNF-induced cell death may be a strategy to prolong allograft survival.

Acknowledgements

We thank Dr. Peter Heeger for insightful discussions into the role of cell death in transplantation.

Financial support and sponsorship

Studies in A.T.T.'s lab has been supported by NIH grants AI052417, AI104521 and AI132405. R.L.A was supported by NIH training grants AI078892 and A1007605.

References

1. Eisen HJ. Immunosuppression-state-of-the-art: anything new in the pipeline? *Curr Opin Organ Transplant* 2014;19(5):500–7. [PubMed: 25160696]
2. Nankivell BJ, Alexander SI. Rejection of the kidney allograft. *N Engl J Med* 2010;363(15):1451–62. [PubMed: 20925547]
3. DeWolf S, Sykes M. Alloimmune T cells in transplantation. *J Clin Invest* 2017;127(7):2473–81. [PubMed: 28628037]
4. Marino J, Paster J, Benichou G. Allorecognition by T Lymphocytes and Allograft Rejection. *Front Immunol* 2016;7:582. [PubMed: 28018349]
5. Maury CP, Teppo AM. Raised serum levels of cachectin/tumor necrosis factor alpha in renal allograft rejection. *J Exp Med* 1987;166(4):1132–7. [PubMed: 3309124]
6. Imagawa DK, Millis JM, Olthoff KM, Derus LJ, Chia D, Sugich LR, et al. The role of tumor necrosis factor in allograft rejection. I. Evidence that elevated levels of tumor necrosis factor-alpha predict rejection following orthotopic liver transplantation. *Transplantation* 1990;50(2):219–25. [PubMed: 2382288]
7. Pizarro TT, Malinowska K, Kovacs EJ, Clancy J Jr., Robinson JA, Piccinini LA. Induction of TNF alpha and TNF beta gene expression in rat cardiac transplants during allograft rejection. *Transplantation* 1993;56(2):399–404. [PubMed: 8356596]
8. Saito R, Prehn J, Zuo XJ, Marchevesky A, Castracane J, Waters P, et al. The participation of tumor necrosis factor in the pathogenesis of lung allograft rejection in the rat. *Transplantation* 1993;55(5):967–72. [PubMed: 8497908]
9. Imagawa DK, Millis JM, Seu P, Olthoff KM, Hart J, Wasef E, et al. The role of tumor necrosis factor in allograft rejection. III. Evidence that anti-TNF antibody therapy prolongs allograft survival in rats with acute rejection. *Transplantation* 1991;51(1):57–62. [PubMed: 1987706]
10. Imagawa DK, Millis JM, Olthoff KM, Seu P, Dempsey RA, Hart J, et al. Anti-tumor necrosis factor antibody enhances allograft survival in rats. *J Surg Res* 1990;48(4):345–8. [PubMed: 2338821]
11. Bolling SF, Kunkel SL, Lin H. Prolongation of cardiac allograft survival in rats by anti-TNF and cyclosporine combination therapy. *Transplantation* 1992;53(2):283–6. [PubMed: 1738921]
12. Lin H, Chensue SW, Strieter RM, Remick DG, Gallagher KP, Bolling SF, et al. Antibodies against tumor necrosis factor prolong cardiac allograft survival in the rat. *J Heart Lung Transplant* 1992;11(2 Pt 1):330–5. [PubMed: 1576139]
13. Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Katsikis P, et al. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum* 1993;36(12):1681–90. [PubMed: 8250987]
14. Targan SR, Hanauer SB, van Deventer SJ, Mayer L, Present DH, Braakman T, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med* 1997;337(15):1029–35. [PubMed: 9321530]

15. Moreland LW, Baumgartner SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL, et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein [see comments]. *N Engl J Med* 1997;337(3):141–7. [PubMed: 9219699]
16. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol* 1996;14:397–440. [PubMed: 8717520]
17. Wallach D The cybernetics of TNF: Old views and newer ones. *Semin Cell Dev Biol* 2016;50:105–14. [PubMed: 26474540]
18. Bradley JR. TNF-mediated inflammatory disease. *J Pathol* 2008;214(2):149–60. [PubMed: 18161752]
19. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat Rev Rheumatol* 2016;12(1):49–62. [PubMed: 26656660]
20. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A* 1975;72(9):3666–70. [PubMed: 1103152]
21. Linkermann A, Stockwell BR, Krautwald S, Anders HJ. Regulated cell death and inflammation: an auto-amplification loop causes organ failure. *Nat Rev Immunol* 2014;14(11):759–67. [PubMed: 25324125]
22. Ting AT, Bertrand MJ. More to Life than NF-kappaB in TNFR1 Signaling. *Trends Immunol* 2016;37(8):535–45. [PubMed: 27424290]
23. O'Donnell MA, Ting AT. Chronicles of a death foretold: dual sequential cell death checkpoints in TNF signaling. *Cell Cycle* 2010;9(6):1065–71. [PubMed: 20237426]
24. O'Donnell MA, Ting AT. RIP1 comes back to life as a cell death regulator in TNFR1 signaling. *FEBS J* 2011;278(6):877–87. [PubMed: 21232018]
25. Dondelinger Y, Jouan-Lanhuet S, Divert T, Theatre E, Bertin J, Gough PJ, et al. NF-kappaB-Independent Role of IKKalpha/IKKbeta in Preventing RIPK1 Kinase-Dependent Apoptotic and Necroptotic Cell Death during TNF Signaling. *Mol Cell* 2015;60(1):63–76. [PubMed: 26344099]
26. Reiley W, Zhang M, Wu X, Granger E, Sun SC. Regulation of the deubiquitinating enzyme CYLD by IkappaB kinase gamma-dependent phosphorylation. *Mol Cell Biol* 2005;25(10):3886–95. [PubMed: 15870263]
27. Beg AA, Baltimore D. An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science* 1996;274(5288):782–4. [PubMed: 8864118]
28. Wang CY, Mayo MW, Baldwin AS Jr. TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappaB [see comments]. *Science* 1996;274(5288):784–7. [PubMed: 8864119]
29. Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM. Suppression of TNF-alpha-induced apoptosis by NF-kappaB. *Science* 1996;274(5288):787–9. [PubMed: 8864120]
30. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* 2003;114(2):181–90. [PubMed: 12887920]
31. Micheau O, Lens S, Gaide O, Alevizopoulos K, Tschopp J. NF-kappaB signals induce the expression of c-FLIP. *Mol Cell Biol* 2001;21(16):5299–305. [PubMed: 11463813]
- *32. O'Donnell MA, Legarda-Addison D, Skountzos P, Yeh WC, Ting AT. Ubiquitination of RIP1 regulates an NF-kappaB-independent cell-death switch in TNF signaling. *Curr Biol* 2007;17(5):418–24. [PubMed: 17306544] This was the first molecular demonstration that blocking RIPK1 ubiquitination converts RIPK1 to become a death-signaling, indicating the existence of a ubiquitin-dependent cell death checkpoint early in the TNF pathway.
- *33. Wang L, Du F, Wang X. TNF-alpha induces two distinct caspase-8 activation pathways. *Cell* 2008;133(4):693–703. [PubMed: 18485876]
- *34. Bertrand MJ, Milutinovic S, Dickson KM, Ho WC, Boudreault A, Durkin J, et al. cIAP1 and cIAP2 Facilitate Cancer Cell Survival by Functioning as E3 Ligases that Promote RIP1 Ubiquitination. *Mol Cell* 2008;30(6):689–700. [PubMed: 18570872] These two reports identified cIAP1 and cIAP2 as the E3 ligases that ubiquitinate RIPK1 to block its death-signaling function.
35. Pasparakis M, Vandenabeele P. Necroptosis and its role in inflammation. *Nature* 2015;517(7534):311–20. [PubMed: 25592536]

36. O'Donnell MA, Perez-Jimenez E, Oberst A, Ng A, Massoumi R, Xavier R, et al. Caspase 8 inhibits programmed necrosis by processing CYLD. *Nat Cell Biol* 2011;13(12):1437–42. [PubMed: 22037414]
37. Lin Y, Devin A, Rodriguez Y, Liu ZG. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. *Genes Dev* 1999;13(19):2514–26. [PubMed: 10521396]
- **38. Vlantis K, Wullaert A, Polykratis A, Kondylis V, Dannappel M, Schwarzer R, et al. NEMO Prevents RIP Kinase 1-Mediated Epithelial Cell Death and Chronic Intestinal Inflammation by NF-kappaB-Dependent and -Independent Functions. *Immunity* 2016;44(3):553–67. [PubMed: 26982364] This study demonstrates that excessive RIPK1-dependent cell death in intestinal epithelial cells caused by the deletion of NEMO led to colonic inflammation.
39. HogenEsch H, Janke S, Boggess D, Sundberg JP. Absence of Peyer's patches and abnormal lymphoid architecture in chronic proliferative dermatitis (cpdm/cpdm) mice. *J Immunol* 1999;162(7):3890–6. [PubMed: 10201907]
- *40. Ikeda F, Deribe YL, Skanland SS, Stieglitz B, Grabbe C, Franz-Wachtel M, et al. SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. *Nature* 2011;471(7340):637–41. [PubMed: 21455181]
- *41. Tokunaga F, Nakagawa T, Nakahara M, Saeki Y, Taniguchi M, Sakata S, et al. SHARPIN is a component of the NF-kappaB-activating linear ubiquitin chain assembly complex. *Nature* 2011;471(7340):633–6. [PubMed: 21455180]
- *42. Gerlach B, Cordier SM, Schmukle AC, Emmerich CH, Rieser E, Haas TL, et al. Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* 2011;471(7340):591–6. [PubMed: 21455173] The above three studies demonstrate that a deficiency in SHARPIN, a component of the LUBAC E3 ubiquitin ligase that catalyzes linear poly-ubiquitination, results in the multi-organ inflammation and immunodeficiency in the cpdm mouse strain.
- **43. Berger SB, Kasparcova V, Hoffman S, Swift B, Dare L, Schaeffer M, et al. Cutting Edge: RIP1 kinase activity is dispensable for normal development but is a key regulator of inflammation in SHARPIN-deficient mice. *J Immunol* 2014;192(12):5476–80. [PubMed: 24821972] This study demonstrates that excessive RIPK1 kinase activity is responsible for the inflammation observed in the cpdm strain of mice.
- **44. Kumari S, Redouane Y, Lopez-Mosqueda J, Shiraishi R, Romanowska M, Lutzmayer S, et al. Sharpin prevents skin inflammation by inhibiting TNFR1-induced keratinocyte apoptosis. *Elife* 2014;3.
- **45. Rickard JA, Anderton H, Etemadi N, Nachbur U, Darding M, Peltzer N, et al. TNFR1-dependent cell death drives inflammation in Sharpin-deficient mice. *Elife* 2014;3. The two studies above provide genetic proof that inappropriate cell death driven by TNF causes inflammation in the cpdm strain of mice.
46. Yatim N, Jusforgues-Saklani H, Orozco S, Schulz O, Barreira da Silva R, Reis e Sousa C, et al. RIPK1 and NF-kappaB signaling in dying cells determines cross-priming of CD8(+) T cells. *Science* 2015;350(6258):328–34. [PubMed: 26405229]
47. Linkermann A, Hackl MJ, Kunzendorf U, Walczak H, Krautwald S, Jevnikar AM. Necroptosis in immunity and ischemia-reperfusion injury. *Am J Transplant* 2013;13(11):2797–804. [PubMed: 24103029]
- *48. Lau A, Wang S, Jiang J, Haig A, Pavlosky A, Linkermann A, et al. RIPK3-mediated necroptosis promotes donor kidney inflammatory injury and reduces allograft survival. *Am J Transplant* 2013;13(11):2805–18. [PubMed: 24103001]
- *49. Pavlosky A, Lau A, Su Y, Lian D, Huang X, Yin Z, et al. RIPK3-mediated necroptosis regulates cardiac allograft rejection. *Am J Transplant* 2014;14(8):1778–90. [PubMed: 24984764] The two studies above demonstrated that renal and cardiac allografts that are deficient in RIPK3 survived longer suggesting that necroptosis is involved in allograft rejection.
- *50. Linkermann A, Brasen JH, Himmerkus N, Liu S, Huber TB, Kunzendorf U, et al. Rip1 (receptor-interacting protein kinase 1) mediates necroptosis and contributes to renal ischemia/reperfusion injury. *Kidney Int* 2012;81(8):751–61. [PubMed: 22237751] This study showed that inhibiting the kinase activity of RIPK1 ameliorated injury in a kidney ischemia reperfusion model.
51. Newton K, Dugger DL, Maltzman A, Greve JM, Hedehus M, Martin-McNulty B, et al. RIPK3 deficiency or catalytically inactive RIPK1 provides greater benefit than MLKL deficiency in

mouse models of inflammation and tissue injury. *Cell Death Differ* 2016;23(9):1565–76. [PubMed: 27177019]

52. Pimentel-Muinos FX, Seed B. Regulated commitment of TNF receptor signaling: a molecular switch for death or activation. *Immunity* 1999;11(6):783–93. [PubMed: 10626900]
53. Li X, Yang Y, Ashwell JD. TNF-RII and c-IAP1 mediate ubiquitination and degradation of TRAF2. *Nature* 2002;416(6878):345–7. [PubMed: 11907583]
- *54. Chun N, Fairchild RL, Li Y, Liu J, Zhang M, Baldwin WM 3rd, et al. Complement Dependence of Murine Costimulatory Blockade-Resistant Cellular Cardiac Allograft Rejection. *Am J Transplant* 2017;17(11):2810–9. [PubMed: 28444847] Using a cold ischemic storage model that mimics deceased donor transplantation, this study showed that TNF is elevated following cardiac allograft transplants.

Figure 1. Schematic of the cell death checkpoints in the TNFR1 signaling pathway.

(A) In most cells, there are two sequential cell death checkpoints in the TNFR1 signaling pathway and ligand binding to TNFR1 does not trigger cell death. The early checkpoint occurs when RIPK1 is modified by K63- and M1-linked poly-ubiquitin chains catalyzed by the TRAF2/cIAP1/2 and LUBAC E3 ligases, respectively. RIPK1 is in a pro-survival mode and is prevented from associating with the FADD/CASPASE 8 death-signaling complex. This checkpoint is reinforced by IKK-mediated phosphorylation of RIPK1 and CYLD to further inhibit their death-signaling capabilities. The late checkpoint occurs when NF κ B translocates to the nucleus to upregulate expression of pro-survival genes. If these checkpoints function properly, cells do not die in response to TNF.

(B) Failure in the early checkpoint occurs when ubiquitination of RIPK1 is impaired. This can happen when expression or activity of the ubiquitin E3 ligases (e.g., TRAF2, cIAP1/2, LUBAC) is blocked, or when the expression or activity of deubiquitinases (e.g., CYLD) is

elevated. A reduction in ubiquitin chains on RIPK1 promotes its interaction with FADD and CASPASE 8 to induce apoptosis, or with RIPK3 to induce necroptosis if apoptosis is inhibited. The RIPK1-dependent death can then lead to inflammation.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. TNF death sensitivity contributes to allograft rejection in multiple ways.

(A) Following extended cold storage and subsequent surgical implantation, the donor organ undergoes ischemia reperfusion, which could cause cell death and injury. We propose that TNF-mediated death contributes to the IR injury and the degree of cell death is determined by the expression level of early checkpoint regulators intrinsic to the donor organ. For example, if cells within the organ from a particular donor express high level of LUBAC but low level of CYLD, those cells would be more resistant to TNF-induced cell death. That organ may be less prone to injury and rejection. Conversely, a donated organ with low LUBAC but high level of CYLD expression would be more sensitive to cell death and more likely to be rejected.

(B) In a susceptible donor organ, debris from cells dying from either RIPK1-dependent apoptosis or necroptosis contain allo-antigens and damage-associated molecular patterns (DAMPs). Pattern-recognition receptors on dendritic cells sense these DAMPs resulting in

their activation and enhanced presentation of allo-antigens to T cells. The activated allo-reactive T cells then kill donor cells by producing IFN γ and releasing cytotoxic granules containing perforin. The initial TNF-induced cell death occurring during IR eventually lead to the induction of T cell effectors that now destroy donor cells.

(C) In addition to perforin-mediated lysis, activated allo-reactive CD8 T cells can also express TNF. In a death-susceptible organ due to lowered expression of pro-survival regulators of the early checkpoint, donor cells can also be killed by the TNF released from the CD8 T cells. Furthermore, other immune cells (e.g., macrophages, monocytes and NK cells) that are also recruited to the donor organ can also produce TNF. These other TNF-producing cells are now cytotoxic if the donor cells have a defective checkpoint and now interpret TNF to be a death signal. The overall balance between pro-survival and pro-death molecules of the TNF pathway determines cell fate and graft survival. Therapeutic agents that reinforce the early checkpoint and block death-signaling proteins could be useful in enhancing allograft survival.