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TNF, Apoptosis and Autoimmunity: A Common Thread?

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ABSTRACT: A subset of cytokine mediators belonging to the tumor necrosis factor (TNF) family cause apoptosis, acting through receptors and signaling pathways that have recently come to light. Further, at least one autoimmune disease results from a defined defect of apoptosis (mutations of the Fas ligand or its receptor). It is offered that many, and perhaps most autoimmune diseases may result from primary defects of apoptosis. Such defects may cause reflexive overproduction of TNF and other pro-apoptotic cytokines. The collateral damage produced by these mediators may be of pathogenetic importance in complex autoimmune disorders such as rheumatoid arthritis and Crohn disease, wherein TNF blockade is known to have ameliorative effects.

Keywords: cytokine, tumor necrosis factor, Fas ligand, receptor, autoimmunity

INTRODUCTION

Conventional wisdom holds that proinflammatory cytokines coordinate, and in fact, cause most of the macroscopic events that are witnessed during inflammation. The swelling, accumulation of leukocytes, tissue damage, and systemic consequences of inflammation are widely and correctly ascribed to the receptor-mediated effects of these cytokines. The casual observation that inflammation attends most forms of autoimmune disease has stimulated the suggestion that cytokines may also be ''involved'' in autoimmunity. In fact, it has been suggested that over-production of certain cytokines, including TNF and IL-1, may be of primary importance in the pathogenesis of autoimmune disease. Numerous studies have been designed to detect correlations between cytokine gene polymorphisms and various forms of autoimmunity, in the thought that overproduction or underproduction of a given cytokine might set the stage for autoimmune disease (1-16). And in recent years, highly specific blockade of TNF, IL-1, and other cytokines has been utilized in the therapy of certain autoimmune diseases, with remarkable success in some selected cases (17-20).

But what is the etiologic link between cytokines and autoimmune disease? Do cytokines merely act to execute the final inflammatory events that lead to disease? Or are they of primary pathogenic importance? Certain clues have emerged from detailed analyses of the TNF receptors, other members of the TNF receptor family, and the signaling molecules that serve these receptors.

The Intertwined History of TNF and Fas Ligand

At least in part, the tumor-necrotizing activity for which TNF was named depends upon its ability to induce programmed cell death (apopto-

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sis) of certain tumor cells (21,22), both *in vitro* and *in vivo*. The isolation of TNF was predicated upon an assay for apoptosis (23-25). An independent line of work, carried out in two separate laboratories, led to the identification of monoclonal antibodies that would induce apoptosis of tumor cells (26-29). These antibodies recognized the Fas antigen (30), also known as APO-1, and later, as CD95. The interaction between monoclonal antibodies and this surface antigen initiated programmed cell death, independent of the presence of complement.

Early speculation held that the Fas antigen might actually constitute the receptor for TNF. Ultimately, however, it emerged that TNF has two separate receptors (31-36), both structurally distinct from the Fas antigen, yet belonging to the same family of receptor molecules. One of the TNF receptors (p55) and the Fas antigen (hereafter called the Fas receptor) share a common mode of signaling; i.e., they induce a proteolytic cascade within target cells that eventually leads to programmed cell death. Moreover, the Fas ligand, identified in 1994 (37), proved to be a cell-anchored homolog of TNF (which is predominantly a secreted protein).

The normal cellular target for TNF's death-promoting activity remains unknown. However, a clear cellular target for the Fas ligand has been inferred through studies of mutant mice, incapable of producing either the Fas ligand (in the case of mice homozygous for the *Gld* mutation (38,39) or the Fas receptor (in the case of mice homozygous for the *Lpr* mutation (40,41). Such animals (and humans with homologous mutations (42) develop a polyclonal lymphoproliferative disease, in which an unusual CD4-, CD8-, T-cell population accumulates to an enormous number in the lymph nodes and spleen. Concommitantly, a lupus-like disorder develops, marked by the presence of anti-nuclear antibodies, renal damage, and other stigmata of systemic autoimmunity (43-46). It may be inferred that defective apoptosis is responsible for the accumulation of the lymphoid cells in animals with germ-line defects of the Fas ligand or its receptor. The mechanistic link between the accumulation of these cells and the development of the autoimmune phenotype remains uncertain. It is not clear that the cells that accumulate are actively autoaggressive. They may, in fact, serve a regulatory role.

Mutations of TNF (47,48) and the p55 TNF receptor (49,50) do not cause overt autoimmune disease. On the contrary, blockade of TNF activity may retard or entirely prevent the subsequent development of at least one autoimmune disease (type I diabetes mellitus) (51,52). Moreover, while the effect is more subtle than that rendered by knockout of the Fas ligand or receptor genes, TNF blockade does lead to the accumulation of lymphoid cells following adoptive transfer (53). As detailed below, this finding may point to a role for TNF in the apoptotic removal of a class of lymphoid cells that normally dampens the immune response.

The Structure and Function of TNF Receptor Family Members

The 17 known members of the TNF receptor family (Figure 1) share a common cysteine-rich motif repeated two to six times in the extracellular domain. The proteins are often depicted as trimeric because their ligands are mostly trimeric, and because co-crystallization of the ligand lymphotoxin and the p55 TNF receptor revealed that three receptor monomers could interact with a single ligand trimer (54). However, there are several excellent reasons to believe that the receptors are actually dimeric. First, the crystal structure of unliganded TNF receptor extracellular domain suggests a dimeric interaction between subunits (55,56). Second, the CD27 molecule, known to be a member of the TNF receptor family, is a dimeric protein, maintained as such in the unliganded state by an intersubunit disulfide bond (57,58). By analogy, it would be expected that other members of the family might be dimers as well. Third, the nerve growth factor receptor (NGFR) engages a dimeric ligand (NGF) (59). It is difficult to

see how binding to a molecule with two-fold axial symmetry might be accomplished by a triad of receptors. Fourth, it is known that certain monoclonal antibodies against TNF have agonist activity without addition of secondary antibodies; hence, two subunits, correctly juxtaposed, can signal the presence of ligand (60). Fifth, obligate dimers of the TNF receptor, produced by fusing the erythropoietin receptor extracellular domain (61) or the platelet-derived growth factor extracellular domain (62) to the ''stem,'' trans-membrane domain, and cytoplasmic domain of the TNF receptor, signal constitutively (in the case of the EpoR chimera) or in a ligand-dependent fashion (in the case of the PDGF chimera). Hence, two subunits are sufficient for signaling. Sixth, secreted versions of the TNF receptor, lacking a transmembrane domain but possessing both extracellular and cytoplasmic domains, are dimeric and are capable of binding TNF with high avidity (63). Similarly, virally encoded versions of the TNF receptor, which arose in the course of evolution as a means of neutralizing TNF, are secreted dimeric proteins (64-68). Based on these observations, it is reasonable to assume that all members of the TNF receptor family exist on the plasma membrane as preformed dimers.

It is frequently asserted that TNF ''crosslinks'' or ''aggregates'' receptor subunits on the cell surface. However, accepting that the TNF receptor is a preformed dimer floating within the plane of the membrane, it stands to reason that coalescence of two receptor subunits is not the critical issue in signal initiation. Rather, TNF binding must induce a change in the physical interaction between subunits that are already paired. In short, a conformational alteration is the basis of signal initiation. The transition between the unliganded and liganded form of the receptor likely involves rotation and displacement of each receptor subunit relative to the other (69).

There are two basic structural divisions within the TNF receptor superfamily. Certain members of the group display a motif commonly known as the

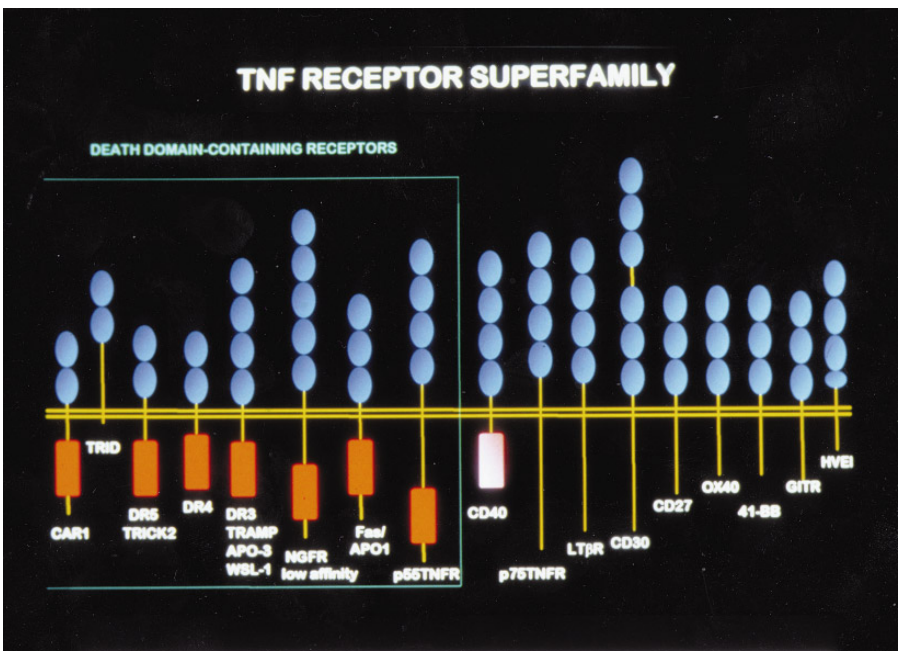

Figure 1. The 17 known members of the TNF receptor family. Repeating units indicate cysteine-rich domains that make up the bulk of the extracellular portion of the molecules. Receptors on the left portion of the illustration bear a cytoplasmic death domain; those on the right do not; the CD40 molecule bears an ambiguous version of this motif.

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''death domain'' on the cytoplasmic side of the membrane (70), whereas other members do not. Receptors bearing death domains are believed to be associated in signal transduction leading to an apoptotic endpoint, while other receptors rely upon a separate category of signaling molecules (most notably proteins with ring-finger and/or zinc-finger motifs, collectively known as TRAFs) (71-82) to achieve their effects, which include induction of proliferation (83-86) and perhaps the production of inflammatory mediators as well as cell death (87-89).

The death domain is apparently a protein interaction domain. It may be assumed, based on the dimeric structure of the receptor itself, that the death domain has a propensity to undergo dimerization. It may, however, form homodimers, maintaining contact with paired receptor subunits, or heterodimers, maintaining contact with signaling molecules that also bear versions of the death domain. Presumably, the exchange of a homotypic association between receptor death domains for a heterotypic association is the cardinal event in proximal signal transduction.

Ring and zinc-finger proteins, collectively known as TRAFs (TNF Receptor Associated Factors) bind directly to the receptor cytoplasmic domain and or to one another in homotypic and/or heterotypic interactions. They are believed to convey certain proliferative and pro-inflammatory responses to members of the ligand family. This is accomplished, at least in large part, through activation of NF-κB, a transcription factor involved in numerous proliferative and pro-inflammatory events.

As a generalization, it may be said that signal transduction leading to programmed cell death has been more fully deciphered than signal transduction leading to proliferation. Further, the probable relationship between apoptosis and autoimmune disease makes a detailed understand-ing of this aspect of signaling initiated by TNF and its homologs absolutely essential. The remainder of this review will focus chiefly upon the apoptotic pathway, offering an hypothesis as to the precise relationship between TNF and autoimmune diseases.

Coupling to Signaling Intermediates

One of the first signaling intermediates utilized by the Fas receptor was a protein, identified through use of the yeast two-hybrid system. It was dubbed FADD (90), or MORT-1, and was found to contain a death domain through which it could interact with the death domain of the receptor. Interestingly, the Lpr^{CG} mutation (91), a structural error in the death domain which permitted surface expression of the Fas antigen but abolished signal transduction through this receptor, was shown to prevent interaction between FADD/MORT-1 and the cytoplasmic domain of the receptor if introduced into the former (90). Hence, the physical basis for a specific autoimmune disease was deciphered in exquisite detail by determination of the most proximal signal transduction intermediate to service the receptor. TRADD (92), RIP (93), and RAIDD (94), related molecules with death domains, were found to engage the TNF receptor, the Fas receptor, and other death domain receptors as well (95).

In the case of FADD, the amino-terminal half of the protein was shown to be of critical importance in further signal transduction events (90). The amino terminus of activated FADD engages MACH (96), or FLICE (97), a cysteine protease of the ICE family of molecules, now termed caspase 8. It may also engage FLICE2 (98), now known as caspase 10. TRADD is believed to effect activation of caspase 2 through coupling with RAIDD, which bears homology to caspase 2 (94). And RIP, which has a kinase domain as well as a death effector domain (93), signals for caspase activation and may also cause phosphorylation of cellular targets yet unknown.

The end effect of caspase activation is the cleavage of various protein targets within the cell. Among these is polyADP ribosylpolymerase (PARP) (99-103), a chromatin-associated enzyme normally concerned with DNA which, when over activated by cleavage at a specific site, can consume NAD reserves, bringing about cell death. Undoubtedly, many targets of the caspase cascade have yet to be identified.

TRAFs, once activated by ligand binding, can cause the dissociation of NF-κB from IκB, leading to nuclear translocation of NF-κB and ensuing gene activation (71,72,74-77,79,104, 105). This is achieved, at least in part, through interaction with additional ring finger proteins of a group known as c-IAPs (80). TRIP (106) and I-TRAF (78), on the other hand, act to inhibit TRAF signaling, each acting through separate mechanisms.

Interactions between members of the ring-finger transducer family and members of the death domain transducer family are known to occur, and may account for the fact that the p75 TNF receptor can, under some conditions, initiate programmed cell death. TRADD has been shown to bind directly to TRAF-1/TRAF-2 heterodimers (92), and a novel caspase termed ''Casper'' has been shown to bind to TRAF-2 as well (107). It has been shown in several studies that proliferative and inflammatory signals may emanate from the p55 receptor as well as the p75 receptor (108-111). The simple assumption that one might dissociate inflammatory from apoptotic effects of TNF by devising receptor-specific agonists has, therefore, become untenable.

Virtually every aspect of the TNF/Fas signal transduction pathway has been manipulated by viruses, which, in the course of evolution, have acquired proteins that allow them to evade the apoptotic and pro-inflammatory effects of the host immune response (65,67,112-119). They include secreted mimics of the TNF receptor which act to neutralize the ligand, direct inhibitors of caspase activity, and proteins that interfere with coupling between the receptor and proximal transducers by virtue of their content of death effector domains. The very fact that viruses have captured and utilized components of the cell death signaling pathway bespeaks the importance of the pathway as a means of countering viral cytopathic effects.

Mutational Basis of Autoimmunity: A "Two Hit" Hypothesis

In autoimmunity, as in neoplasia, unwanted cell proliferation lies at the heart of the dysfunction that is macroscopically observed. Within the immune system, control of cell proliferation is an issue of central importance. The normal response to a pathogen entails transient cell proliferation, followed by removal of the expanded clones that were called upon to deal with host invasion. ''Expansion'' likely involves not only cells with specificity for the pathogen, but an array of supportive cells as well. The same may be said of the process of clonal elimination. Hence, the immune system is constantly in a state of flux, with various components undergoing expansion and contraction in response to environmental demands. This plasticity carries with is a certain risk. It is likely that failure of mechanisms for expansion or elimination of specifically reactive cells, or their supporting cohorts, might occur on a clonal basis. The consequences of such malfunction might vary greatly, depending upon the number of cells that are involved, their specific targets, or their precise regulatory functions. However, it is not unreasonable to suppose that failure of clonal expansion or elimination might be an essential aspect of autoimmune disease.

It is know with certainty that at least one autoimmune disease: systemic lupus erythematosus, is faithfully modeled by a monogenic germline defect, which prevents signal transduction through the Fas ligand/Fas receptor axis. Hence, as noted above, *Gld* or *Lpr* mice, lacking functional ligand or receptor molecules, respectively, develop lympha-denopathy and autoimmune disease as a result of the failure to clear an unusual class of CD4-CD8- TCR+ cells. The unwanted accumulation of cells is, in this case, polyclonal. But what, one might ask, would happen if only a fraction of the lymphoid population was burdened with the mutation? Would an autoimmune phenotype result? And if so, what characteristics might it have?

A tantalizing glimpse at the question is offered by a single human patient with SLE, in whom heterozygosity for a gross mutation of the Fas receptor was observed (120). It might be inferred that a second mutation, occurring in critical somatic cells, inactivated the second locus, and was the causative event in the observed disease. Unfortunately, no verification of this possible explana-

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tion could be obtained on the basis of family studies or analysis of peripheral blood leukocytes since neither analysis could be performed (J. Mountz, personal communication).

It is now universally accepted that many human and animal cancers result from ''recessive oncogenes''; i.e., from mutations that destroy both copies of a critical growth-regulatory gene within a single cell. Typically, familial cancers result from germline transmission of a defective gene of this sort (e.g., transmission of a mutation of the Rb gene), with superimposed acquisition of a second, somatic mutation that disrupts function of the remaining, intact allele. In the a tissue that requires the regulatory function that the gene conveys, neoplasia may develop in the absence of the protein concerned. A ''two hit'' mechanism may account for many sporadic cancers as well as familial ones. Familiarity is an indisputable characteristic of autoimmune disease. Yet strict Mendelian inheritance is unusual, consistent with the fact that only one form of autoimmunity- that caused by mutations of the Fas ligand or its receptor- is monogenic. Indeed, even in identical twins, concordance rates for many autoimmune diseases, from type I diabetes to multiple sclerosis, seldom exceed 50%. It may therefore be accepted that stochastic or environmental events influence the development of autoimmunity, yet it is also obvious that autoimmune diseases are primarily genetic.

With regard to environmental factors that permit the development of an autoimmune disease, the possible role of viruses, and the putative importance of epitopic structures shared by pathogens and host tissues are frequently cited. However, no specific agent has been identified as a causative factor in *any* human autoimmune disease. Indeed, it is not certain that any infectious agent is of importance, and the element of chance may have an entirely different explanation.

As to the genetic propensity toward the development of certain autoimmune diseases, linkage studies have implicated components of the major histocompatability complex (MHC) as probable participants in the process. However, hundreds of genes reside within the MHC region or in close proximity to it. The identity of those genes that confer genetic predisposition to autoimmunity remains to be determined. A preponderance of opinion suggests that structural variation in the class I and/or class II genes, which act to present antigens to T lymphocytes, is of decisive importance in the development of autoimmune disease.

It has often been suggested that overproduction of TNF or its homologs might be of primary importance in autoimmune disease. And in the context of the ''two hit'' model of autoimmunity introduced above, it might be thought that mutational inactivation of TNF or its homologs might create an autoimmune phenotype. In this connection, it is worth noting that at least three MHC-linked genes are members of the TNF ligand family (those encoding TNF itself, LT-α, and LT-β). However, the direct involvement of these genes in autoimmune processes remains a tenuous proposition. First, no naturally occurring mutations that clearly affect the structure or expression of any of the ligand genes have ever been identified, either in patients with autoimmune diseases or in any other individuals. Second, in two specific autoimmune diseases of mice (the NZW/NZB model of SLE, and the NOD model of type I diabetes mellitus), differences in TNF mRNA production arising from the autoimmune-prone allele as compared with a control allele were effectively discounted (121). And third, targeted germline mutations of the genes encoding these ligands fail to create an autoimmune phenotype, at least in the absence of other genetic lesions. Given that mutations affecting the structure or antigen-presenting ability of MHC antigens are of permissive importance in the development of autoimmune diseases, it is still granted that other genetic factors are at work. As such, in murine models of autoimmunity, several unlinked loci confer susceptibility to the development of type I diabetes or SLE. Hence, there is still room for the possibility that genes affecting the involution of lymphoid cells might be essential to the autoimmune phenotype.

From the foregoing discussion, it is clear that a collection of proteins participate to sense an external signal mandating cell death, and to bring

this signal to a productive conclusion. The entire cascade includes ligands (e.g., TNF, lymphotoxin, Fas ligand, and others), receptors for these ligands, primary transducers (e.g., FADD, RIP, RAIDD, and TRADD), secondary transducers (e.g., FLICE, FLICE2, and other caspases), and end targets of the proteolytic cascade (e.g., poly ADP ribosylpolymerase (PARP), laminin, and other proteins within the cell). Components of the death cascade act in series- and in some cases, in parallel with one another- to avert autoimmune disease by conveying an apoptotic signal within lymphocytes that are in some way ''intended'' for death. Mutations affecting any of the components of the apoptotic cascade might possibly eventuate autoimmune disease.

In this scheme (Figure 2), the target tissue in an autoimmune disorder would depend upon several factors. First, the gene that is inactivated might specify a protein that is of critical importance in only a limited subset of lymphoid cells. Hence, loss of gene function might have consequences only in the development of a restricted group of autoimmune disorders. Given that a germline mutation is required for the development of a certain autoimmune disease, the ''second hit'' might occur in one cell or in many; its significance might depend upon whether the cell ever undergoes antigen-driven clonal expansion. In this manner, chance events (as well as heredity) might be brought to bear in the ''decision'' as to whether a certain autoimmune disease is to occur.

The role played by TNF and its signaling cascade might be a direct or indirect one. Specifically, mutational inactivation of TNF or its signaling intermediates within a certain subset of somatic cells might be primarily responsible for the development of an autoimmune disorder, much as germline mutations of the Fas ligand or receptor are responsible for the development of an autoimmune disorder. On the other hand, overproduction of TNF, occurring as a reflexive response to mutations affecting other death pathways, might constitute an indirect, or secondary lesion in an autoimmune disease. These two scenarios are not mutually exclusive; it is possible that each might occur in separate autoimmune diseases.

To elaborate on the second scenario, it may be imagined that a population of cells that is destined for apoptosis normally emits signals that lead to its demise. If this were not so, then the involution of lymphoid cells that occurs under many physiologic circumstances might prove non-specific. Whatever the nature of the signal, it would be anticipated that it would evoke the production of death-inducing cytokines such as TNF, Fas ligand, and other members of the ligand family. These, in turn, would bind to receptors on the surface of the targeted lymphoid cells, normally causing their elimination. However, if a lesion in the death signaling pathway prevents removal of these cells, they would reasonably be expected to increase in number (as occurs in lymphoproliferative disease), or at least, to remain static in number. The perpetuation of feedback signals for production of pro-inflammatory cytokines would logically lead to the development of an inflammatory lesion, as is characteristic of all autoimmune disorders (Figure 3).

The fact that reagents which neutralize TNF can ameliorate rheumatoid arthritis and Crohn disease is consistent with this secondary model of TNF function in autoimmunity. It may be imagined that TNF is overproduced as the end result of a futile feedback loop, intended to assure the destruction of lymphoid clones that can no longer be eliminated through activation of the apoptotic pathways normally in place. Presumably for this very reason, TNF may be detected in plasma or synovial fluid obtained from patients with rheumatoid arthritis (122-128), whereas it is absent from the joint fluid of normal individuals, or those with degenerative joint disease. The finding that anti-TNF therapy leads to an elevated leukocyte count in the peripheral blood (17,18,129,130)might be taken as evidence that, to a limited extent, TNF is capable of holding the defective lymphocyte population in check. The observation that anti-TNF therapy yields a therapeutic effect that long outlasts the presence of inhibitory antibody (17,18,129,130) may be taken to indicate that a relatively long period of time is required for the downstream pro-inflammatory effects of TNF to be felt in the setting of this disease.

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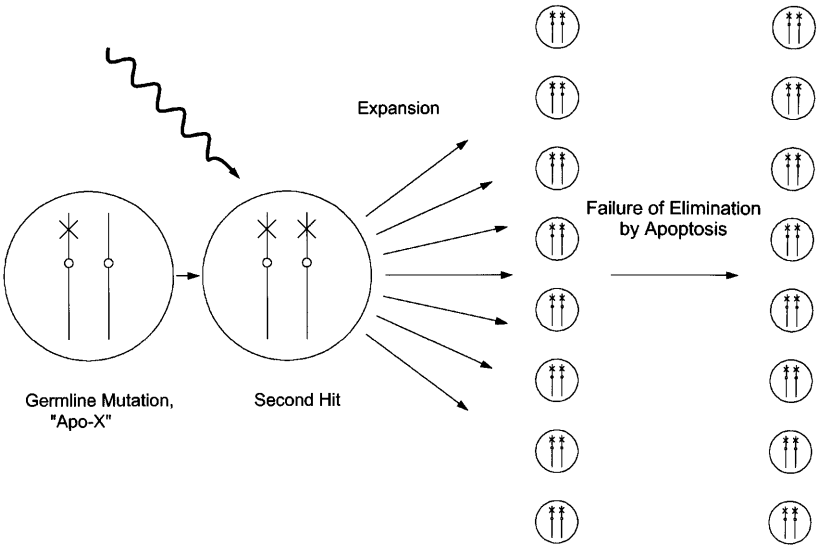

Figure 2. Two-hit model of autoimmunity. A germline mutation of a gene encoding a protein required for cell death might, in some lymphoid clones, be complemented by a second mutation affecting the intact allele. Under those conditions, a lymphocyte called upon to undergo clonal expansion might not be eliminated as normally required. Such cells could, in the case of epitopic similarity to host tissues, be responsible for autoimmune disease.

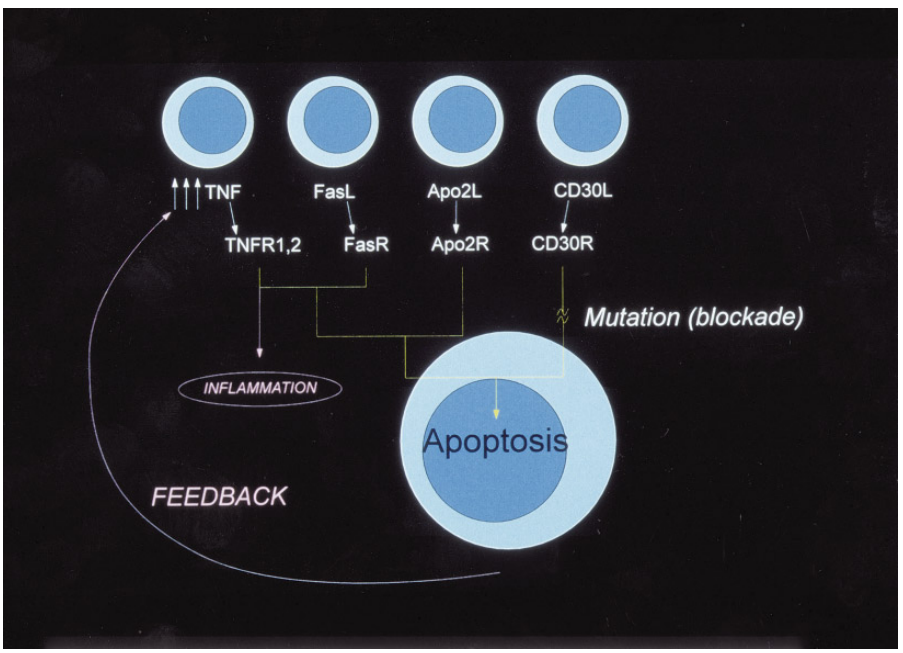

Figure 3. Feedback loop model of autoimmunity. Lesions in the cell death pathway, caused by germline and/or somatic mutation, would lead to a failure of programmed cell death. The cells that accumulate might emit signals that normally lead to their own clearance through induction of death-promoting ligands like TNF. These ligands, while powerless to remove the cells concerned, might trigger inflammatory events in the surrounding environment; hence, the tissue injury and inflammation associated with autoimmunity. Choice of the lesion, ligands, and receptors is, in this example, arbitrary and speculative.

In an entirely different disease model, application of anti-TNF antibody seems to exert a preemptive effect on the development of autoimmunity (51,52). When NOD-scid mice are injected with lymphocytes derived from NOD donors, they develop insulitis and over diabetes over a well-defined time course. The first cases of diabetes are observed after about 100 days; all animals develop diabetes within 120 days. However, early exposure to a chimeric TNF inhibitor (131)seems to entirely prevent diabetes (53). The window of time during which TNF blockade is enforced antedates the development of insulitis, and protection, once established, is essentially permanent (53). Therefore, TNF seems to be required for the development of an effective immune response to auto-antigens, and this requirement may be temporally ascribed to a discrete interval between 40 and 80 days post-adoptive transfer. Concommitant with the protective effect, TNF blockade permits the accumulation of far more adoptively transferred lymphocytes, so that the spleen of mice protected with the TNF inhibitor is twice the size of the spleen of control mice. These cells are predominantly CD4+, and bear antigenic characteristics of Th2 lymphocytes (i.e., they are CD45Rb^{high}). It may be surmised that TNF normally elicits the apoptotic removal of these cells at an early stage after adoptive transfer. When no TNF is available during this stage of lymphoid reconstitution, the cells remain, and in some way interfere with subsequent development of autoimmunity (Figure 4).

TNF, Fas ligand, and other proteins that induce apoptosis may therefore have very different- and even opposite- end effects on immune function. In one case (exemplified by Fas ligand or Fas receptor mutations), disruption of an apoptosis-promoting gene leads to the accumulation of cells that cause autoimmunity. In a second instance (perhaps exemplified by blockade of TNF or mutations affecting the TNF ligand/receptor pair), disruption of an apoptosis-promoting gene might cause the accumulation of cells that prevent autoimmunity (hence, type I diabetes is averted by timely impairment of TNF activity). In yet a third scenario (here proposed as a basic mechanism of rheumatoid arthritis), disruption of an apoptosis-promoting gene might lead to the accumulation of cells that stimulate TNF production, along with the production of other ligands responsible for the initiation of apoptosis; hence, collateral inflammatory damage, treatable by imposition of TNF blockade, may be the result.

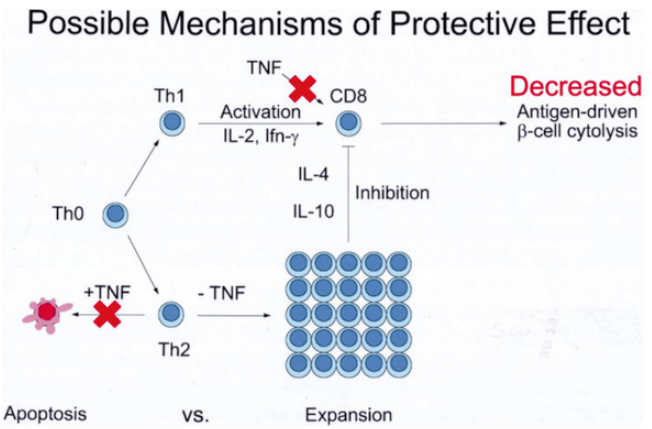

Figure 4. Prevention of autoimmune diabetes by TNF blockade. Inhibition of TNF activity leads to an accumulation of splenocytes with Th2 antigenic characteristics, and may block the function of Th1 cells as well. Through both actions, it may block the final assault on β-cells, thought to be accomplished largely by cytolytic (CD8+) T lymphocytes.

Candidate Genes and Determinants of the Autoimmune Phenotype

A number of predictions follow from the hypotheses offered above. First (and foremost), it is to be expected that mutations will actually be found in heritable autoimmune diseases. The *sine qua non* of a two-hit mechanism is the presence of a germline mutation associated with the disease. Quite possibly, multiple mutations (and even mutations of genes encoding several different death proteins) could yield a common autoimmune phenotype. This is particularly predictable in the case of autoimmune diseases that have broad specificity with respect to the tissues affected. SLE, pan-systemic sclerosis (PSS), and scleroderma, which have many overlapping features and which may, in fact, represent a con-

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tinuum of disease, each affect diverse tissues and organ systems. As noted above, an excellent model of SLE is created by Fas or Fas ligand germline mutations. A two-hit mechanism would call for a second hit rather early in ontogeny, or for the occurrence of several second hits. On the other hand, an autoimmune disease affecting a single, anatomically and histologically restricted tissue (e.g., the thyroid or the β cells of the pancreatic islets) might depend upon a second hit within a very specific lymphoid clone, already endowed with the ability to recognize the target antigen, or otherwise capable of supporting only one type of autoimmune assault.

To a large extent, the phenotype of a mutation is dependent upon the redundancy of the gene in which it occurs: if other genes can replace the function of the gene that was destroyed, the phenotype associated with the mutation may be subtle or inapparent. At present, the degree of redundancy within cell death pathways remains poorly understood. At the level of receptor/ ligand interaction, rather little redundancy is apparent. There is a single ligand for the Fas receptor, for example, and while two ligands may serve the p55 and p75 TNF receptors, these ligands (TNF and lymphotoxin) are produced under rather different conditions. Beneath the membrane, it has been shown that both FADD and TRADD can interact with the p55 TNF receptor. As such, a mutation of either proximal transducer might be partially compensated. Caspase 8 (MACH or FLICE) and caspase 10 (FLICE-2) may each be coupled to the same upstream signaling intermediates. On the other, they may have different target specificities; hence the loss of either might have different end effects.

The polygenic character of most autoimmune diseases suggests that targeted deletion of genes encoding cell death proteins may be inadequate to expose their participation in autoimmunity. As an alternative approach, it might be preferable to analyze such genes in patients with authentic autoimmune disorders, or in animal models. The mapping of susceptibility loci in several such diseases (132,133) might make permit a more focused effort toward this end.

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