

CD95's deadly mission in the immune system

Peter H. Krammer

Tumorimmunology Program, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

Apoptosis in the immune system is a fundamental process regulating lymphocyte maturation, receptor repertoire selection and homeostasis. Thus, death by apoptosis is as essential for the function of lymphocytes as growth and differentiation. This article focuses on death receptor-associated apoptosis and the role of CD95 (Apo-1/Fas)-mediated signalling in T-cell and B-cell development and during the course of an immune response. Gaining an insight into these processes improves our understanding of the pathogenesis of diseases such as cancer, autoimmunity and AIDS, and opens new approaches to rational treatment strategies.

The immune system is a society of interacting cells consisting of T and B lymphocytes, natural killer (NK) cells, macrophages and professional antigen-presenting cells (APCs) and their various subclasses. Most cellular components of the immune system are born in the bone marrow. B lymphocytes, NK cells and macrophages mature in the bone marrow and in the fetal liver. T lymphocytes mature in the bone marrow and in the thymus. T-cell and B-cell development share many features, yet differ in others (Fig. 1).

B cells

B cells express cell membrane receptors (antibodies) with a single antigen specificity. Millions of different B cells produce antibodies and can potentially capture millions of antigens. The sum of all antibody specificities is called 'the antibody repertoire'¹. B cells are selected in the bone marrow on the basis of the affinity of their antibodies: cells with high affinity for proteins derived from 'self' tissues are eliminated. Mature B lymphocytes leave the bone marrow and populate the secondary lymphoid organs, spleen and lymph nodes and the gut-associated lymphoid tissue. Once activated by an antigen, B cells undergo a second round of selection in the follicles of secondary lymphoid organs, after which they mature into plasma cells that produce and secrete antigen-specific antibodies, and then recirculate to the bone marrow².

T cells

Pre-T lymphocytes emigrate from the bone marrow into the thymus. In the thymus, they mature and are positively or negatively selected, depending on the affinity of their T-cell antigen receptors (TCRs) for self major histocompatibility (MHC) antigens. MHC class I and II antigens are molecules that sample peptide fragments from foreign and self proteins, respectively. They display these peptides at the cell's surface for scrutiny by T cells — a process called 'antigen presentation'. Each MHC class I or II protein presents a different fragment; thousands of MHC molecules protrude from every cell. Most of the peptides presented in the thymus are derived from self proteins. T cells with a high affinity for self MHC molecules and peptide are eliminated to ensure tolerance to normal tissues and to prevent autoimmunity. T cells that interact with MHC class II molecules develop into cells that express the CD4 molecule on their surface (CD4⁺), and those with affinity for MHC class I molecules turn into T lymphocytes that carry the CD8 antigen (CD8⁺). Only

mature T cells that produce a functional TCR leave the thymus and populate the secondary lymphoid organs. Mature CD4⁺ T cells function as helper T cells and secrete cytokines that regulate either cellular immune responses (T helper 1 cells) or antibody responses (T helper 2 cells). Mature CD8⁺ T cells function as cytotoxic effector (killer) cells³ (Fig. 2).

Cells of the immune system work as a team. After an attack by infectious agents, professional APCs, dendritic cells in particular, present antigenic peptides of these infectious agents to T cells⁴. Antigen–MHC complexes, co-stimulating cell-surface molecules on APC and cytokines drive T cells into clonal expansion. These T cells, in turn, then communicate with other T or B cells to regulate their responses. After a peak phase with the highest clonal expansion of reactive cells, the immune response undergoes a down phase. Most lymphocytes are eliminated; the few that survive constitute the pool of memory cells⁵.

Life and death in the immune system

Several features of the immune system are unique. One is its specificity: the repertoire of T and B lymphocytes, initially built from randomly selected antibody and TCR variable-region genes, is shaped by selection to cope, on the one hand, with the vast universe of antigens and, on the other hand, with the danger of autoimmunity³. Another distinctive feature is its homeostatic control: after a clonal expansion phase, antigen-reactive lymphocytes must be titrated back until the pool of lymphoid cells reaches the baseline level again⁶. This is achieved by balanced fine-tuning between growth/expansion and death by apoptosis; generally, the immune system produces more cells than finally needed, and extra cells are eliminated by apoptosis.

Apoptosis is the most common form of death in cells of the immune system. It is astounding how many different pathways immune cells can choose from to die. In principle, death can be by neglect when the antigen-specific receptors of the lymphoid cells are not stimulated or the lymphocytes are deprived of trophic cytokines. In a more active form, death can involve the death-receptor/death-ligand systems^{7–9}. Apoptosis is such a central regulatory feature of the immune system that it is not surprising that too little or too much apoptosis results in severe diseases.

The CD95–CD95L death system

A subset of tumour necrosis factor receptor (TNF-R) family members is involved in death transducing signals and are

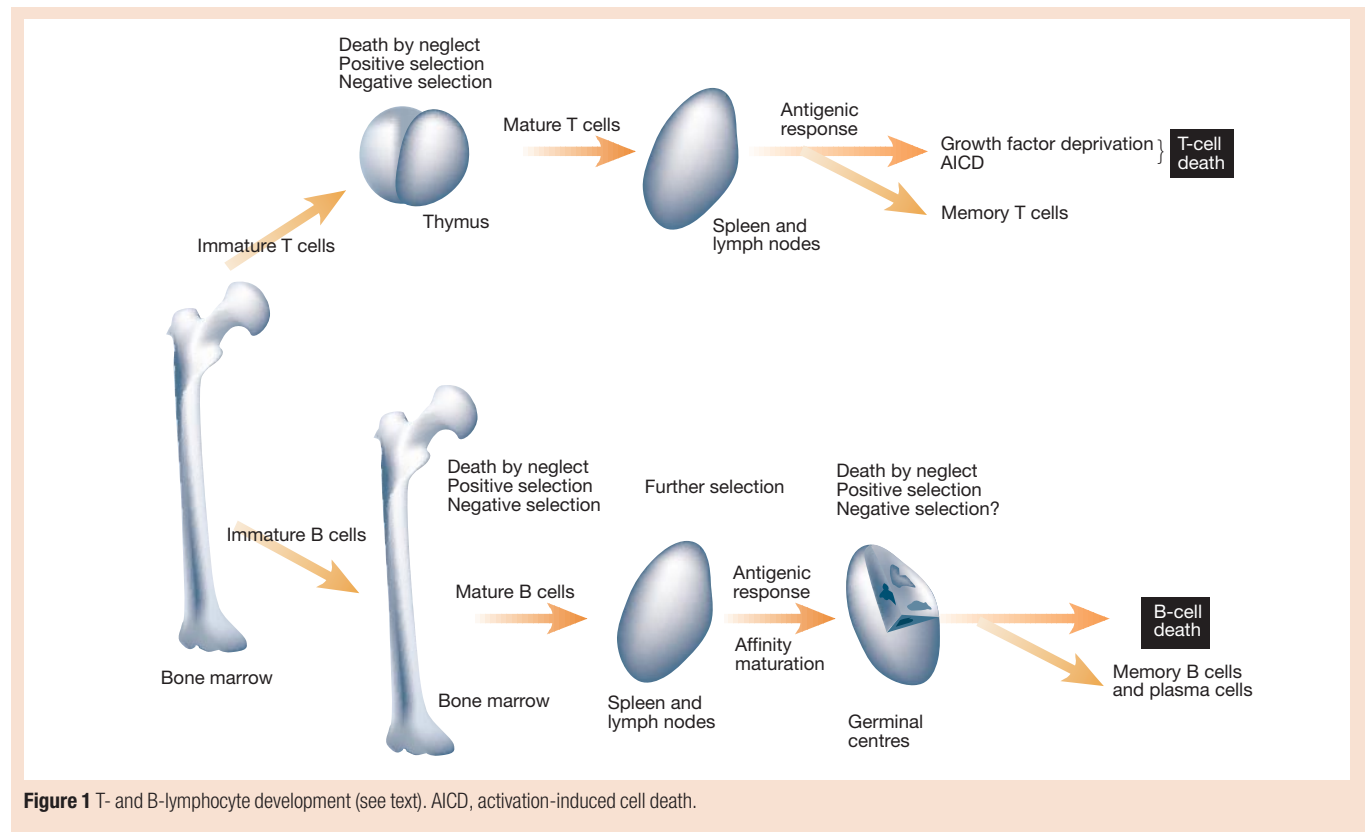


Figure 1 T- and B-lymphocyte development (see text). AICD, activation-induced cell death.

therefore referred to as the ‘death receptors.’ Members of this family contain one to five cysteine-rich repeats in their extracellular domain, and a death domain in their cytoplasmic tail. The death domain is essential for transduction of the apoptotic signal. CD95 is one such family member and has a significant role in the immune system and outside. It is a widely expressed glycosylated cell-surface molecule of relative molecular mass ~45,000–52,000 (335 amino-acid residues). It is a type I transmembrane receptor, but alternative splicing can result in a soluble form, the function of which is unclear¹⁰. CD95 expression can be boosted by cytokines such as interferon- γ and TNF but also by the activation of lymphocytes^{11,12}. CD95-mediated apoptosis is triggered by its natural ligand, CD95L, which is a TNF-related type II transmembrane molecule⁷ and expressed in a far more restricted way than the receptor. Killer cells (so-called cytotoxic T lymphocytes) remove, for example, virus-infected cells, and those that express CD95L can do so by interacting with the CD95 receptor^{13,14} on their targets (Fig. 2). CD95L is seen on killer cell-derived vesicles^{15,16}, but can also be cleaved from the membrane by a metalloprotease^{17–19}; whereas soluble human CD95L can induce apoptosis²⁰, soluble mouse CD95L cannot²¹.

The CD95 death-inducing signalling complex

The oligomerization, most probably the trimerization, of CD95 is required for transduction of the apoptotic signal. A complex of proteins associates with activated CD95 (refs 8, 22). This death-inducing signalling complex (DISC) forms within seconds of receptor engagement. First, the adaptor FADD (Fas-associated death domain protein, also known as Mort1) binds via its own death domain to the death domain in CD95. FADD also carries a so-called death-effector domain (DED), and, again by homologous interaction, recruits the DED-containing procaspase-8 (also known as FLICE) into the DISC. Next, procaspase-8 is activated proteolytically and active caspase-8 is released from the DISC into the cytoplasm in the form of a heterotrimer of two small subunits and two large subunits²³. Active caspase-8 cleaves various proteins in the cell including procaspase-3, which results in its activation and the completion of the cell death

programme. Various other proteins have been described to bind to activated CD95 and the DISC, but their precise role and importance in the regulation of apoptosis remain to be defined.

Recently, with the use of fluorescence resonance energy transfer, another model of CD95 signalling has been worked out. Extracellular pre-ligand-binding assembly domains (PLADs) were described for CD95 and TNF-R, which are supposed to aggregate the receptors before ligand binding. To prevent the premature signalling of pre-associated receptors, which is a dangerous situation, intracellular receptor-associated apoptosis blockers were postulated^{24,25}. On the basis of the PLAD model it is not entirely clear how ligand binding interferes with PLAD association and leads to receptor association, which initiates apoptosis. More structural work is needed to resolve these issues. It is also unclear whether the DISC model and the PLAD model complement each other to describe initial signalling events *in vivo*.

Whereas some cytotoxic T lymphocytes kill their target cells by turning on the death receptor, others use perforin and granzyme B (GrB) to eliminate infected cells. With the help of perforin, GrB finds its way into the target cell and can kill it by directly cleaving and activating caspase-8 (ref. 14) (Fig. 2).

Two different pathways downstream of CD95

In so-called type I cells²⁶, the death signal is propagated by a caspase cascade initiated by the activation of large amounts of caspase-8 at the DISC, followed by a rapid cleavage of caspase-3 and other caspases, which in turn cleave vital substrates in the cell. But in type II cells²⁶ hardly any DISC is formed, so the caspase cascade cannot be propagated directly but has to be amplified via the mitochondria. Caspase-8 cuts the Bcl-2 family member Bid; truncated Bid ‘activates’ the mitochondria^{27,28}. Mitochondria are ‘activated’ in both type I and type II cells, but are not strictly necessary for the death of type I cells. On ‘activation’, mitochondria release pro-apoptotic molecules such as cytochrome *c*²⁹ and Smac/DIABLO^{30,31}. Together with the apoptosis protease-activating factor Apaf-1 and procaspase-9 in the cytoplasm, these molecules form the so-called apoptosome, the second initiator complex of apoptosis (see review in this issue by Hengartner,

pages 770–776). Caspase-9 activates further downstream caspases and the end result, again, is apoptosis (Fig. 3). The reason for the differences between type I and type II cells is unclear at present, but perhaps biochemical differences at the receptor level hold the answer. Recently, Bid-deficient mice were generated³² that are resistant to CD95-induced hepatocellular apoptosis. In Bid^{-/-} cells from these mice, mitochondrial dysfunction was delayed, cytochrome *c* was not released, effector caspase activity was reduced and the cleavage of apoptosis substrates was altered. Taken together, these data support the type-I/type-II concept of CD95 signalling.

FLIPs (FLICE-inhibitory proteins)

Additional DED-containing proteins have been found in a certain class of herpes viruses. These proteins contain two DEDs and also bind to the CD95–FADD complex. This inhibits the recruitment and activation of caspase-8, formerly known as FLICE; hence their name FLIPs (for FLICE-inhibitory proteins). In transfected cells, v-FLIP inhibited the apoptosis induced by several apoptosis-inducing receptors (CD95, TNF-R1, TRAMP/DR3 and TRAIL-R1), indicating that these receptors use similar signalling pathways^{33–35}. Two human homologues of v-FLIP have been identified by several groups at the same time and are known under a variety of names. Although it is widely assumed that the cellular FLIPs block apoptosis as well, the data are ambiguous and c-FLIP might be pro-apoptotic or anti-apoptotic depending on the cellular context. Recent results with cells from c-FLIP-deficient mice support the role of c-FLIP as an anti-apoptotic molecule³⁶.

Signalling by other death receptors

Signalling of apoptosis by other members of the death receptor subfamily seems to follow the same basic rules and is initiated by the same sequential steps^{37–39}: (1) ligand binding, (2) receptor trimerization and DISC formation, (3) attraction of the adapter molecule FADD into the DISC, (4) association of procaspase-8, and (5) auto-catalytic cleavage of the procaspase and the formation of active caspase-8 (a heterotetramer). Active caspase-8 then serves as the initiator caspase, activating other further downstream executioner caspases that cleave cellular death substrates, resulting in the morphological and biochemical catastrophe termed apoptosis. The following paragraphs discuss briefly how these signalling pathways are used in the immune system.

T-lymphocyte death in the thymus

The T-cell repertoire is shaped in the thymus by apoptosis and survival signals. A young adult mouse with (1–2) × 10⁸ thymocytes generates between 20 and 40 million new T cells per day⁴⁰. But the number of T cells that leave the thymus and enter the peripheral T-cell pool is only about 2–3% of the number initially generated.

Despite the high death rate of T cells in the thymus, only a limited number of apoptotic cells can be observed in histological sections. Thus, apoptotic thymocytes are removed efficiently and, most importantly, this is achieved without signs of inflammation (see refs 41–43 and the review in this issue by Savill and Fadok, pages 784–788).

Pre-T lymphocytes, after entry into the thymus, differentiate and rearrange their TCR genes. Those T cells that fail to rearrange their TCR genes productively and thus cannot be stimulated by self-MHC–peptide complexes die by neglect. In T lymphocytes of FADD dominant-negative transgenic mice the requirement for pre-TCR signals is bypassed⁴⁴. In these mice, T-cell survival and differentiation are promoted. Because FADD is an essential adaptor of several death-receptor DISCs, these data suggest a role for death receptors at this early stage of T-cell development. Thymocytes that have successfully passed pre-TCR selection mature further, develop into CD4⁺CD8⁺ (double-positive) T cells and undergo further TCR-affinity-driven positive and negative selection on thymic stromal cells. After these selection processes, mature single-positive CD4⁺ MHC class-II-restricted and CD8⁺ MHC class-I-restricted T cells leave the thymus and generate the peripheral T-cell pool. Like crossing several borders, the T cell crosses several checkpoints to ensure self-MHC restriction and self-tolerance.

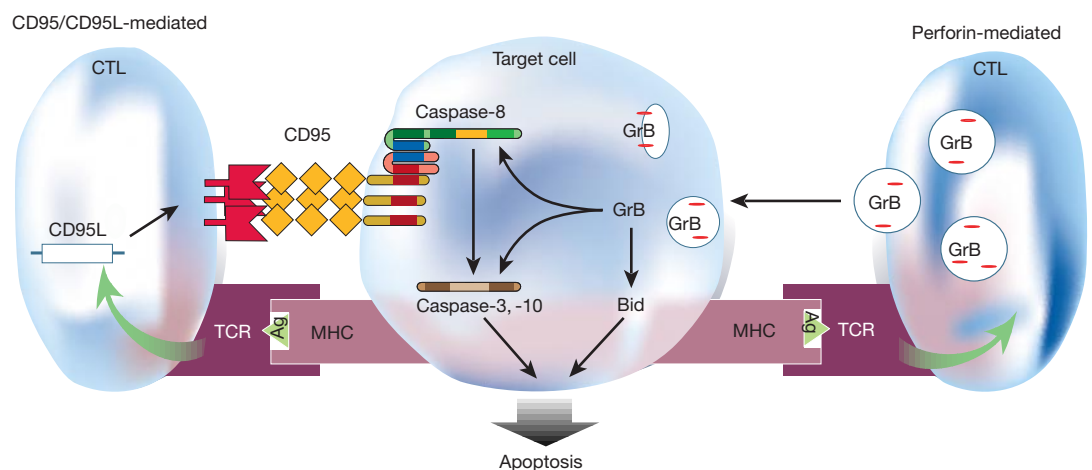
Initially, most investigators agreed that the CD95 system is not involved in negative selection because the TCR repertoire in mice with a defect in this system (*lpr*, *lpr^{g3}* and *gld* mice) was not altered⁴⁵. But on closer inspection it was found that negative selection might involve the CD95 system when T cells encounter high antigen concentrations⁴⁶. The role of other members of the TNF-R superfamily, TNF-R1 and TNF-R2, CD30 and CD40, remains controversial. Similarly, survival signals of thymocytes at different maturation stages remain ill-defined. Numerous data suggest that members of the Bcl-2 family influence survival of immature T lymphocytes, that is, positive selection, but not negative selection³.

Finally, a modulating role in thymocyte survival and apoptosis has been ascribed to several different molecules such as glucocorticoid hormones, cytokines, co-stimulating cell-surface receptors, signalling molecules, transcription factors³ and nitric oxide⁴⁷. In view of the available data, our understanding of the molecular basis of apoptosis and selection of T lymphocytes in the thymus still remains fragmentary.

Deletion of peripheral T cells by apoptosis

Deletion by apoptosis is also observed in mature peripheral T cells. It occurs by neglect in those T cells that are not sufficiently stimulated by growth signals⁴⁸ and, importantly, it occurs at the peak or the down phase of the immune response (Fig. 4)^{49–54} to downregulate the number of reactive cells and to terminate the immune response. This

Figure 2 Cytotoxic T lymphocytes (CTLs) can kill target cells by the CD95 (yellow)/CD95L (red) system (left) or by the perforin/granzyme B (GrB) system (right). CD95 signalling involves a caspase cascade (see text). The entry of GrB into target cells and its release involve perforin. GrB cleaves and activates caspases in the target cell. The target cell dies by apoptosis through either a CD95 or a GrB signalling event.



so-called activation-induced cell death (AICD) might also serve as a second line of defence against autoimmunity by deleting autoreactive cells in the periphery.

After activation, T cells go through several phases (Fig. 4): (1) an interleukin (IL)-2-dependent clonal expansion and effector phase after challenge with antigen, (2) a down phase in which most antigen-specific T cells are eventually eliminated, and (3) a phase in which certain T cells that survive the down phase enter the memory T-cell pool. In the first phase, T cells are resistant to apoptosis; memory cells are also thought to be relatively resistant to apoptosis⁵⁵. But in the down phase T cells become progressively more sensitive to apoptosis in the presence of IL-2. Thus, IL-2 serves a dual role: it is initially mandatory for clonal expansion and later for sensitizing T cells towards apoptosis⁵⁶. *In vitro*, T-cell activation leads to the expression of CD95L and permits T cells to eliminate neighbouring CD95-positive cells; those cells that secrete CD95L can commit suicide^{11,54}. But whereas the CD95–CD95L system seems to be used at the initiation of AICD, the TNF-R2/TNF- α system is important at a later phase^{57,58}. Furthermore, reactive oxygen species and perforin/GrB are also involved in AICD^{59,60} and can also occur in a caspase-independent manner⁶¹. Apparently, activated T cells have various ways of dying. The molecular basis for their choice and the identity of the pathways used *in vivo* remain to be determined.

Sensitive T cells behave like type I cells, form a CD95–DISC complex and initiate a caspase cascade that results in apoptosis. But resistant T cells have decreased DISC formation, and amplification via the mitochondria is blocked by upregulation of the anti-apoptotic molecule Bcl-x_L. The role of c-FLIP in this is controversial⁶². Taken together, T cells can die by different routes and the shift from apoptosis resistance to sensitivity coincides with a shift from a type II to a type I pathway of apoptosis.

Co-stimulation and T-cell survival

T cells activated by one signal by means of the TCR can be saved from AICD by a second signal from co-stimulatory molecules, adhesion molecules or cytokine receptors. CD28 is a major co-stimulating co-receptor expressed on T cells; it is stimulated by CD80 and CD86 expressed on APCs and functions to increase cytokine production and cytokine receptor induction. Under certain conditions CD28 can sensitize T cells towards apoptosis, but generally CD28 enhances the cell proliferation and viability of T cells.

The effect of co-stimulation was observed on three levels: (1) a strong upregulation of c-FLIP_s (refs 63, 64), (2) the upregulation of Bcl-x_L (ref. 65), and (3) the downregulation of CD95L messenger RNA and protein at a defined time (8–12 h after stimulation). Thus, co-stimulation blocks both the type I and type II pathways in T cells and, at least temporarily, also blocks CD95L expression⁶³. At present it is not clear how antigen-activated T cells with an apoptosis-resistant phenotype are turned into memory T cells, and whether memory T cells are locked in the apoptosis-resistant state, but molecules such as c-FLIP and Bcl-x_L might hold some of the answers.

B-lymphocyte death

Three cell-surface molecules are key elements in the regulation of B-cell life and death: B-cell receptor (BCR), CD40 and CD95. The stage of maturation and activation of the B cell, the quantity and quality of the signal provided, and the context of cytokines and other components of the cellular environment are all key factors in whether triggering the BCR, for example by antigens, induces survival or death^{66,67}. Evidence from studies of normal and malignant B cells suggests that BCR activation induces apoptosis by the mitochondrial pathway. But many components of the signalling pathway are still elusive. It is therefore unclear which signals link BCR stimulation to mitochondrial activation^{68,69}.

As in T cells co-stimulated by CD28, BCR-activated B cells can be rescued from apoptosis by co-stimulation by way of CD40 that has been activated by CD40L expressed on T cells and macrophages. This

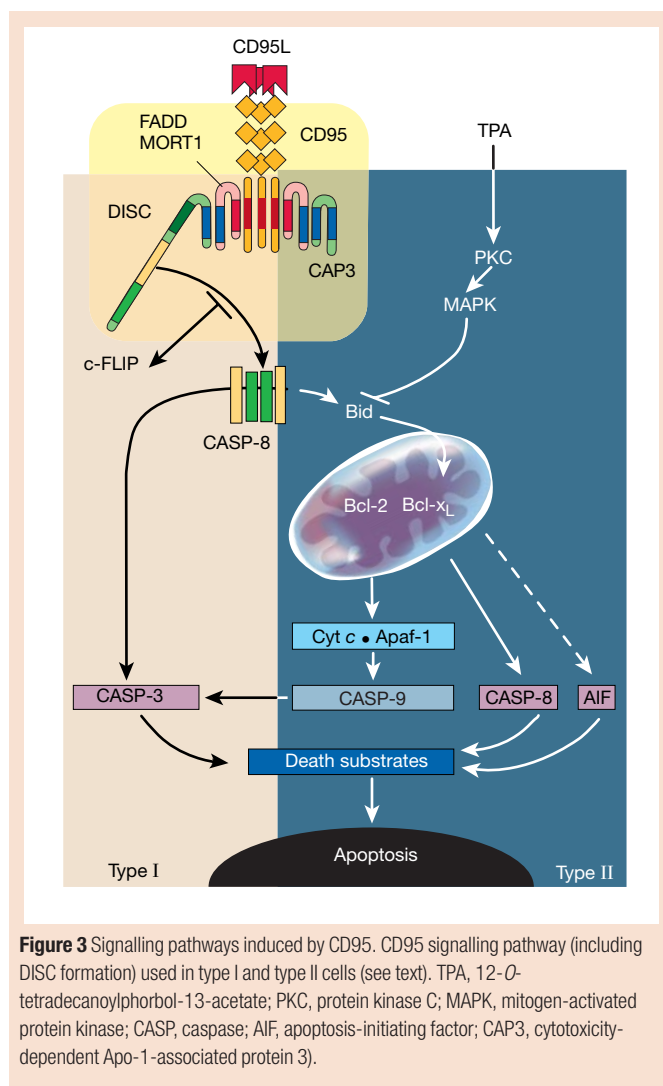


Figure 3 Signalling pathways induced by CD95. CD95 signalling pathway (including DISC formation) used in type I and type II cells (see text). TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; CASP, caspase; AIF, apoptosis-initiating factor; CAP3, cytotoxicity-dependent Apo-1-associated protein 3).

stimulus might represent the most important survival signal for B cells even though such signals at a different maturation stage might also prepare B cells for death^{2,70}. Although it has been noted that transgenic *bcl-2* prevents death and impairs affinity maturation in germinal centres, it is unclear, for example, in which other situations Bcl-2 and other family members and the inhibitor-of-apoptosis (IAP) proteins block apoptosis⁷¹, and in which situations IL-4 and other cytokines act as survival signals². In addition, it is unclear how plasma cells die and which anti-apoptotic signals regulate their survival^{2,72}.

Thus, the principles of B-cell and T-cell development, repertoire selection and involvement of apoptosis in death by neglect and negative selection are similar. However, there are some fundamental B-cell-specific characteristics (Fig. 1).

Autoreactive B cells are eliminated in the bone marrow but, in response to antigenic stimulation, B cells undergo a second diversification and affinity maturation step in the germinal centres of the secondary lymphoid organs by a process called somatic hypermutation: low-affinity or autoreactive B-cell mutants are eliminated by apoptosis and the rest mature into memory B cells and long-lived plasma cells². Plasma cells might constitute an important component of B-cell memory, in particular those that recirculate to the bone marrow, where they are kept alive by as yet undefined stromal signals⁷³.

Although T cells can use CD95L to commit activation-induced suicide⁷, B cells generally do not express CD95L and die from a direct BCR-mediated signal. This opens the possibility that T cells kill CD95-positive B cells. This might apply to susceptible tolerant B cells

or to B cells insufficiently stimulated by survival signals or those whose BCRs are unoccupied by antigen⁷⁴.

Recently, the discovery of new receptor–ligand pairs within the TNF-R/TNF superfamilies has shed further light on the regulation of B-cell life and death^{9,72,75–77}. Blys (TALL-1, THANK, BAFF, zTNF4) and APRIL expressed on T cells and dendritic cells were found to bind to the receptors TACI and BCMA expressed on B cells upregulating nuclear factor (NF)- κ B, B-cell proliferation and immunoglobulin production. The receptor–ligand systems seem to act in concert to regulate B-cell function. Overstimulation of these systems can lead to autoimmunity and autoantibody formation, as in systemic lupus erythematosus. Blocking these systems might be used as a new treatment method in such diseases.

Interactions between APCs, T cells and B cells

T and B cells influence each other and influence persistence, clonal expansion and apoptosis of other cells. But it is the APCs that prime the T cells and initiate T-cell-dependent immunity⁴. APCs are able to engulf apoptotic and necrotic cells, and present their antigens to T cells (see review in this issue by Savill and Fadok, pages 784–788). But at present it is undecided whether material from apoptotic or necrotic cells activates or soothes the T cells^{78,79}. APCs are not passive bystander cells. Activated APCs synthesize CD95L, TRAIL, TNF and other factors that modulate the activity and function of T cells⁸⁰. In turn, activated T cells influence APC function and thereby affect the course of the immune response. At the initiation of the immune response, APCs must be resistant to apoptosis to exert their function⁸¹. Thus, switching these cells off to downregulate the response becomes an important issue. Two members of the TNF-R superfamily, CD40 and CD95, have adversarial roles in this context: the CD40–CD40L system allows the survival of APCs and the CD95–CD95L system induces their death⁸². The plasticity of the immune system might require that the cells can give and receive life and death signals at the same time and that it is the cellular context that determines which signal dictates the cellular response.

Diseases of the immune system involving apoptosis

Apoptosis is a fundamental process of regulation of the immune system; its derailment leads to severe diseases. Several examples of such diseases with either too little or too much apoptosis are discussed below.

Genetic defects in the CD95–CD95L system

Several mouse mutations have been identified that cause complex disorders of the immune system, manifested as lymphadenopathy and autoimmunity. One is the recessive *lpr* (lymphoproliferation) mutation. The symptoms of the disease arising from *lpr* are similar to those in systemic lupus erythematosus. The mutations *lpr^{gk}* (allelic to *lpr*) and *gld* (generalized lymphoproliferative disease) cause a very

similar disease. In all three cases, aberrant T cells accumulate; in *lpr* mice a splicing defect results in the greatly decreased expression of CD95. In *lpr^{gk}* mice a point mutation in the intracellular death domain of CD95 abolishes the transmission of the apoptotic signal. In *gld* mice a point mutation in the carboxy terminus of CD95L impairs its ability to interact successfully with its receptor. Thus, a failure of apoptosis accounts for the complex immune disorder in *lpr* and *gld* mutant mice⁸³.

In humans a similar disease with a dysfunction of the CD95 (type Ia ‘autoimmune lymphoproliferative syndrome’ (ALPS))–CD95L (type Ib ALPS) system has been reported. Children with ALPS (or Canale Smith syndrome) show massive, non-malignant lymphadenopathy, an altered and enlarged T-cell population and a severe autoimmunity. Many of these children show a crippling mutation in the death domain of CD95 but are heterozygous for this defect. Because the parents are unlike the children in being physically unaffected, a secondary, as yet unknown, defect must exist that is responsible for the symptoms^{24,25,84,85}.

In some cases (type II ALPS), defective CD95-mediated apoptosis is observed without mutations in CD95 or CD95L (ref. 86). This suggests that other defects that affect CD95 signalling exist; it has recently been suggested that mutations in caspase-10 might cause such a complementing defect.

Thus, the inability of the immune system to eliminate self-reactive lymphocytes by apoptosis can cause autoimmunity. It is possible that autoantigen-driven prolonged T-cell activation might lock the cells into an AICD-resistant phenotype. Alternatively, apoptosis might produce changes in cellular constituents that affect antigen processing and self-tolerance. Increased resistance to apoptosis and persistence of autoreactive activated T cells have in fact been found in models of experimental autoimmunity. In these systems a clinically beneficial effect of drugs was observed that resensitized such T cells towards apoptotic deletion⁸⁷.

Apoptosis and lymphoid tumours

Apoptosis, or rather the lack of it, is important in the generation of tumours. Follicular lymphomas result from a translocation of *bcl-2* into the immunoglobulin heavy-chain locus and the deregulated expression of *bcl-2* under the influence of the immunoglobulin enhancer. Overexpression of *bcl-2* suppresses apoptosis and favours tumour cell proliferation⁸⁸. This is supported by the increased tumour incidence in, for example, *bcl-2* transgenic animals⁸⁹. Similarly, c-FLIP and most other apoptosis inhibitors might have oncogenic potential. In contrast, proapoptotic molecules could, in principle, act as tumour suppressors. Furthermore, resistance to chemotherapy might result from similar anti-apoptotic mechanisms to those known to block apoptosis in normal cells, and future therapeutic strategies will be aimed at sensitizing tumour cells to apoptosis while sparing normal tissue from drug damage⁹⁰ (see review in this issue by Nicholson, pages 810–816).

Tumours develop multiple mechanisms to evade elimination by the immune system. These mechanisms comprise a lack of expression of co-stimulatory or MHC molecules and active strategies such as the production of immunosuppressive cytokines. In addition, CD95L might have an immunosuppressive function. A number of tumours, including lymphoid tumours, are resistant to apoptosis and express functional CD95L constitutively or after chemotherapy. This situation might enable tumour cells to delete anti-tumour lymphocytes and to suppress anti-tumour immune responses, a phenomenon called ‘tumour counter-attack’.

CD95L is expressed constitutively in immune-privileged sites such as the testis and the eye and might contribute to the immune-privileged status by inducing apoptosis in infiltrating lymphocytes; this might be exploited in trying to delay the rejection of allografts. But it has also been reported that the overexpression of CD95L in grafts does not simply confer immune privilege; instead it induces a granulocytic response that accelerates rejection. Thus, the consequences of CD95L expression *in vivo* are far from clear, and further

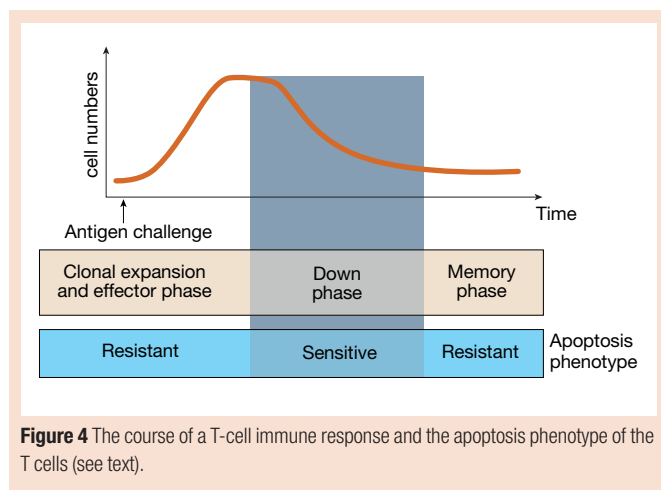


Figure 4 The course of a T-cell immune response and the apoptosis phenotype of the T cells (see text).

experiments are needed to establish whether the counter-attack by tumours underlies their escape from the immune system *in vivo*. Once established, counter-attack could possibly be exploited for therapeutic use by either inhibiting it for cancer therapy or setting it up in organ transplantation⁹¹.

Apoptosis in AIDS

AIDS, characterized by a depletion of CD4⁺ T helper cells, is a disease with too much apoptosis. For example, the number of CD4⁺ T cells in the peripheral blood of individuals productively infected with HIV is low (in the range of one in several thousand). This implies that T-cell depletion in this disease also affects non-infected CD4⁺ T cells. How do they die? As discussed before, T cells can choose between several death-signalling pathways. These different signalling pathways might all be affected in AIDS. Further experiments are urgently needed to determine the contribution of such pathways to CD4⁺ T-cell depletion in this disease. The experiments described below discuss initial attempts to answer this question.

The regulatory viral gene products (for example, HIV-1 Tat) produced by HIV-infected cells penetrates non-infected cells and renders these cells hypersensitive to TCR-induced CD95-mediated apoptosis. HIV Tat induces a pro-oxidative state in the affected cells, increases CD95L expression and facilitates TCR-triggered CD95-mediated suicide. Further sensitization of the CD4⁺ T cells results from the binding of HIV gp120 to CD4 and from the cross-linking of bound gp120 by anti-gp120 antibodies^{92–98}. In addition to CD95-mediated apoptosis, a novel and rapid type of apoptosis induced by both HIV-binding cell-surface receptors, CD4 and CXCR4 (a chemokine receptor), was found in T-cell lines, human peripheral blood lymphocytes and CD4–CXCR4 transfectants. The potency of this phenomenon and its specificity for CD4⁺ T cells suggest that it might have a significant role in T helper cell depletion in AIDS. On the basis of these data, the use of antichemokine-receptor antibodies meant to prevent HIV-1 infection might be dangerous. But the use of the natural ligand of CXCR4, SDF-1 α , or its derivatives could be considered for therapy, because it inhibits infection as well as CXCR4-mediated apoptosis. Studying the apoptotic signalling cascade triggered by CD4 and CXCR4 might prove to be useful in the identification of therapeutic strategies aimed at intervening with the progressive loss of CD4⁺ T cells in HIV-1-infected individuals⁶¹.

Conclusion

Without death by apoptosis, the life of cells of the immune system and their precise and specific function would not be possible. We have begun to understand the signalling pathways of apoptosis in lymphocytes and the rules that determine lymphocyte interactions. We have also begun to understand the survival signals that counteract apoptosis and we might be capable, eventually, of manipulating the cells therapeutically (see review in this issue by Nicholson, pages 810–816). Finally, apoptosis adds new insight into the pathogenesis of diseases; this might be of therapeutic benefit in the future. □

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