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ANTIGEN-INDUCED DEATH OF T-LYMPHOCYTES

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1. ABSTRACT

Resting mature T-lymphocytes are activated when they are triggered *via* their antigen-specific T-cell receptor (TCR) molecule or the associated CD3 antigen. In contrast, preactivated T-cells can undergo activation-induced cell death (AICD) in response to the same signals. Stimulation of activated T-cells upregulates the expression of the Fas-ligand, and the interaction of Fas-ligand with the corresponding Fas receptor triggers an apoptosis program that culminates in cellular suicide usually associated with the fragmentation of DNA into oligonucleosomal bands. Molecular evidence indicates that proteases related to interleukin-1-β converting enzyme play an essential role in the execution of cell death. AICD of mature T-lymphocytes can be efficiently triggered by monoclonal antibodies against the CD3/TCR complex, or by superantigens such as bacterial enterotoxins. Although it is more difficult to induce AICD by conventional peptide antigens, it is now clear that antigen-induced AICD is a powerful means of eliminating antigen-reactive T-cells. Therefore, AICD contributes to the regulation (i.e., termination) of cellular immune responses. In addition, AICD might play a role in the establishment of peripheral immune tolerance. Increased knowledge of the molecular mechanisms of AICD opens new immunotherapeutical perspectives for the treatment of certain autoimmune diseases, and will have implications in other areas such as transplantation medicine.

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2. INTRODUCTION

2.1 Features of apoptosis

There are essentially two ways how cells can die, necrosis and apoptosis. Necrosis is characterized by irreversible swelling and lysis of the cell, which usually occurs in response to a damage of rather unphysiological nature. Due to the disruption of the cell membrane, the cellular content is released, resulting in an inflammatory reaction in the neighbouring tissue. In contrast, apoptosis proceeds *via* an ordered sequence of events that culminates in the suicide of the cell. Hence, apoptosis is also referred to as programmed cell death. Apoptosis is associated with characteristic morphological features which have been studied in great detail (1; see ref. 2 for review). Briefly, cells undergoing apoptosis separate from neighbouring cells and display a characteristic condensation of the nucleus, as well as blebbing of the plasma membranes and formation of the apoptotic bodies containing cell organelles and chromatin fragments. The content of the apoptotic bodies is not released into the environment. Therefore, in contrast to necrosis, apoptosis does not provoke an inflammatory reaction in the surrounding tissue. *In vivo*, apoptotic cells are rapidly removed by

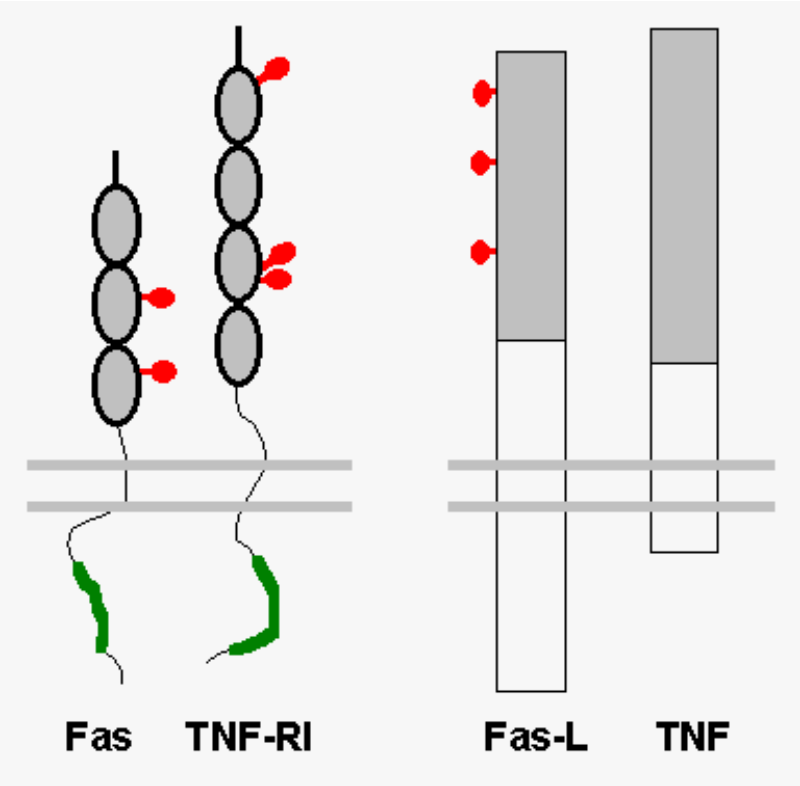

Figure 1: Schematic diagram of the structure of Fas and Fas-L. The extracellular domain of Fas contains three cysteine-rich subdomains (shaded areas). The intracellular death domain is shown as bold line. The black symbols indicate N-glycosylation sites. The Fas-Ligand is a type II membrane protein. The shaded area is the extracelluar region which shares significant homolgy with TNFα. For comparison, the schematic structure of TNF receptor type I and TNFα are also shown. Adapted and modified from ref. 17.

macrophages, possibly through vitronectin receptor-mediated phagocytosis (3). Thus, it is difficult to demonstrate the presence of apoptosis *in vivo*, due to the rapid clearance of such cells. Due to the action of endonucleases during the apoptotic process, the cellular DNA is cut into oligonucleosomal fragments of approximately 200 bp in length, and multiples thereof. Upon electrophoresis in an agarose gel and staining with ethidium bromide, the fragmented DNA gives rise to a characteristic "apoptotic DNA ladder" (4). Probably all cells (except erythrocytes and thrombocytes) have the intrinsic capacity to undergo apoptosis. While it has been obvious for a long time that apoptosis plays a central role in fetal development and organogenesis (2), its significance in the immune system has been recognized only in recent years (5). For some time, the term apoptosis has been used for the morphological description of a specific way of cell death (1). Only recently has it been possible to decipher major pathways of the molecular mechanisms of apoptosis. In this regard, *Caenorhabditis elegans* has been widely studied. However, major breakthroughs in the identification of molecules involved in apoptosis have been made recently in immunological systems. In the following sections, we will review the recent evidence that programmed cell death plays an important role in the regulation of T-cell mediated immune responses. While programmed cell death of T-cells is frequently associated with the above described typical features of apoptosis including low molecular weight DNA fragmentation, there are well-documented instances where DNA fragmentation is dispensable (6, 7). Moreover, the central role of the apoptosis-inducing interaction between the Fas molecule and its corresponding ligand, Fas-Ligand (see below), is beyond any doubt. Nevertheless, there are examples where signal-induced lymphocyte death occurs in the apparent absence of Fas/Fas-Ligand interaction (8-11). We have coined the term activation-induced cell death (AICD) to describe the signal-induced programmed cell death of T-lymphocytes (12). Apoptosis may also be triggered by the lack of stimuli required for survival or lack of growth factors ("death by neglect"), as is the case with hematopoietic precursor cells in the absence of appropriate colony-stimulating factors (13). In this article, we will concentrate on the induction of AICD *via* the antigen-specific T-cell receptor (TCR) molecule.

2.2 Role of death molecules

Apoptosis can be initiated by triggering of cell surface receptors such as Fas (APO-1) with monoclonal antibodies (mAb) or by interaction with its specific ligand (Fas-L, CD154). Fas is a member of the nerve growth factor (NGF)/tumor necrosis factor (TNF) receptor gene family. The mature protein has 319 amino acids (aa) and a calculated molecular weight of 36 kD (14). The Fas molecule consists of an extracellular domain of 157 aa, a hydrophobic transmembrane domain containing 17 aa, and a cytoplasmic tail of 145 aa at its carboxyl terminal (14). The Fas apoptosis signaling domain is contained within a stretch of 68 amino acids in the intracellular part. Fas shares this characteristic "death domain" with the TNF receptor type I (15). Conversely, the natural ligand of the Fas molecule, Fas-L, belongs to the TNF gene family (16, 17). Figure 1 shows a diagram indicating the schematic structure of the Fas and Fas-L molecules. In addition to Fas-L and TNFα, other recently identified members of the TNF family such as TRAIL (TNF-related apoptosis-inducing ligand) can trigger apoptosis in susceptible cells (18). The induction of apoptosis *via* the Fas molecule requires the close proximity of the cytoplasmic tails of several Fas molecules, as it is induced by crosslinking anti-Fas antibodies or by Fas-L in its trimeric form. The Fas apoptosis pathway does not depend on extracellular Ca^{2+} (19, 20) and does not require macromolecular synthesis (14, 21-23). Interestingly, cell death displaying all characteristic morphological features of apoptosis can be triggered *via* Fas in enucleated cells, indicating that apoptosis can proceed in the absence of a nucleus (6). The signaling through Fas involves the activation of an acidic sphingomyelinase (24) giving rise to the generation of ceramide, a complex lipid which induces rapid apoptosis when added in a membrane-permeable form to cell cultures (25). Ceramide mediates Fas-induced apoptosis through the activation of the Ras signaling pathway (26). In turn, the activation of Ras is associated with the rapid and transient synthesis of reactive oxygen intermediates (ROI), pointing to an important role of ROI in Fas-mediated apoptosis (27, 28). In addition, other components of the Fas-dependent death pathway (FADD, RIP) have been identified by searching for proteins that interact with the intracellular death domain of Fas (29, 30). Other potential regulators of Fas-dependent apoptosis include the FAP-1 phosphatase and the serine/threonine kinase FAST (31, 32). Although it has been reported that the activation of a protein tyrosine kinase is involved in Fas signaling (33), we have established experimental systems where Fas-mediated apoptosis clearly proceeds in the absence of detectable tyrosine phosphorylation (34; and Oberg *et al*., submitted for publication). Following coimmunoprecipitation with anti-Fas mAb, four cell death-associated proteins (CAP; cytotoxicity-dependent APO-1-associated proteins) have been identified, which form a death-inducing signaling complex (DISC) (35). One of the CAP encodes a 55 kDa protein termed FLICE which shares homology to Interleukin-1β-converting enzyme (ICE) -like cysteine proteases (36); this molecule has been independently cloned by Boldin *et al* and has been termed MACH by this group (37). The cleavage of cellular substrates including poly (ADP-ribose) polymerase (PARP) and by ICE/CED-3 like proteases is a central event in the execution of apoptosis (38-40). Accordingly, apoptosis can be inhibited by peptides that act as competitive substrates for ICE-like proteases (38, 40, 41).

The Fas/Fas-L system plays a major role in the induction and regulation of programmed cell death in T-lymphocytes. A commonly considered scenario proceeds as follows (see Figure 2). Upon stimulation of activated T-cells *via* the CD3/TCR complex, Fas-L mRNA and cell surface expression are rapidly upregulated. Fas-L binds to the surface-expressed Fas molecule on the same or on neighbouring cells and triggers apoptosis (42-44). Since Fas-L is rapidly cleared from the cell surface through the action of matrix metalloproteases (45), both "suicide" and "fratricide" can be initiated (Figure 2). Although the role of the Fas/Fas-L system in the induction of T-cell apoptosis is unambiguous, it is evident that other molecules are perhaps equally important. Under certain circumstances, TNFα can act as a mediator of apoptosis in mature T-lymphocytes (46, 47). The potential role of the recently described TRAIL molecule (18) in T-cell apoptosis has not yet been precisely defined. It is unlikely that all types ofT-cell apoptosis involve the Fas/Fas-L system (8, 9, 11). Fas-independent apoptosis triggered through the cell surface CD2 molecule has been described (10). Moreover, immobilized mAb against the CTLA4 costimulatory molecule has been reported to induce apoptosis in antigen-specific human T-lymphocytes (48). It is unknown whether the Fas system is involved in this case. The possibility that AICD can take place in T-lymphocytes without the involvement of Fas/Fas-L, will be further discussed (see below).

2.3 Apoptosis in intrathymic T-cell development

Immature precursor cells lacking CD3/TCR enter the thymus. During intrathymic development, TCR gene rearrangement takes place, and cell surface-expressed heterodimeric TCRs are randomly generated. The vast majority (> 95 %) of developing thymocytes do not leave the thymus, and die by apoptosis. Only a minority of thymocytes is rescued from programmed cell death, due to positive selection of cells expressing TCR molecules of appropriate affinity/avidity for self MHC molecules to allow for the differentiation into mature self

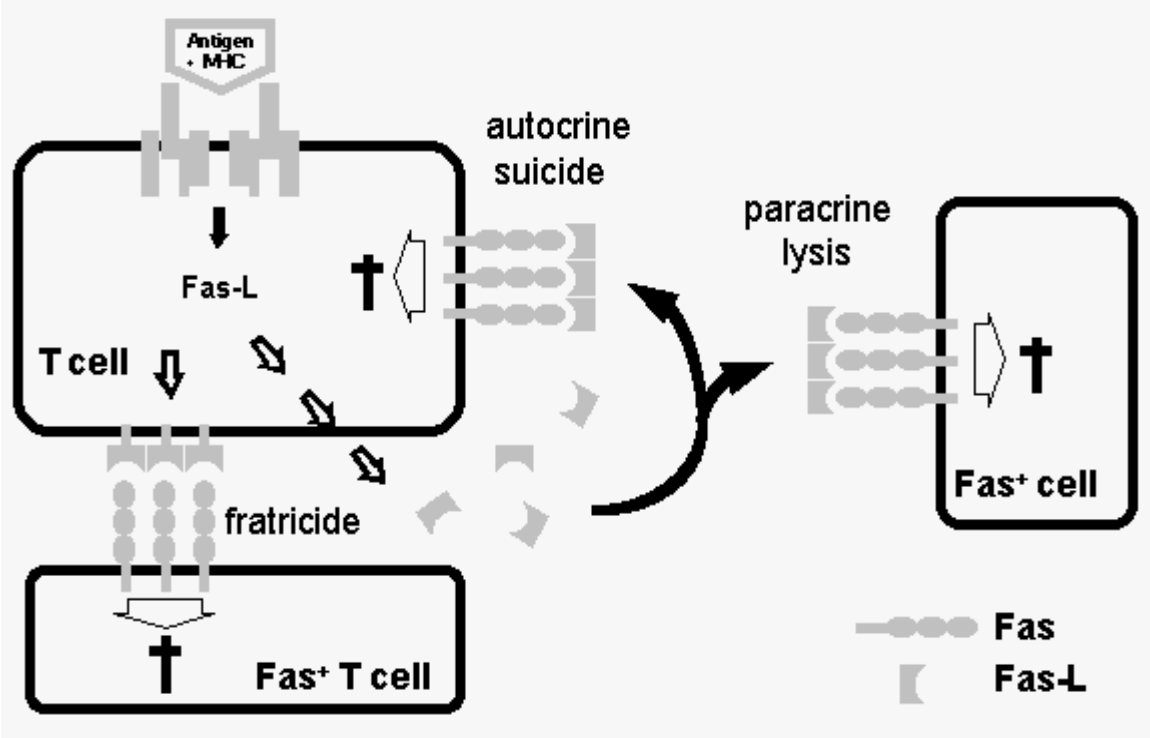

Figure 2: Role of the Fas/Fas-Ligand system in the induction of programmed cell death in T-lymphocytes and their targets. Activated T-cells stimulated *via* the CD3/T-cell receptor complex through the recognition of antigen presented by MHC molecules rapidly express Fas-L molecules. In different scenarios, Fas-L molecules cleaved from the T-cell surface by matrix metalloproteases, or Fas-L molecules still anchored in the cell membrane, can induce apoptosis either in the same cell (autocrine suicide), or in a neighbouring T-cell (fratricide), or in a Fas^+ target cell (paracrine lysis).

MHC-restricted T-cells (see ref. 49 for review). The significance of the Fas/Fas-L system in the on thymocytes (50), there is only low intrathymic expression of the corresponding Fas-L (16). More importantly, negative intrathymic T-lymphocyte selection (by apoptosis) seems to develop to a normal extent in Fas-defective *lpr* or Fas-L-defective *gld* mice (51, 52). However, there is evidence that the Fas/Fas-L interaction is involved in the modulation of thymocyte apoptosis (53). In addition, there are synergistic interactions between the Fas molecule and the CD3/TCR complex in the induction of programmed cell death in thymocytes (54). Immature thymocytes readily undergo apoptosis following treatment with glucocorticoids or anti-CD3 mAb, both *in vivo* and *in vitro* (4, 55-57). While these stimuli induce apoptosis irrespective of the antigen specificity of the TCR, selective depletion (by apoptosis) of thymocytes expressing specifically reactive TCR can be triggered by exogenous superantigens such as *staphylococcus aureus* enterotoxins (58) or by endogenous superantigens encoded by e.g. mouse mammary tumor virus (59). Taken together, there is no doubt that apoptosis is an important physiological process that contributes to the ordered development of the immune system and the developmental shaping of the TCR repertoire. More intrathymic apoptosis is not entirely clear. Although the Fas antigen is expressed
recent evidence indicates, however, that apoptosis also regulates immune responses of peripheral T-lymphocytes in the mature immune system.

3. ACTIVATION-INDUCED CELL DEATH (AICD) OF MATURE T-LYMPHOCYTES

AICD can be triggered in activated T-lymphocytes *via* the CD3/TCR molecular complex and certain other surface molecules involved in T-cell activation such as CD2. Monoclonal anti-CD3/TCR antibodies have been widely used to study AICD in T-cells. It is assumed that this system adequately mimics the activation of T-cells through TCR-mediated recognition of antigen, but the strength (and perhaps the quality) of the signal generated by anti-CD3/TCR mAb *versus* antigenic peptide presented by appropriate MHC molecules may be quite different. Examples of mAb-and antigen-induced AICD of mature T-cells will be discussed in the following sections.

3.1 Induction via the CD3/T-cell receptor molecular complex

The heterodimeric $\alpha\beta$ (or $\gamma\delta$) TCR molecule is closely associated with the CD3 polypeptide

complex on the surface of T-lymphocytes. While the clonally distributed αβ chains of the TCR serve to recognize short antigenic peptides presented by antigen-presenting cells in the context of MHC class I or class II molecules, the signal transduction occurs *via* the TCR ξ chain and the ε, γ and δ subunits of the CD3 molecule (see ref. 60 for review). Anti-CD3 mAb (as well as anti-TCR mAb) have been shown to exert antigen-like effects on T-cells, and thus stimulate Ca^{2+} influx, cytokine synthesis and interleukin-2 (IL-2)-dependent proliferation (61, 62). As mentioned above, anti-CD3/TCR mAb induce apoptosis in immature thymocytes (55-57, 63). However, T-cells at other stages of differentiation are also susceptible to anti-CD3-induced growth arrest, again associated with the induction of programmed cell death. The spontaneous proliferation of transformed T-cells at various stages of differentiation as well as of T-cell hybridomas was inhibited by anti-CD3 mAb, and cell death associated with DNA fragmentation was observed (64-69). More recently, it was realized that the induction of growth arrest and cell death by anti-CD3/TCR mAb was not restricted to immature thymocytes and transformed T-cells, but could be similarly triggered in mature, non-transformed T-lymphocytes. Early studies indicated that such anti-CD3/TCR mAb inhibited the IL-2-driven *in vitro* proliferation of activated peripheral murine and human T-lymphocytes (70-73). It was confirmed by several groups that cross-linking of the CD3/TCR complex by mAb triggered AICD in these instances (74-76). The early observation that anti-CD3/TCR mAb can initiate AICD in normal T-lymphocytes raised a great deal of interest in the question of how AICD might contribute to the regulation of cellular immune responses. Moreover, the molecular mechanisms controlling the induction or prevention of AICD have developed into a scientific topic of utmost interest to immunologists. It is obvious that a precise understanding of the underlying rules is a prerequisite for successful attempts to modulate AICD, *e.g*. by pharmacological intervention (77). As would be expected, resting peripheral T-lymphocytes are generally resistant to AICD triggered by anti-CD3/TCR mAb; instead, these cells respond to CD3/TCR signaling by the induction of a proliferative response. Under *in vitro* culture conditions, it takes seveal days of stimulation before freshly isolated mature peripheral T-cells acquire sensitivity towards AICD (22, 78-80). In response to anti-TCR mAb, AICD is induced both in T-cells expressing the conventional αβ TCR as well as in T-cells expressing the γδ TCR (81). In contrast to resting mature T-cells of adults, neonatal murine T-cells display enhanced sensitivity to AICD and undergo apoptosis in response to primary TCR-mediated stimulation (82). The induction of AICD sensitivity of mature T-cells is influenced by several parameters. Surprisingly, the T-cell growth factor IL-2 has been implicated in the priming of mature T-lymphocytes for AICD (83, 84). It was suggested that the role of growth factors in the induction of apoptosis is to drive T-cells into the S phase of the cell cycle where they are sensitive to TCR-triggered AICD (85). In addition to cytokines, the ligation of cell surface molecules can modulate AICD. Thus, cross-linking of the CD4 T-cell antigen by immobilized anti-CD4 mAb or by the Human Immunodeficiency Virus (HIV) envelope protein, gp120, has been shown to prime $CD4^+$ T-lymphocytes for subsequent apoptosis triggered *via* the CD3/TCR complex (86, 87). This mechanism might contribute to the continuous decline of CD4 T-lymphocytes in HIV-infected individuals. Only a minor fraction of circulating $CD4^+$ T-cells is actually infected with HIV; it has been suggested that the disappearance of non-infected CD4 T-cells is due to priming by gp120/anti-gp120 immune complexes and subsequent encounter of antigen through the TCR (88, 89). It appears, however, that the role of CD4 in the regulation of AICD is more complex. While cross-linking of the CD4 molecule primes resting T-cells for apoptosis, we have recently observed opposite effects on activated T-lymphocytes. Thus, ligation of CD4 by anti-CD4 mAb or HIV-1 gp120 drastically inhibited subsequent AICD triggered through CD3/TCR, due to the prevention of upregulation of Fas-L (Oberg *et al*., submitted for publication). Based on these *in vitro* observations, we would postulate that gp120 present in the serum of HIV-infected individuals might actually inhibit AICD of circulating activated $CD4^+$ T-cells. Independent of the seemingly different role of the CD4 molecule for the regulation of AICD in resting *versus* activated T-cells, it is obvious that the sensitivity to AICD is not limited to $CD4^+$ T-cells (90) but is shared by $CD8^+$ T-cells (74). Importantly, functionally distinct subpopulations of $CD4^+$ T-cells are equally susceptible to anti-CD3-stimulated programmed cell death, independent of their capacity to exert anti-CD3 mAb-mediated redirected lysis of Fc-receptor-positive target cells (90). While the molecular mechanisms underlying the differential outcome of CD3 signaling (proliferative response or AICD) are not precisely understood, mutagenesis of the immunoreceptor tyrosine-based activation motifs (ITAM) that are the principle signaling components revealed a significant role of the TCR ξ chain in the signaling of AICD (91). The conserved 18 amino acid ITAM sequence is found three times in the TCR ξ chain and once each in the CD3 subunits γ, δ and ε. The induction of efficient AICD required the ITAM motifs of the ξ chain, particularly the membrane proximal Z1 ITAM (91). Taken together, there are multiple examples where AICD has been successfully triggered in normal preactivated T-lymphocytes by mAb against CD3 or TCR. In the following sections, we will briefly review the current evidence that AICD can be also initiated by TCR ligands that are naturally encountered by T-lymphocytes.

3.2 AICD triggered by superantigens

In contrast to conventional antigens, superantigens need not be processed by antigen-presenting cells. They activate T-cells by directly bridging the MHC class II molecules on antigen-presenting cells with the variable region of the TCR β chain (92). Most superantigens identified to date are bacterial proteins such as the *staphylococcus aureus* enterotoxins (SE) or products of endogenous or exogenous (retro)viruses. The injection of SEB into BALB/c mice leads to a transient increase in SEB-reactive Vβ8-expressing T-cells, followed by selective depletion of these cells, due to the induction of programmed cell death (93). Although not all Vβ8 cells are deleted following the injection of SEB into mice, the treated animals are tolerant to subsequent challenge with SEB (93, 94). In addition to the deletion of SEB-reactive cells due to apoptosis, other mechanisms such as the induction of anergy in the remaining Vβ8 cells contribute to the functional status of tolerance in such mice (95-101). The deletion of $V\beta8^+$ $CD4^+$ T-cells in response to SEB occurs in the absence of $CD8^+$ T-cells, in support of the notion that activated $CD4^+$ T-cells undergo suicide rather than being killed by $CD8^+$ T-cells (102). AICD following exposure to SEB can be modulated by additional signals. *In vivo*, clonal deletion of $V\beta8^+$ T-cells is significantly increased when mice are injected with hydrocortisone before the application of SEB (103). Under *in vitro* conditions, the induction of AICD in sensitized lymph node T-cells by restimulation with SEB is inhibited by mAb directed against lymphocyte function-associated antigen-1 (LFA-1) or intercellular adhesion molecule-2 (ICAM-2), indicating that cell interactions mediated through adhesion molecules can play a role in AICD (104).

Like activated murine T-cells, human CD4 and CD8 T-lymphocytes are susceptible to superantigen-induced AICD (105-107). In contrast to murine T-cells, activated human T-cells express MHC class II antigens. Therefore, AICD of cloned human $CD4^+$ T-cells is triggered by SE superantigens in the absence of additional antigen-presenting cells (105). In the presence of antigen-presenting cells, AICD proceeds to a similar extent, but a proliferative response is simultaneously initiated in the fraction of surviving clone cells (105). Again, the induction of AICD by SE superantigens was inhibited by mAb directed against LFA-1 (CD11a/CD18; ref. 106). While the analysis with human lymphocytes is largely restricted to *in vitro* experimental systems, clonal deletion of SE-reactive human T-lymphocytes also occurs *in vivo* in the model of SCID mice reconstituted with human fetal liver and thymus (107).

In addition to bacterial superantigens, endogenous or exogenous retroviral superantigens also delete peripheral T-cells expressing the adequate TCR Vβ elements by induction of AICD. Intrathymic deletion of Vβ14-bearing thymocytes occurs in mice transgenic for mouse mammary tumor virus (MMTV) (59). Upon transfer of lymphocytes expressing endogenous MMTV, or of lymphocytes maternally infected with MMTV, into recipients lacking this retroviral superantigen, deletion of T-cells expressing the reactive TCR Vβ elements occurs *in vivo* (108, 109). Therefore, superantigens of both bacterial and viral origin are potent inducers of AICD in activated T-lymphocytes from various species.

3.3 AICD triggered by conventional antigens

It has been known for some time that specific antigen can inhibit the proliferative response of murine mature T-lymphocytes (110-112). The recognition of the specific antigenic peptide presented by appropriate MHC class I molecules triggers the self-destruction of cytotoxic T-cells, due to the induction of AICD (113-117). *In vivo*, such a mechanism seems to contribute to the termination of an ongoing cellular immune response. Systemic infection with lymphocytic choriomeningitis virus (LCMV) is accompanied by cellular expansion of virus-specific $CD8^+$ T-cells. Following virus control, the number of $CD8^+$ T-cells decreases, due to the induction of apoptosis (118). In transgenic mice expressing a transgenic TCR specific for LCMV glycoprotein peptide 33-41, the injection of this peptide induced deletion of transgenic T-cells by AICD (119). Similarly, the exposure to specific antigen induces peripheral $CD8^+$ T-cell deletion also in other TCR transgenic mice with different TCR specificity, e.g. for the male-specific H-Y antigen (120-122) or the nucleoprotein of influenza virus (123). Transgenic mice where the majority of peripheral T-cells express one single TCR molecule with known antigen specificity, provide convenient models to study antigen-triggered AICD *in vivo*. However, there are clear-cut examples that antigen can trigger AICD also in activated non-transgenic, normal T-lymphocytes. Both CD8 and CD4 T-cells are susceptible to AICD induced through TCR-mediated recognition of allogeneic MHC class I (124-127) or MHC class II molecules (128, 129), respectively. In addition, AICD is triggered in $CD4^+$ T-cells by the specific antigenic peptide, e.g. tetanus toxoid or myelin basic protein, presented by the appropriate MHC class II molecules (130-132). Thus, a large body of evidence supports the notion that AICD can be triggered in activated cells through the TCR-mediated recognition of antigen. The extent of AICD is in part controlled by the antigen dosage. Early studies have demonstrated that the antigen-induced suppression of the *in vitro* growth of antigen-specific T-lymphocytes requires high concentrations of antigen (110, 111). More recently, the antigen dosage has been correlated with the TCR avidity and the extent of apoptosis. The studies of Alexander-Miller and coworkers revealed that in $CD8^+$ T-lymphocytes, AICD is preferentially triggered by high concentrations of peptide antigen in cytotoxic T-cells

displaying high avidity TCR (133). Similarly, the induction of apoptosis in antigen-specific CD4 T-cells occurs preferentially at high antigen concentrations (134, 135). Taken together, it appears that antigen dosage is a major factor controlling the induction of AICD in activated mature T-lymphocytes.

3.4 Role of Fas/Fas-Ligand interaction in antigen-induced AICD

AICD triggered in transformed T-cells and T-cell hybridomas by stimulation of the CD3/TCR complex is mediated *via* the induction of Fas-L expression and subsequent interaction of Fas-L with the Fas/CD95 antigen (42-44). Similarly, the superantigen-induced AICD of activated normal T-lymphocytes seems to depend on Fas/Fas-L interactions and can be inhibited by reagents that interfere with this interaction, e.g. some anti-Fas mAb or Fas-Fc fusion proteins (42, 136, 137). Several lines of evidence indicate that the Fas/Fas-L system is also operational *in vivo* in the course of deletion of peripheral T-lymphocytes. Thus, apoptotic T-cells isolated from the peripheral blood or the draining lymph nodes of mice injected with bacterial superantigen express high levels of Fas and Fas-L (138, 139). Moreover, *lpr* mice carrying a genetic defect in the Fas gene (140) are unable to normally delete peripheral T-cells following the injection of bacterial superantigen (138, 141) or conventional peptide antigen (51), and have a defect in AICD triggered *in vitro* by superantigen or anti-CD3 mAb (141-143). There is evidence, however, that AICD can also take place *in vivo* in the absence of a functional Fas molecule. Thus, it was observed by Tucek-Szabo and coworkers that the injection of high concentrations of anti-CD3 mAb triggered apoptosis to a comparable degree in lymph node T-cells from wild-type and *lpr* mice (144). In addition, the *in vivo* elimination of male-antigen (H-Y)-specific T-cells occurs normally when lymph node cells from female H-Y TCR transgenic *lpr/lpr* mice are injected into B6 *nu/nu* male mice (145). Moreover, the peripheral $CD4^+$ T-cell deletion following the injection of influenza hemagglutinin into TCR transgenic mice was similar in mice of normal or Fas defective *lpr* background (47). In the latter case, the peripheral deletion of transgenic T-cells following the *in vivo* administration of the specific antigenic peptide was prevented by a neutralizing anti-TNFα mAb (47), suggesting that TNFα might be involved as an effector molecule in AICD of mature T-lymphocytes (46, 47). Despite the clear-cut evidence for the important role of the Fas/Fas-L system in AICD triggered *via* the CD3/TCR complex, it is obvious that, at least under certain conditions, AICD can proceed in the absence of a functional Fas molecule. It is conceivable that TNFα, the related TRAIL molecule (18), or other ligand-receptor interactions might transduce death signals in these situations.

4. CLINICAL IMPLICATIONS

The elimination of potentially self-reactive thymocytes during intrathymic T-cell development involves apoptotic mechanisms. The above summarized observations indicating that antigen can also trigger AICD in mature peripheral T-lymphocytes suggest that programmed cell death also plays a role in the establishment of peripheral tolerance (12). Moreover, the intentional induction of AICD might form the basis for novel immunotherapeutical strategies.

4.1 Role of AICD in peripheral tolerance

While it is clear that programmed cell death is the major mechanism of the intrathymic elimination of self-reactive lymphocytes, it is less obvious that apoptotic deletion contributes to the establishment of tolerance to foreign antigens in the peripheral immune system. The establishment and maintenance of peripheral tolerance is a multi-step process that may involve functional "anergy" (146, 147), downmodulation of cell surface TCR expression (147), as well as physical elimination of antigen-reactive T-cells (93, 149, 150; see ref. 151 for review). In the setting of allograft transplantation, it is highly desirable to induce tolerance in the transplant recipient towards donor MHC and non-MHC antigens. Pearson and coworkers reported that brief treatment of C3H/He recipient mice with anti-CD4 mAb together with C57/BL10 donor cells induced specific tolerance of subsequent C57/BL10 cardiac allografts (152). It is possible (although not proven in this study) that CD4 cross-linking primed the recipient T-cells for AICD following TCR-mediated recognition of donor alloantigens, in accordance with the *in vitro* studies (86). In support of this possibility, it was reported by Markmann and coworkers that the clonal deletion of antigen-reactive T-cells following the intrathymic inoculation with Mls-disparate lymphoid cells required the additional application of anti-CD4 mAb (153). In experimental models of organ transplantation, the intrathymic injection of donor lymphoid cells has been successfully used to prolong cardiac and liver allograft survival, possibly through the induction of AICD in alloreactive T-cells (154, 155). Furthermore, some immunosuppressive drugs that are used in clinical transplantatiom medicine might in part exert their effect through the modulation of AICD. While cyclosporin A (CsA) and FK506 inhibit AICD triggered through CD3 or CD2 *in vitro* (156, 157), both drugs can actually enhance apoptosis of immature thymocytes *in vivo* (158, 159). Interestingly, FK506 but not CsA, also augmented superantigen-induced AICD of peripheral T-lymphocytes following the injection of SEB into mice (159). Such a mechanism might contribute to the clinical efficacy of FK506. Finally, it is noteworthy that the clinically used anti-CD3 mAb is a potent inducer of *in vitro* AICD in activated human T-lymphocytes (76). Therefore, AICD might contribute

to the immunosuppressive effect of anti-CD3 treatment, in addition to other mechanisms such as modulation of cell surface CD3 expression.

Recent studies suggest that the Fas/Fas-L system also plays a role in the prevention of graft rejection. Bellgrau and coworkers reported that testicular allografts expressing Fas-L escaped rejection by inducing apoptosis in Fas-expressing recipient T-cells activated by graft antigens (160). It should be noted, however, that the expression of Fas-L might not always be beneficial for graft survival. In contrast to Bellgrau *et al*. (160), Yagita and coworkers observed a severe inflammatory rejection of Fas-L expressing baby hamster kidney (BHK) fibroblasts, apparently mediated by neutrophils (161). Nevertheless, it appears that the constitutive expression of Fas-L forms the basis for the maintenance of immunological tolerance in so-called "immune privileged sites" such as the anterior chamber of the eye (162).

4.2 Therapeutical perspectives in autoimmune diseases

The discovery that antigen can delete antigen-reactive mature T-lymphocytes through the induction of AICD has raised great interest in the potential clinical application of this approach in the treatment of certain autoimmune diseases. A widely studied model of T-cell mediated autoimmune disease is experimental autoimmune encephalomyelitis (EAE). The injection of the relevant autoantigen, myelin basic protein (MBP), or the transfer of MBP-specific T-cells, triggers EAE in susceptible rats and mice. *In situ*, apoptotic T-cells can be detected in the central nervous system of Lewis rats suffering from EAE (163). During the spontaneous recovery from EAE, antigen-specific down-regulation of MBP-reactive T-cells occurs, due to the selective apoptotic elimination of autoreactive T-lymphocytes from the target organ (164, 165). These results suggest the possibility that the application of specific (auto)antigen may be used to delete unwanted autoreactive T-cells by the induction of AICD. In fact, several experimental autoimmune diseases can be prevented by the injection of large doses of the relevant autoantigen (166, 167). Although it was not reported in these studies whether apoptosis was involved in the induction of tolerance, there are well-documented examples that high doses of autoantigen can delete autoreactive T-cells *via* AICD. Critchfield and coworkers induced EAE in mice by the adoptive transfer of MBP peptide-reactive T-cells. In contrast to mice injected with a control antigen, mice injected with the relevant MBP peptide had a dramatic deletion of T-cells expressing the MBP-reactive TCR, and did not develop disease (168). Peripheral deletion of antigen-reactive T-lymphocytes can also be achieved by other routes of antigen application. In the studies of Chen and coworkers, the oral application of high concentrations of ovalbumin triggered the deletion of antigen-reactive T-cells in the Peyer's patches of mice transgenic for the ovalbumin-specific TCR (169). Thus, the deletion of antigen-reactive T-cells *via* the induction of AICD is a promising new approach in the treatment of autoimmune and certain other diseases (170, 171). However, in contrast to experimental models, the relevant autoantigen in human disease is frequently unknown, and antigen-independent strategies of modulation of AICD and apoptosis need to be pursued.

5. Concluding remarks

It is obvious that the induction or prevention of AICD in peripheral T-lymphocytes is a complex process that is regulated by multiple signals, only some of which have been discussed here. Depending on the experimental system, AICD is modulated by cytokines (46, 83-85, 172-175) and costimulatory signals (141, 176). In this review, we have focussed on AICD of peripheral T-cells triggered through signaling *via* the CD3/TCR complex. There are other clinically important situations where a disturbance of apoptosis is suspected to contribute to the pathogenesis of the disease. Several reviews have recently discussed the possible role of apoptosis in the progressive loss of $CD4^+$ T-cells during HIV infection (e.g., ref. 177). Therefore, this aspect has not been discussed here. Moreover, there is increasing evidence that apoptosis is an important parameter contributing to the regulation of the interaction between tumor cells and the immune system. It is obvious that a defective capacity to undergo physiological programmed cell death could form the basis for uncontrolled growth of tumor cells. Perhaps more importantly, tumors may also actively prevent T-cells from cell-mediated tumor cell attack through the exploration of the Fas/Fas-L system. This hypothesis is supported by the recent demonstration that some tumor cells constitutively express the Fas-L, thus inducing apoptosis in tumor-infiltrating Fas^+ T-lymphocytes (178, 179). This may be an important mechanism of immune escape of tumor cells. In conclusion, there are various lines of evidence that antigen-induced cell death contributes to the regulation of cellular immune responses as well as to the establishment of peripheral immune tolerance. The continued elucidation of the molecular mechanisms involved in the control of AICD will hopefully provide exciting perspectives for the treatment of certain diseases.

6. REFERENCES

1. J. F. R. Kerr, A. H. Wyllie & A. R. Currie: Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26, 239-257 (1972)

2. J F R Kerr & B V Harmon: Definition and Incidence of Apoptosis: An Historical Perspective. In:

Apoptosis: The molecular basis of cell death. Eds: Tomei L D, Cope F O, Cold Spring Harbor Laboratory Press (1991)

3. J. Savill, I. Dransfied, N. Hogg & C. Haslett. Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. *Nature* 343, 170-173 (1990)

4. A. H. Wyllie: Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 184, 555-56 (1980)

5. G Kroemer, C Martinez-A: Apoptosis in immunology. In: Curr Top Microbiol Immunol, vol. 200, Springer-Verlag, Heidelberg Berlin New York (1995)

6. K. Schulze-Osthoff, H. Walczak, W. Dröge & P. H. Krammer: Cell nucleus and DNA fragmentation are not required for apoptosis. *J Cell Biol* 127, 15-20 (1994)

7. G. M. Cohen, X.-M. Sun, R. T. Snowden, D. Dinsdale & D. N. Skiletter: Key morphological features of apoptosis may occur in the absence of internucleosomal DNA fragmentation. *Biochem J* 286, 331-4 (1992)

8. M. E. Peter, J. Dhein, A. Ehret, S. Hellbardt, H. Walczak, G. Moldenhauer & P. H. Krammer: APO-1(CD95)-dependent and -independent antigen receptor-induced apoptosis in human T and B cell lines. *Int Immunol* 7, 1873-7 (1995)

9. C. L. Tucek-Szabo, S. Andrjelic, E. Lacy, K. B. Elkon & J. Nikolic-Zugic: Surface T cell Fas receptor/CD95 regulation, in vivo activation, and apoptosis. *J Immunol* 156, 192-200 (1996)

10. B. Mollereau, M. Deckert, O. Déas, F. Rieux-Laucat, F. Hirsch, A. Bernard, A. Fischer, D. H. Lynch, B. Charpentier, F. Le Deist & A. Senik: CD2-induced apoptosis in activated human peripheral T cells. A Fas-independent pathway that requires early protein tyrosine phosphorylation. *J Immunol* 156, 3184-90 (1996)

11. P. D. Katsikis, M. E. García-Ojeda, E. S. Wunderlich, C. A. Smith, H. Yagita, K. Okumura, N. Kayagaki, M. Alderson, L. A. Herzenberg & L. A. Herzenberg: Activation-induced peripheral blood T cell apoptosis is Fas independent in HIV-infected individuals. *Int Immunol* 8, 1311-7 (1996)

12. D. Kabelitz, T. Pohl & K. Pechhold: Activation-induced cell death (apoptosis) of mature peripheral T lymphocytes. *Immunol Today* 14, 338-9 (1993)

13. G. T. Williams, C. A. Smith, E. Spooncer, T. M. Dexter & D. R. Taylor: Haemopoietic colony stimulating factors promote cell survival by suppressing apoptosis. *Nature* 343, 76-9 (1990)

14. N. Itoh, S. Yonehara, A. Ishii, M. Yonehara, S.-I. Mizushima, M. Sameshima, A. Hase, Y. Seto & S. Nagata: The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 66, 233-43 (1991)

15. N. Itoh, S. Nagata: A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen. *J Biol Chem* 268, 10932-7 (1993)

16. T. Suda, T. Takahashi, P. Golstein & S. Nagata: Molecular cloning and expression of the Fas ligand: A novel member of the tumor necrosis factor family. *Cell* 75, 1169-78 (1993)

17. S. Nagata & P. Golstein: The Fas death factor. *Science* 267, 1449-56 (1995)

18. S. R. Wiley, K. Schooley, P. J. Smolak, W. S. Din, C.-P. Huang, J. K. Nicholi, G. R. Sutherland, T. D. Smith, C. Rauch, C. A. Smith & R. G. Goodwin: Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 3, 673-82 (1995)

19. E. Rouvier, M.-F. Luciani & P. Golstein: Fas involvement in Ca^{2+}-independent T cell-mediated cytotoxicity. *J Exp Med* 177, 195-200 (1993)

20. S.-T. Ju, H. Ciu, D. J. Panka, R Ettinger & A. Marshak-Rothstein: Participation of target Fas protein in apoptosis pathway induced by $CD4^+$ Th1 and $CD8^+$ T cells. *Proc Natl Acad Sci USA* 91, 4185-4189 (1994)

21. S. Yonehara, A. Ishii & M. Yonehara: A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with the receptor for tumor necrosis factor. *J Exp Med* 169, 1747-1756 (1989)

22. C. Klas, K. M. Debatin, R. Jonker & P. H. Krammer: Activation interferes with the APO-1 pathway in mature human T cells. *Int Immunol* 5, 625-630 (1993)

23. T. Stalder, S. Hahn & P. Erb: Fas antigen is the major target molecule for $CD4^+$ T cell mediated cytotoxicity. *J Immunol* 152, 1127-1133 (1994)

24. M.G. Cifone, R De Maria, P. Roncaioli, M. R. Rippo, M. Azuma, L. L. Lanier, A. Santoni & R. Testi: Apoptotic signaling through CD95 (Fas/Apo-1) activates an acidic sphingomyelinase. *J Exp Med* 177, 1547-1552 (1993)

25. L. M. Obeid, C. M. Linardic, L. A. Karolak & Y. A. Hannun: Programmed cell death induced by ceramide. *Science* 259, 1769-71 (1993)

26. E. Gulbins, R. Bissonette, A. Mahboubi, S. Martin, W. Nishioka, T. Brunner, G. Baier, G. Baier-Bitterlich, C. Byrd, F. Lang, R. Kolesnick, A. Altman & D. Green: Fas-induced apoptosis is mediated via a ceramide-initiated Ras signaling pathway. *Immunity* 2, 341-351 (1995)

27. E. Gulbins, B. Brenner, K. Schlottmann, J. Welsch, H. Heinle, U. Koppenhoefer, O. Linderkamp, K. M. Coggeshall & F. Lang: Fas-induced programmed cell death is mediated by a Ras-regulated O_2^- synthesis. *Immunology* 89, 205-12 (1996)

28. H.-D. Um, J. M. Orenstein & S. M. Wahl: Fas mediates apoptosis in human monocytes by a reactive oxygen intermediate dependent pathway. *J Immunol* 156, 3469-77 (1996)

29. A. M. Chinnaiyan, K. O'Rourke, M. Tewari & V. M. Dixit: FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 81, 505-512 (1995)

30. B. Z. Stanger, P. Leder, T.-H. Lee, E. Kim & B. Seed: RIP: a novel protein containing a death domain that interacts with Fas/Apo-1 (CD95) in yeast and causes cell death. *Cell* 81, 513-523 (1995)

31. T. Sato, s. Irie, S. Kitada & J. C. Reed. FAP-1: a protein tyrosine phosphatase that associates with Fas. *Science* 268, 411-415 (1995)

32. Q. Tian, J. L. Taupin, S. Elledge, M. Robertson & P. Anderson: Fas-activated serine/threonine kinase (FAST) phosphorylates TIA-1 during Fas-mediated apoptosis. *J Exp Med* 182, 865-874 (1995)

33. C. M. Eischen, C. J. Dick & P. J. Leibson: Tyrosine kinase activation provides an early and requisite signal for Fas-induced apoptosis. *J Immunol* 153, 1947-1954 (1994)

34. O. Janssen, B. Lengl-Janßen, H.-H. Oberg, M. J. Robertson & D. Kabelitz: Induction of cell death via Fas (CD95, Apo-1) may be associated with but is not dependent on Fas-induced tyrosine phosphorylation. *Immunol Letters*, 49, 63-69 (1996)

35. F. C. Kischkel, S. Hellbardt, I. Behrmann, M. Germer, M. Pawlita, P. H. Krammer & M. E. Peter: Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *EMBO J* 14, 5579-5588 (1995)

36. M. Muzio, A. M. Chinnaiyan, F. C. Kischkel, K. O'Rourke, A. Shevchenko, J. Ni, C. Scaffidi, J. D. Bretz, M. Zhang, R. Gentz, M. Mann, P. H. Krammer, M. E. Peter & V. M. Dixit: FLICE, a novel FADD homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/Apo-1) death-inducing signaling complex. *Cell* 85, 817-27 (1996)

37. M. P. Boldin, T. M. Goncharov, Y. V. Goltsev & D. Wallach: Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. *Cell* 85, 803-15 (1996)

38. D. W. Nicholson, A. Ali, N. A. Thornberry, J. P. Vaillancourt, C. K. Ding, M. Gallant, Y. Gareau, P. R. Griffin, M. Labelle, Y A. Lazebnik, N. A. Munday, S. M. Raju, M. E. Smulson, T.-T. Yamin, V. L. Yu & D. K. Miller: Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature* 376, 37-43 (1995)

39. T. Fernandes-Alnemri, M. G. Litwack & E. S. Alnemri: CPP32, a novel human apoptotic protein with homology to *Caenorhabditis elegans* cell death protein Ced-3 and mammalian interleukin-1β-converting enzyme. *J Biol Chem* 269, 30761-4 (1994)

40. L. Casciola-Rosen, D. W. Nicholson, T. Chong, K. R. Rowan, N. A. Thornberry, D. K. Miller & A. Rosen: Apopain/CPP32 cleaves proteins that are essential for cellular repair: a fundamental principle of apoptotic death. *J Exp Med* 182, 1957-64 (1996)

41. A. Sarin, M.-L. Wu & P. A. Henkart: Different interleukin-1β converting enzyme (ICE) family protease requirements for the apoptotic death of T lymphocytes triggered by diverse stimuli. *J Exp Med* 184, 2445-50 (1996)

42. J. Dhein, H. Walczak, C. Bäumler, K.-M. Debatin & P. H. Krammer: Autocrine T cell suicide mediated by Apo-1/(Fas/CD95). *Nature* 373, 438-441 (1995)

43. T. Brunner, R. J. Mogil, D. LaFace, N. J. Yoo, A. Mahboubi, F. Echeverri, S. J. Martin, W. R. Force, D. H. Lynch, C. F. Ware & D. R. Green: Cell-autonomous Fas(CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. *Nature* 373, 441-444 (1995)

44. S.-T. Ju, D. J. Panka, H. Cui, R. Ettinger, M. El-Khatib, D. H. Sherr, B. Z. Stanger & A. Marshak-Rothstein: Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature* 373: 444-448 (1995)

45. S. M. Mariani, B. Matiba, C. Bäumler, P. H. Krammer: Regulation of cell surface APO-1/Fas (CD95) ligand expression by metalloproteases. *Eur J Immunol* 25, 2302-7 (1995)

46. L. Zheng, G., Fisher, R. E. Miller, J. Peschon, D. H. Lynch & M. J. Lenardo: Induction of apoptosis in

mature T cells by tumour necrosis factor. *Nature* 377, 348-51 (1995)

47. H. K. Sytwu, R. S. Liblau, H. O. McDevitt: The roles of Fas/APO-1 (CD95) and TNF in antigen-induced programmed cell death in T cell receptor transgenic mice. *Immunity* 5: 17-30 (1996)

48. J. G. Gribben, G. J. Freeman, V. A. Boussiotis, P. Rennert, C. L. Jellis, E. Greenfield, M. Barber, V. A. Restivo, Jr., X. Ke, G. S. Gray & L. M. Nadler: CTLA4 mediates antigen-specific apoptosis of human T cells. *Proc Natl Acad Sci USA* 92: 811-15 (1995)

49. P. Kisielow & H. von Boehmer: Development and selection of T cells: Facts and puzzles. *Adv Immunol* 58: 87-209 (1995)

50. J. Drappa, N. Brot & K. B. Elkon: The Fas protein is expressed at high levels on $CD4^+CD8^+$ thymocytes and activated mature lymphocytes in normal mice but not in the lupus-prone strain, MRL *lpr/lpr*. *Proc Natl Acad Sci USA* 90, 10340-44 (1993)

51. G. G. Singer & A. K. Abbas: The Fas antigen is involved in peripheral but not thymic deletion of T lymphocytes in T cell receptor transgenic mice. *Immunity* 1, 365-71 (1994)

52. C. L. Sidman, J. D. Marshall, H. von Boehmer: Transgenic T cell receptor interactions in the lymphoproliferative and autoimmune syndromes of *lpr* and *gld* mutant mice. *Eur J Immunol* 22, 499-504 (1992)

53. J. E. Castro, J. A. Listman, B. A. Jacobson, Y. Wang, P. A. Lopez, S. Ju, P. W. Finn & D. L. Perkins. Fas modulation of apoptosis during negative selection of thymocytes. *Immunity* 5, 617-27 (1996)

54. G. H. Fisher, M. J. Lenardo & J. C. Zúñiga-Pflücker: Synergy between T cell receptor and Fas (CD95/APO-1) signaling in mouse thymocyte death. *Cell Immunol* 169, 99-106 (1996)

55. C. A. Smith, G. T. Williams, R. Kingston, E. J. Jenkinson & J. J. T. Owen: Antibodies to CD3/T-cell receptor complex induce death by apoptosis in immature T cells in thymic cultures. *Nature* 337: 181-4 (1989)

56. Y. Shi, R. P. Bissonnette, N. Parfrey, M. Szalay,, R. T. Kubo & D. R. Green: In vivo administration of monoclonal antibodies to the CD3 T cell receptor complex induces cell death (apoptosis) in immature thymocytes. *J Immunol* 146, 3340-6 (1991)

57. D. J. McConkey, P. Hartzell, J. F. Amador-Pérez, S. Orrenius & M. Jondal: Calcium-dependent killing of immature thymocytes by stimulation via the CD3/ T cell receptor complex. *J Immunol* 143: 1801-6 (1989)

58. E. J. Jenkinson, R. Kingston, C. A. Smith, G. T. Williams & J. J. T. Owen: Antigen-induced apoptosis in developing T cells: a mechanism for negative selection of the T cell receptor repertoire. *Eur J Immunol* 19, 2175-7 (1989)

59. H. Acha-Orbea, A. N. Shakhov, L. Scarpellino, E. Kolb, V. Müller, A. Vessaz-Shaw, R. Fuchs, K. Bölchlinger, P. Rollini, J. Billotte, M. Sarafidou & H. R. MacDonald: Clonal deletion of Vβ14-bearing T cells in mice transgenic for mammary tumour virus. *Nature* 350, 207-11 (1991)

60. R. L. Wange & L. E. Samelson: Complex complexes: Signaling at the TCR. *Immunity* 5, 197-205 (1996)

61. S. C. Meuer, J. C. Hodgdon, R. E. Hussey, J. P. Protentis, S. F. Schlossman & E. L. Reinherz: Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J Exp Med* 158, 988-993 (1983)

62. S. C. Meuer, R. E. Hussey, D. A. Cantrell, J. C. Hodgdon, S. F. Schlossman, K. A. Smith & Ellis L. Reinherz: Triggering of the T3-Ti antigen-receptor complex results in clonal T-cell proliferation through an interleukin 2-dependent autocrine pathway. *Proc Natl Acad Sci USA* 81, 1509-13 (1984)

63. T. Tadakuma, H. Kizaki, C. Odaka, R. Kubota, Y. Ishimura, H. Yagita & K. Okumura: $CD4^+CD8^+$ thymocytes are susceptible to DNA fragmentation induced by phorbol ester, calcium ionophore and anti-CD3 antibody. *Eur J Immunol* 20: 779-84 (1990)

64. M. Mercep, J. A. Bluestone, P. D. Noguchi & J. D. Ashwell: Inhibition of transformed T cell growth in vitro by monoclonal antibodies directed against distinct activating molecules. *J Immunol* 140, 324-35 (1988)

65. S. Takahashi, H. T. Maecker & R. Levy: DNA fragmentation and cell death mediated by T cell antigen receptor/CD3 complex on a leukemia T cell line. *Eur J Immunol* 19, 1911-19 (1989)

66. D. S. Ucker, J. D. Ashwell & G. Nickas: Activation-driven T cell death. I. Requirements for de novo transcription and translation and association with genome fragmentation. *J Immunol* 143, 3461-69 (1989)

67. C. Odaka, H. Kizaki & T. Tadakuma. T cell receptor-mediated DNA fragmentation and cell death in T cell hybridomas. *J Immunol* 144, 2096-2101 (1990)

68. S. Vukmanovic & R. Zamoyska: Anti-CD3-induced cell death in T cell hybridomas: mitochondrial failure and DNA fragmentation are distinct event. *Eur J Immunol* 21: 419-24 (1991)

69. Y. Shi, M. G. Szalay, L. Paskar, M. Boyer, B. Singh & D. Green: Activation-induced cell death in T cell hybridomas is due to apoptosis. Morpholog aspects and DNA fragmentation *J. Immunol* 144, 3326-33 (1990)

70. G. J. Nau, R. L. Moldwin, D. W. Lancki, D.-K. Kim & F. W. Fitch: Inhibition of IL 2-driven proliferation of murine T lymphocyte clones by supraoptimal levels of immobilized anti-T cell receptor monoclonal antibody. *J Immunol* 139, 114-22 (1987)

71. S. Webb & J. Sprent: Downregulation of T cell responses by antibodies to the T cell receptor. *J Exp Med* 165, 584-9 (1987)

72. G. J. Nau, D.-K. Kim & F. W. Fitch: Agents that mimic antigen receptor signaling inhibit proliferation of cloned murine T lymphocytes induced by IL-2. *J Immunol* 141, 3557-63 (1988)

73. J. B. Breitmeyer, S. O. Oppenheim, J. F. Daley, H. B. Levine & S. F. Schlossman: Growth inhibition of human T cells by antibodies recognizing the T cell antigen receptor complex. *J Immunol* 138, 726-31 (1987)

74. J. J. Russell, C. L. White, D. Y. Loh & P. Meleedy-Rey: Receptor-stimulated death pathway is opened by antigen in mature T cell. *Proc Natl Acad Sci USA* 88, 2151-55 (1991)

75. A. Bensussan, G. Leca, N. Corvaïa & L. Boumsell. Selective induction of autocytotoxic activity through the CD3 molecule. *Eur J Immunol* 20, 2615-19 (1990)

76. O. Janssen, S. Wesselborg & D. Kabelitz: Immunosuppression by OKT3:-induction of programmed cell death (apoptosis) as a possible mechanism of action. *Transplantation* 53, 233-4 (1992)

77. G. Kroemer: The pharmacology of T cell apoptosis. *Adv Immunol* 58, 211-96 (1995)

78. S. Wesselborg, O. Janssen & D. Kabelitz: Induction of activation-driven death (apoptosis) in activated but not resting peripheral blood T cells. *J Immunol* 150: 4338-45 (1993)

79. L. G. Radvanyi, G. B. Mills & R. G. Miller: Religation of the T cell receptor after primary activation of mature T cells inhibits proliferation and induces apoptotic cell death. *J Immunol* 150: 5704-15 (1993)

80. M. Salmon, D. Pilling, N. J. Borthwick, N. Viner, G. Janossy, P. A. Bacon & A. N. Akbar: The progressive differentiation of primed T cells is associated with an increasing susceptibility to apoptosis. *Eur J Immunol* 24: 892-9 (1994)

81. O. Janssen, S. Wesselborg, B. Heckl-Österreicher, K. Pechhold, A. Bender, S. Schondelmaier, G. Moldenhauer & D. Kabelitz: T cell receptor/CD3-signaling induces death by apoptosis in human T cell receptor $\gamma\delta^+$ T cells. *J Immunol* 146, 35-9 (1991)

82. B. Adkins, K. Chun, K. Hamilton & M. Nassiri: Naive murine neonatal T cells undergo apoptosis in response to primary stimulation. *J Immunol* 157, 1343-9 (1996)

83. M. J. Lenardo: Interleukin-2 programs mouse $\alpha\beta$ T lymphocytes for apoptosis. *Nature* 353, 858-861 (1991)

84. R. Wang, A. M. Rogers, B. J. Rush & J. H. Russell: Induction of sensitivity to activation-induced death in primary $CD4^+$ cells: a role for interleukin-2 in the negative regulation of responses by mature $CD4^+$ T cells. *Eur J Immunol* 26, 2263-70 (1996)

85. S. A. Boehme & M. J. Lenardo: Propriocidal apoptosis of mature T lymphocytes occurs at S phase of the cell cycle. *Eur J Immunol* 23, 1552-1560 (1993)

86. M. K. Newell, L. J. Haughn, C. R. Maroun & M. H. Julius: Death of mature T cells by separate ligation of CD4 and the T-cell receptor for antigen. *Nature* 347, 286-9 (1990)

87. N. K. Banda, J. Bernier, D. K. Kurahara, R. Kurrle, N. Haigwood, R.-P. Sekaly & T. H. Finkel: Crosslinking CD4 by human immunodeficiency virus gp120 primes T cells for activation-induced apoptosis. *J Exp Med* 176, 1099-1106 (1992)

88. H. Groux, G. Torpier, D. Monté, Y. Mouton, A. Capron & J.-C. Ameisen: Activation-induced death by apoptosis in $CD4^+$ T cells from human immunodeficiency virus-infected asymptomatic individuals. *J Exp Med* 175: 331-40 (1992)

89. M.-L. Gougeon, L. Montagnier: Apoptosis in AIDS. *Science* 260, 1269-72 (1993)

90. J. H. Russell, B. J. Rush, S. I. Abrams, R. Wang: Sensitivity of T cells to anti-CD3-stimulated suicide is independent of functional phenotype. *Eur J Immunol* 22: 1655-8 (1992)

91. B. Combadière, M. Freedman, L. Chen, E. W. Shores, P. Love & M. J. Lenardo: Qualitative and quantitative contributions of the T cell receptor ζ chain to mature T cell apoptosis. *J Exp Med* 183, 2109-17 (1996)

92. P. Marrack & J. Kappler: The staphylococcal enterotoxins and their relatives. *Science* 248, 705-711 (1990)

93. Y. Kawabe & A. Ochi: Programmed cell death and extrathymic reduction of Vβ8$^+$ CD4$^+$ T cells in mice tolerant to *Staphylococcus aureus* enterotoxin B. *Nature* 349: 245-8 (1991)

94. J. A. Gonzalo, I. M. de Alborán, J. E. Alés-Martínez, C. Martínez-A. & G. Kroemer: Expansion and clonal deletion of peripheral T cells induced by bacterial superantigen is independent of the interleukin-2 pathway. *Eur J Immunol* 22: 1007-11 (1992)

95. Y. Kawabe & A. Ochi: Selective anergy of Vβ8$^+$,CD4$^+$ T cells in Staphylococcus enterotoxin B-primed mice. *J Exp Med* 172: 1065-70 (1990)

96. B. L. Rellahan, L. A. Jones, A. M. Kruisbeek, A. M. Fry & L. A. Matis: In vivo induction of anergy in peripheral Vβ8$^+$ T cells by staphylococcal enterotoxin B. *J Exp Med* 172: 1091-1100 (1990)

97. H. R. MacDonald, S. Baschieri & R. K. Lees: Clonal expansion precedes anergy and death of V$_\beta$8$^+$ peripheral T cells responding to staphylococcal enterotoxin B in vivo. *Eur J Immunol* 21: 1963-6 (1991)

98. D. E. Scott, W. J. Kisch & A. D. Steinberg: Studies of T cell deletion and T cell anergy following in vivo administration of SEB to normal and lupus-prone mice. *J Immunol* 150, 664-72 (1993)

99. S. Baschieri, R. K. Lees, A. R. Lussow & H. R. MacDonald: Clonal expansion to staphylococcal enterotoxin B *in vivo*: selective effects on T cell subsets and lymphokines. *Eur J Immunol* 23: 2661-6 (1993)

100. K. Heeg, S. Bendigs, T. Miethke & H. Wagner: Induction of unresponsiveness to the superantigen staphylococcal enterotoxin B: cyclosporin A resistant split unresponsiveness unfolds *in vivo* without preceding clonal expansion. *Int Immunol* 5, 929-37 (1993)

101. M. E. Hamel, E. E. Eynon, H. F. J. Savelkoul, A. van Oudenaren & A. M. Kruisbeek: Activation and re-activation potential of T cells responding to staphylococcal enterotoxin B. *Int Immunol* 7, 1065-77 (1995)

102. O. Williams, J. A. Gonzalo & C. Martínez-A: Staphylococcal enterotoxin B promotes deletion and functional inactivation of CD4 Vb8-positive cells in the absence of CD8 T cells. *Scand J Immunol* 38, 254-58 (1993)

103. A. R. Lussow, T. Crompton, O. Karapetian & H. R. MacDonald: Peripheral clonal deletion of superantigen-reactive T cells is enhanced by cortisone. *Eur J Immunol* 23, 578-81 (1993)

104. E. Z. Tchilian, G. Anderson, N. C. Moore, J. J. T. Owen & E. J. Jenkinson: Involvement of LFA-1/ICAM-2 adhesive interactions and PKC in activation-induced cell death following SEB rechallenge. Immunology 87, 566-72 (1996)

105. D. Kabelitz & S. Wesselborg: Life and death of a superantigen-reactive human CD4$^+$ T cell clone: staphylococcal enterotoxins induce death by apoptosis but simultaneously trigger a proliferative response in the presence of HLA-DR$^+$ antigen-presenting cells. *Int Immunol* 4, 1381-88 (1992)

106. N. K. Damle, G. Leytze, K. Klussman & J. A. Ledbetter: Activation with superantigens induces programmed death in antigen-primed CD4$^+$ class II$^+$ major histocompatibility complex T lymphocytes via a CD11a/CD18-dependent mechanism. *Eur J Immunol* 23, 1513-22 (1993)

107. R. Baccala, B. A. E. Vandekerckhove, D. Jones, D. H. Kono, M.-G. Roncarolo & A. N. Theofilopoulos: Bacterial superantigens mediate T cell deletions in the mouse severe combined immunodeficiency-human liver/thymus model. *J Exp Med* 177, 1481-5 (1993)

108. G. A. Waanders, A. N. Shakhov, W. Held, O. Karapetian, H. Acha-Orbea & H. R. MacDonald: Peripheral T cell activation and deletion induced by transfer of lymphocyte subsets expressing endogenous or exogenous mouse mammary tumor virus. *J Exp Med* 177, 1359-66 (1993)

109. H. Wang, R. F. Gill, D. Lichlyter, A. Iglesias, H. Bluethmann & W.-Z. Wei: Deletion of CD4$^+$ T cells and thymocytes by apoptosis in mouse mammary tumor virus (C4)-infected Vb2 transgenic mice. *Eur J Immunol* 24, 2950-6 (1994)

110. J. H. Russell, D. E. Manning, D. E. McCulley & P. Meleedy-Rey: Antigen as a positive and negative regulator of proliferation in cytoxic lympohocytes. A model for the differential regulation of proliferation and lytic activity. *J Immunol* 140, 1796-1801 (1988)

111. G. Suzuki, Y. Kawase, S. Koyasu, I. Yahara, Y. Kobayashi & R. H. Schwartz: Antigen-induced suppression of the proliferative response of T cell clones. *J Immunol* 140, 1359-65 (1988)

112. S. Brocke, M. Dayan, L. Steinman, J. Rothbard & E. Mozes: Inhibition of T cell proliferation specific for acetylcholine receptor epitopes related to myasthenia gravis with antibody to T cell receptor or with competitive synthetic polymers. *Int Immunol* 2, 735-742 (1990)

113. D. J. Moss, S. R. Burrows, G. D. Baxter & M. F. Lavin: T cell-T cell killing is induced by specific epitopes: Evidence for an apoptotic mechanism. *J Exp Med* 173, 681-86 (1991)

114. P. R. Walden & H. N. Eisen: Cognate peptides induce self-destruction of $CD8^+$ cytolytic T lymphocytes. *Proc Natl Acad Sci USA* 87, 9015-9019 (1990)

115. R. M. Pemberton, D. C. Wraith & B. A. Askonas: Influenza peptide-induced self-lysis and down-regulation of cloned cytotoxic T cells. *Immunology* 70, 223-5 (1990)

116. J. P. Dutz, P. R. Walden & H. N. Eisen: Effects of cognate peptides on cytolytic and proliferative activities of cloned cytotoxic T lymphocytes. *Int Immunol* 4, 571-80 (1992)

117. A. Suhrbier, S R. Burrows, A. Fernan, M. F. Lavin, G. D. Baxter & D. J. Moss: Peptide epitope induced apoptosis of human cytotoxic T lymphocytes. Implications for peripheral T cell deletion and peptide vaccination. *J Immunol* 150, 2169-78 (1993)

118. J. P. Christensen, C. Röpke & A. R. Thomsen: Virus-induced polyclonal T cell activation is followed by apoptosis: partitioning of $CD8^+$ T cells based on $\alpha 4$ integrin expression. *Int Immunol* 8, 707-15 (1996)

119. D. Kyburz, P. Aichele, D. E. Speiser, H. Hengartner, R. M. Zinkernagel & H. Pircher: T cell immunity after a viral infection versus T cell tolerance induced by soluble viral peptides. *Eur J Immunol* 23, 1956-62 (1993)

120. D. A. Carlow, S. J. Teh, N. S. C. van Oers, R. G. Miller & H.-S. Teh: Peripheral tolerance through clonal deletion of mature $CD4^-CD8^+$ T cells. *Int Immunol* 4, 599-610 (1992)

121. J. Kirberg, L. Bruno & H. von Boehmer: $CD4^+8^-$ help prevents rapid deletion of $CD8^+$ cells after a transient response to antigen. *Eur J Immunol* 23, 1963-7 (1993)

122. L. Zhang, D. R. Martin, W.-P. Fung-Leung, H.-S. Teh & R. G. Miller: Peripheral deletion of mature $CD8^+$ antigen-specific T cells after in vivo exposure to male antigen. *J Immunol* 148, 3740-5 (1992)

123. C. Mamalaki, Y. Tanaka, P. Corbella, P. Chandler, E. Simpson & D. Kioussis: T cell deletion follows chronic antigen specific T cell activation *in vivo*. *Int Immunol* 5, 1285-92 (1993)

124. S. R. Sambhara & R. G. Miller: Programmed cell death of T cells signaled by the T cell receptor and the $\alpha 3$ domain of class I MHC. *Science* 252, 1424-7 (1991)

125. T. Pohl, K. Pechhold, H.-H. Oberg, O. M. Wilbert & D. Kabelitz: Antigen-induced death of alloreactive human T-lymphocytes occurs in the absence of low molecular weight DNA fragmentation. *Cell Immunol* 166, 187-95 (1995)

126. L. Zhang. The fate of adoptively transferred antigen-specific T cells *in vivo*. *Eur J Immunol* 26: 2208-14 (1996)

127. N. Zavazava & M. Krönke: Soluble HLA class I molecules induce apoptosis in alloreactive cytotoxic T lymphocytes. *Nature Med* 2, 1005-10 (1996)

128. D. Kabelitz, H.-H. Oberg, T. Pohl & K. Pechhold: Antigen-induced death of mature T lymphocytes: Analysis by flow cytometry. *Immunol Rev* 142, 157-74 (1994)

129. N. K. Damle, K. Klussman, G. Leytze, A. Aruffo, P. S. Linsley & J. A. Ledbetter: Costimulation with integrin ligands intercellular adhesion molecule-1 or vascular cell adhesion molecule-1 augments activation-induced death of antigen-specific $CD4^+$ T lymphocytes. *J Immunol* 151, 2368-79 (1993)

130. F. Bettens, E. Frei, K. Frutig, D. Mauri, W. J. Pichler & T. Wyss-Coray: Noncytotoxic human CD4+ T-cell clones presenting and simultaneously responding to an antigen die of apoptosis. *Cell Immunol* 161, 72-8 (1995)

131. C. M. Pelfrey, L. R. Tranquill, S. A. Boehme, H. F. McFarland & M. J. Lenardo: Two mechanisms of antigen-specific apoptosis of myelin basic protein (MBP)-specific T lymphocytes derrived from multiple sclerosis patients and normal individuals. *J Immunol* 154, 6191-6202 (1995)

132. B. Nag, T. Kendrick, S. Arimilli, S. C. T. Yu & S. Sriram: Soluble MHC II-peptide complexes induce antigen-specific apoptosis in T cells. *Cell Immunol* 170, 25-33 (1996)

133. M. A. Alexander-Miller, G. R. Leggatt, A. Sarin & J. A. Berzofsky: Role of antigen, CD8, and cytotoxic T lymphocyte (CTL) avidity in high dose antigen induction of apoptosis of effector CTL. *J Exp Med* 184, 485-92 (1996)

134. J. M. Critchfield, J. C. Zúñiga-Pflücker & M. J. Lenardo: Parameters controlling the programmed death of mature mouse T lymphocytes in high-dose suppression. *Cell Immunol* 160, 71-8 (1995)

135. R. S. Liblau, C. I. Pearson, K Shokat, R. Tisch, X.-D. Yang & H. O. McDevitt: High-dose soluble antigen: Peripheral T-cell proliferation or apoptosis. *Immunol Rev* 142, 193-208 (1994)

136. M. R. Alderson, T. W. Tough, T. Davis-Smith, S. Braddy, B. Falk, K. A. Schooley, R. G. Goodwin, C. A. Smith, F. Ramsdell & D. H. Lynch. Fas ligand mediates activation-induced cell death in human T lymphocytes. *J Exp Med* 181, 71-7 (1995)

137. M. Boshell, J. McLeod, L. Walker, N. Hall, Y. Patel & D. Sansom: Effects of antigen presentation on superantigen-induced apoptosis mediated by Fas/Fas ligand interactions in human T cells. *Immunology* 87, 586-92 (1996)

138. R. J. Mogil, L. Radvanyi, R. Gonzalez-Quintial, R. Miller, G. Mills, A. N. Theofilopoulos & D. R. Green: Fas (CD95) participates in peripheral T cell deletion and associated apoptosis in vivo. *Int Immunol* 7, 1451-8 (1995)

139. T. Renno, M. Hahne, J. Tschopp & H. R. MacDonald: Peripheral T cells undergoing superantigen-induced apoptosis in vivo express B220 and upregulate Fas and Fas ligand. *J Exp Med* 183, 431-7 (1996)

140. S. Nagata & T. Suda: Fas and Fas ligand: *lpr* and *gld* mutations. *Immunol Today* 16, 39-43 (1995)

141. L. Van Parijs, A. Ibraghimov & A. K. Abbas: The roles of costimulation and Fas in T cell apoptosis and peripheral tolerance. *Immunity* 4, 321-328 (1996)

142. J. H. Russell, B. Rush,, C. Weaver & R. Wang: Mature T cells of autoimmune *lpr/lpr* mice have a defect in antigen-stimulated suicide. *Proc Natl Acad Sci USA* 90, 4409-13 (1993)

143. P. Bossu, G. G. Singer, P. Andres, R. Ettinger, A. Marshak-Rothstein & A. K. Abbas: Mature $CD4^+$ T lymphocytes from MRL/*lpr* mice are resistant to receptor-mediated tolerance and apoptosis. *J Immunol* 151, 7233-39 (1993)

144. C. L. Tucek-Szabo, S. Andjelic, E. Lacy K. B. Elkon & J. Nikolic-Zugic: Surface T cell Fas receptor/CD95 regulation, *in vivo* activation and apoptosis. *J Immunol* 156, 192-200 (1996)

145. S.-J. Teh, J. P. Dutz, B. Motyka & H.-S. Teh: Fas (CD95)-independent regulation of immune responses by antigen-specific $CD4^-CD8^+$ T cells. *Int Immunol* 8, 675-81 (1996)

146. R. E. O'Hehir & J. R. Lamb: Induction of specific clonal anergy in human T lymphocytes by *Staphyloccus aureus* enterotoxins. *Proc Natl Acad Sci USA* 87, 8884-8 (1990)

147. D. Falb, T. J. Briner, G. H. Sunshine, C. R. Bourque, M. Luqman, M. L. Gefter & T. Kamradt: Peripheral tolerance in T cell receptor-transgenic mice: Evidence for T cell anergy. *Eur J Immunol* 26, 130-5 (1996)

148. G. Schönrich, U. Kalinke, F. Momburg, M. Malissen, A.-M. Schmitt-Verhulst, B. Malissen, G. J. Hämmerling & B. Arnold: Down-regulation of T cell receptors on self-reactive T cells as a novel mechanism for extrathymic tolerance induction. *Cell* 65, 293-304 (1991)

149. S. Webb, C. Morris & J. Sprent: Extrathymic tolerance of mature T cells: Clonal elimination as a consequence of immunity. *Cell* 63, 1249-1256 (1990)

150. L. D'Adamio, K. M. Awad & E. L. Reinherz: Thymic and peripheral apoptosis of antigen-specific T cells might cooperate in establishing self tolerance. *Eur J Immunol* 23, 747-53 (1993)

151. B. Arnold, G. Schönrich & G. J. Hämmerling: Multiple levels of peripheral tolerance. *Immunol Today* 14, 12-4 (1993)

152. T. T. Pearson, C. R. Darby, A. R. Bushell, L. J. West, P. J. Morris & K. J. Wood: The assessment of transplantation tolerance induced by anti-CD4 monoclonal antibody in the murine model. *Transplantation* 55, 361-7 (1993)

153. J. F. Markmann, J. S. Odorico, H. Bassiri, N. Desai, J. I. Kim & C. F. Barker. Deletion of donor-reactive T lymphocytes in adult mice after intrathymic inoculation with lymphoid cells. *Transplantation* 55, 871-7 (1993)

154. Y. Nakafusa, J. A. Goss, T. Mohanakumar & M. W. Flye: Induction of donor-specific tolerance to cardiac but not skin or renal allografts by intrathymic injection of splenocyte alloantigen. *Transplantation* 55, 877-82 (1993)

155. L. Campos, E. J. Alfrey, A. M. Posselt, J. S. Odorico, C. F. Barker & A. Naji: Prolonged survival of rat orthotopic liver allografts after intrathymic inoculation of donor-strain cells. *Transplantation* 55, 866-70 (1993)

156. Y. Shi, B. M. Sahai & D. R. Green: Cyclosporin A inhibits activation-induced cell death in T-cell hybridomas and thymocytes. *Nature* 339, 625-6 (1989)

157. S. Wesselborg, U. Prüfer, M. Wild, B. Schraven, S. C. Meuer & D. Kabelitz: Triggering via the alternative CD2 pathway induces apoptosis in activated human T lymphocytes. *Eur J Immunol* 23, 2707-10 (1993)

158. S. A. McCarthy, R. N. Cacchione, M. S. Mainwaring & J. S. Cairns: The effects of immunosuppressive drugs on the regulation of activation-induced apoptotic cell death in thymocytes. *Transplantation* 54, 543-7 (1992)

159. K. Migita, K. Eguchi, Y. Kawabe, T. Tsukada, A. Mizokami & S. Nagataki: FK506 augments activation-induced programmed cell death of T lymphocytes in vivo. *J Clin Invest* 96, 727-32 (1995)

160. D. Bellgrau, D. Gold, H. Selawry, J. Moore, A. Franzusoff & R. C. Duke: A role for CD95 ligand in preventing graft rejection. *Nature* 377, 630-2 (1995)

161. H. Yagita, K.-I. Seino, N. Kayagaki & K. Okumura: CD95 ligand in graft rejection. *Nature* 379, 682 (1996)

162. T. S. Griffith, X. Yu, J. M. Herndon, D. R. Green & T. A. Ferguson: CD95-induced apoptosis of lymphocytes in an immune privileged site induces immunological tolerance. *Immunity* 5, 7-16 (1996)

163. M. P. Pender, P. A. McCombe, G. Yoong & K. B. Nguyen: Apoptosis of αβ T lymphocytes in the nervous system in experimental autoimmune encephalomyelitis: Its possible implications for recovery and acquired tolerance. *J Autoimmunity* 5, 401-10 (1992)

164. Z. Tabi, P. A. McCombwe & M. P. Pender: Apoptotic elimination of $V\beta8.2^+$ cells from the central nervous system during recovery from experimental autoimmune encephalomyelitis induced by the passive transfer of $V\beta8.2^+$ encephalitogenic T cells. *Eur J Immunol* 24, 2609-17 (1994)

165. Z. Tabi, P. A. McCombe & M. P. Pender: Antigen-specific down-regulation of myelin basic protein-reactive T cells during spontaneous recovery from experimental autoimmune encephalomyelitis: further evidence of apoptotic deletion of autoreactive T cells in the central nervous system. *Int Immunol* 7, 967-73 (1995)

166. M. A. Cremer, A. D. Hernandez, A. S. Townes, J. M. Stuart & A. H. Kang. Collagen-induced arthritis in rats: antigen-specific suppression of arthritis and immunity by intravenously injected native type II collagen. *J Immunol* 131, 2995-3000 (1983)

167. D. L. Kaufman, M. Clare-Salzler, J. Tian, T. Forsthuber, G. S. P. Ting, P. Robinson, M. A. Atkinson, E.E. Sercarz, A. J. Tobin & P. V. Lehmann: Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature* 366, 69-72 (1993)

168. J. M. Critchfield, M. K. Racke, J. C. Zúñiga-Pflücker, B. Cannella, C. S. Raine, J. Goverman, M. J. Lenardo. T cell deletion in high antigen dose therapy of autoimmune encephalomyelitis. *Science* 263, 1139-1143 (1994)

169. Y. Chen, J.-I. Inobe, R. Marks, P. Gonnella, V. K. Kuchroo & H. L. Weiner: Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* 376, 1277-80 (1995)

170. J. M. Critchfield & M. J. Lenardo: Antigen-induced programmed T cell death as a new approach to immune therapy. *Clin Immunol Immunopathol* 75, 13-9 (1995)

171. C. B. Thompson: Apoptosis in the pathogenesis and treatment of disease. *Science* 267, 1456-62 (1995)

172. H. Groux, D. Monte, B. Plouvier, A. Capron & J.-C. Ameisen: CD3-mediated apoptosis of human medullary thymocytes and activated peripheral T cells: respective roles of interleukin-1, interleukin-2, interferon-γ and accessory cells. *Eur J Immunol* 23, 1623-9 (1993)

173. Y. Liu & C. A. Janeway Jr.: Interferon γ plays a critical role in induced cell death of effector T cell: A possible third mechanism of self-tolerance. *J Exp Med* 172, 1735-9 (1990)

174. X. Zhang, L. Giangreco, H. E. Broome, C. M. Dargan & S. L. Swain: Control of CD4 effector fate: Transforming growth factor β1 and interleukin 2 synergize to prevent apoptosis and promote effector expansion. *J Exp Med* 182, 699-709 (1995)

175. M. Radrizzani, P. Accornero, A. Amidei, A. Aiello, D. Delia, R. Kurrle & M. P. Colombo: IL-12 inhibits apoptosis induced in a human Th1 clone by gp120/CD4 cross-linking and CD3/TCR activation or by IL-2 deprivation. *Cell Immunol* 161, 14-21 (1995)

176. L. G. Radvanyi, Y. Shi, H. Vaziri, A. Sharma, R. Dhala, G. B. Mills & R. G. Miller: CD28 costimulation inhibits TCR-induced apoptosis during a primary T cell response. *J Immunol* 156: 1788-98 (1996)

177. J. C. Ameisen, J. Estaquier, T. Idiorek & F. De Bels: Programmed cell death and AIDS pathogenesis: Significance and potential mechanisms. *Curr Top Microbiol Immunol* 200: 195-211 (1995)

178. M. Hahne, D. Rimoldi, M. Schröter, P. Romero, M. Schreier, L. E. French, P. Schneider, T. Bornand,

A. Fontana, D. Lienard, J.-C. Cerottini & J. Tschopp: Melanoma cell expression of Fas (Apo-1/CD95) ligand: Implications for tumor immune escape. *Science* 274, 1363-6 (1996)

179. S. Strand, W. J. Hofmann, H. Hug, M. Müller, G. Otto, D. Strand, S. M. Mariani, W. Stremmel, P. H. Krammer & P. R. Galle: Lymphocyte apoptosis induced by CD95 (APO-1/Fas) ligand-expressing tumor cells - a mechanism of immune evasion? *Nature Med* 2, 1361-6 (1996)