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PROSPECT

Role of Apoptosis in the Regulation of Virus-Induced T Cell Responses, Immune Suppression, and Memory

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Abstract Apoptosis is an important mechanism enabling the selection of the non-self-reactive T cell repertoire and for maintaining homeostasis in the immune system after it has expanded to combat infections. Highly activated, proliferating T cells become susceptible to apoptosis driven by a number of stimuli, and T cells activated during a viral infection become susceptible to "activation induced cell death" after repeated stimulation through the T cell receptor (TcR). This is a major mechanism for the immune deficiencies observed during many viral infections. During infections with a high antigen load this can lead to a selective deletion of virus-specific cytotoxic T lymphocytes (CTL) and to the establishment of persistent infection. More commonly, the CTL control the infection first, and high levels of apoptosis in the expanded lymphocyte population occur after antigen and growth factors become limiting. This cell death does not seem to depend on TcR specificity, as the residual population contains a remarkably stable population of memory CTL precursors that approximate the frequency per CD8 cell of that seen during the peak of the acute infection. Subsequent infections with heterologous viruses result in an expansion and then an apoptotic elimination of T cells, with the consequence being a reduction in precursor CTL specific for the first virus. Thus, apoptosis shapes the quality and quantity of T cell memory.

Key words: apoptosis, T cells, cytotoxic T lymphocytes, memory, viral infections

Self/non-self discrimination, rapid proliferation and differentiation in response to foreign antigens, and an effective response to negative stimuli orchestrating the silencing of an immune response are all characteristics of T lymphocytes. Abnormal regulation of T cell responses could lead to the development of autoimmunity, to overproduction of toxic cytokines such as TNFβ, or, upon further dysregulation, to the development of lymphomas and leukemias. T cell proliferation is therefore tightly regulated, and each time T cells traverse the cell cycle, a decision must be made to continue proliferation, to stop proliferation and enter a resting phase, or to undergo programmed cell death. In fact, the choice is often apoptosis, a form of programmed cell death used to regulate lymphocyte responses in ways that, in the context of a viral infection, can result in virus-induced immune suppression and can contribute to the qualitative and quantitative modulation of the memory T cell pool after the infection has subsided [Selin and Welsh, 1994; Razvi and Welsh, 1995].

APOPTOSIS IN THE THYMUS

The thymus is the primary site for the education of T cells to distinguish self from non-self antigens. T cell antigens are short peptides presented to the T cell receptors (TcR) in the context of Class I or Class II major histocompatibility complex (MHC) molecules [Monaco, 1992]. Immature T cells passing through the thymus must engage self-MHC-peptide complexes with sufficient affinity to avoid elimination by apoptosis (positive selection); these positively-selected T cells will display a TcR repertoire capable of interacting with various peptide modifications of self-MHC molecules [Ashton-Rickhardt et al., 1994; Blackman et al., 1988]. However, those T cells interacting with self peptide-MHC complexes at high affinity are also eliminated by apoptosis by a process known as negative selection, which leads to "central tolerance" [Blackman et al., 1988; Jenkinson et al., 1989]. These conclusions are supported by experiments with TcR-transgenic mice, where apoptosis in the

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dominant T cell fraction can be observed in different thymic environments. For example, intrathymic apoptosis, as demonstrated by DNA fragmentation and by electron microscopy, occurs in thymocytes expressing an ovalbumin peptide-specific transgenic TcR upon inoculation of mice with the specific peptide [Murphy et al., 1990]. Thymocytes are therefore very sensitive to apoptosis driven through the TcR by peptide/MHC complexes or by antibodies to TcR or to CD3, and in the case of intrathymic virus infections, such as that of lymphocytic choriomeningitis virus (LCMV) in the mouse, infection early in life can lead to a deletion in virus-reactive T cells, resulting in a persistent infection as a consequence of central tolerance [King et al., 1992]. Thymocytes are also quite sensitive to apoptosis driven by other agents, such as glucocorticoids or γ-irradiation [Arends and Wyllie, 1991]. During acute viral infections there can be considerable thymic atrophy [Hamaoka et al., 1969], and this may be the consequence of stress-related steroids inducing thymocyte apoptosis.

PERIPHERAL TOLERANCE

Not all potentially self-reactive T cells can be eliminated in the thymus, because some tissue-specific antigens would not be presented to the T cells in the thymic environment. Thus, a back-up system to tolerize T cells in the periphery (i.e., outside of the thymus) is needed to prevent autoimmunity. T cells that leave the thymus can be inactivated or "anergized" by exposure to peptide/MHC complexes on cells that do not have appropriate costimulatory factors to fully drive T cell activation and proliferation. These costimulatory factors, which are expressed on "professional" antigen-presenting cells such as dendritic cells, activated macrophages, and activated B cells, include the surface molecules B7.1 and B7.2, which engage the CD28 molecule on T cells and help stimulate the production of IL-2 [Hathcock et al., 1994]. Inappropriate stimulation of cells can lead to a state of anergy that renders T cells unable to respond to appropriate stimulation for an indeterminant length of time. This state is associated with a failure to produce IL-2 and can sometimes be overcome by the addition of IL-2 from another source [Mueller et al., 1989]. In some cases, peripheral tolerance is achieved by this functional inactivation or "anergy," whereas in other situations clonal death may be the dominant mechanism. It is not known what factors determine this outcome.

Some tissues are relatively sequestered from the immune system, except under conditions of infection or tissue injury; thus, their antigens may normally be ignored by the mature T cell pool. Under conditions of strong T cell responses to viral infections, it is possible that helper factors are produced by the T cell response to viral antigens and that exposure of T cells to normally sequestered sites could respectively stimulate either anergic or "ignorant" self-reactive T cells infiltrating that tissue. The inflammatory response during an acute viral infection has been shown to break tolerance to normally sequestered viral transgenes expressed in tissues such as the pancreas [Ohashi et al., 1991; Oldstone et al., 1991]. T cell autoimmunity to normal host cell antigens can clearly be shown to be stimulated by viral infections in animal models, such as the parvovirus-induced diabetes in rats [Guberski et al., 1991]. Futhermore, human autoimmune diseases have also been associated with viral infections [Simpson et al., 1984].

APOPTOSIS IN MATURE T LYMPHOCYTES

TcR occupancy in the presence of appropriate costimulatory factors induces resting T cells to undergo a series of events leading to proliferation, cytokine release, and cytolytic capacity. During viral infections this can lead to a massive lymphocytic expansion in need of down-regulatory control mechanisms. Activated proliferating mature T cells become very susceptible to apoptotic death induced by a number of stimuli, including triggering through the TcR in a process known as activation-induced cell death (AICD) [Russell et al., 1991]. A variety of approaches have shown that activated T cells and resting T cells respond quite differently to TcR-stimulation. IL-2 receptor-expressing $CD4^+$ T cell clones cycling in IL-2 underwent apoptosis upon TcR-triggering by anti-CD3 or by antigen, whereas resting clones underwent proliferation under the same stimulatory conditions [Lenardo, 1991]. IL-2 induces high levels of T cell proliferation and causes, among other changes, expression of the *c-myc* gene [Shibuya et al., 1992]. Antisense oligonucleotides to *c-myc* block the sensitivity of T cells to AICD [Shi et al., 1992], and dysregulated or enforced ectopic overexpression of *c-myc* can sensitize various cell types to apoptosis, providing that the function of the *p53* tumor suppressor gene is normal [Hermeking and Eick, 1994]. Work in various systems has shown the importance of p53 in

programmed cell death, and T cells from *p53*-knockout mice are resistant to apoptosis elicited by certain stimuli [Lowe et al., 1993].

Similar observations on AICD in vitro and in vivo have been made with monoclonal T cell populations from mice carrying rearranged TcR transgenes. Transgenic H-2L^d-specific T cells when first activated with antigen in vitro underwent apoptosis upon subsequent triggering with anti-TcR or anti-CD3 antibodies, whereas resting T cells proliferated in response to those activating stimuli [Russell et al., 1991]. Adoptive transfer of male antigen (H-Y)-specific transgenic CD8$^+$ T cells into male mice led to first an expansion and then a decline in number of the transgenic T cells; DNA fragments were found in the reisolated transgenic T cells, indicating that they were undergoing apoptosis in this antigen-excess environment [Rocha and von Boehmer, 1991]. T cell peptide-infusion of mice bearing transgenic TcR specific either for an influenza virus or an LCMV epitope caused first a stimulation and then an elimination by apoptosis of the transgenic T cells [Mamalaki et al., 1993; Kyburz et al., 1993]. These experiments lead to the conclusion that resting T cells will respond to antigen in a productive proliferative manner but may eventually respond to further stimulation by undergoing apoptosis. This developing sensitivity to AICD was correlated with the extent of cellular proliferation, as measured by population doubling time and incorporation of ^{3}H-thymidine or 2-bromodeoxyuridine, and cells arrested by pharmacologic cell cycle-blocking agents were more resistant to AICD, whereas S-phase cells were more sensitive [Boehme and Lenardo, 1993].

The reasons why highly activated cells are more likely to undergo apoptosis upon receptor stimulation are not known, but cell cycle-dependent enzymes, such as p34^{cdc2} kinase and cyclins A and B, which are involved in the dissolution of nuclear membrane integrity and in the condensation of chromatin during mitosis, may also be important in similar events that occur during apoptosis [Shi et al., 1994]. Indeed, actively cycling cells are better targets for apoptosis mediated by CTL, and the activity of p34^{cdc2} kinase in the target cell is required for the fragmentation of its DNA [Nishioka and Welsh, 1994; Shi et al., 1994]. It may therefore be important that the expression of cell cycle-associated proteins be rigorously controlled and down-regulated at different stages in the cell cycle in a timely fashion. Under conditions of vigorous lymphocyte proliferation, which can occur in vivo as rapidly as every 8–12 h, residual levels of these nuclear dissolution factors might shunt cells into the apoptotic pathway upon TcR stimulation. Alternatively, it remains possible that this sensitivity to apoptosis is not directly related to the cell cycle per se but instead is due to concominant but independently occurring biochemical changes in the cycling cells.

Activated lymphocytes are sensitive not only to AICD but to other mechanisms of apoptosis induction as well. Activated lymphocytes tend to be susceptible to apoptosis upon growth factor withdrawal [Duke and Cohen, 1986] and may express on their membranes the Fas molecule and its ligand [Crispe, 1994]. These proteins are members of the TNF receptor and TNF families, respectively, and engagement of Fas can deliver an apoptotic signal to the activated lymphocytes. Induction of apoptosis in influenza virus-specific TcR-transgenic T cells by peptide infusion in vivo is impaired in Fas-deficient mice bearing the *lpr* mutation [Singer and Abbas, 1994]. Highly active T cell responses are also associated with the production of cytokines and other factors which may participate in driving T cells into apoptosis [Biron, 1994]. These include TGFβ, TNFα and β, glucocorticoids, and prostaglandins, and some or all of these factors may contribute to the down-regulation of the immune response.

APOPTOSIS IN THE DECLINE OF THE T CELL RESPONSE TO VIRAL INFECTIONS

Acute infections with viruses such as Epstein-Barr virus (EBV) in man and LCMV in the mouse can lead to major increases in the number and activity of T cells [Akbar et al., 1993; Selin and Welsh, 1994]. Activated T cells induced during acute EBV infections express high levels of the Fas protein but low levels of Bcl-2, an anti-oxidative protein that associates with mitochondria and inhibits apoptosis driven by a number of stimuli, perhaps by reducing the free radical load in cells [Akbar et al., 1993; Hockenbery et al., 1993; Kane et al., 1993]. The acute LCMV infection of the mouse elicits a 10-fold increase in the number of CD8$^+$ T cells in the spleen. Between days 4 and 7 postinfection the LCMV-specific CTL precursors increase in frequency at a rate consistent with an 8–12 h division time. Under most conditions of acute LCMV infection, antigen is cleared by day 7, the

T cells expand in number for another day or two, and then they rapidly decrease in number over the next 5 days. Using the in situ nucleotidyl transferase assay, which is a new histological technique designed to detect apoptotic cells in tissue sections [Gavrieli et al., 1992], we have found major increases in the number of apoptotic T and B cells in the spleen at days 9–14 postinfection [Razvi and Welsh, 1995; Razvi et al., 1995a]. Thus, the down-regulation in lymphocyte number at late stages of infection is associated with high levels of apoptosis in this lymphoid organ (Fig. 1).

The factors that drive lymphocyte apoptosis in viral infections are unknown and may involve a number of mechanisms. One might predict that the apoptosis could be due to growth factor-deprivation of activated lymphocytes, as IL-2 and other growth factors become limiting shortly before this apoptosis occurs. However, T cells expressing the *bcl-2* transgene are resistant to apoptosis upon growth factor deprivation in vitro, yet apoptosis of lymphocytes proceeds normally after acute LCMV infection of *bcl-2*-transgenic mice, arguing for another mechanism [Razvi et al., 1995a]. Activated lymphocytes express Fas and its ligand [Crispe, 1994], but there is significant apoptosis in lymphocytes after LCMV infection of Fas-deficient *lpr* mice. Therefore, this apoptosis might be driven by other stimuli, such as steroids, cytokines such as TGFβ, or other unidentified factors, but this needs to be further clarified.

The apoptosis in T cells under conditions where the antigen has been previously cleared occurs across the board among the activated lymphocytes, which comprise over 90% of the T cell population, and is apparently not driven by the TcR. This conclusion is based on the observation that the LCMV-specific CTL precursor frequency per $CD8^+$ T cell is approximately the same (within a factor of 2) several months postinfection as it is during the peak of the acute CTL response [Selin and Welsh, 1994]. Similar stabilities in pCTL frequencies are found for each of three immunodominant LCMV-encoded T cell peptides (Selin et al., unpublished data). This argues that there is no selective elimination or sparing of the virus-specific T cells, which continue to be maintained in a remarkably stable memory T cell pool.

CLONAL EXHAUSTION OF T CELLS UNDER CONDITIONS OF HIGH DOSE INFECTION

In contrast to the unselective cross-the-board apoptosis discussed above, a selective apoptosis of virus antigen-specific T cells can be achieved under conditions of high dose viral infection. Highly activated T cells can be driven into apoptosis by continued stimulation through the TcR,

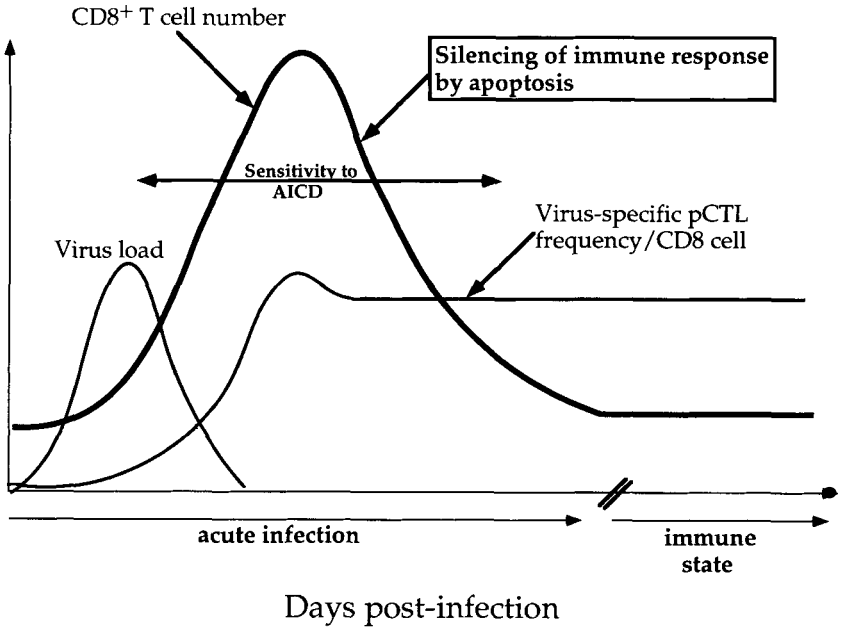

Fig. 1. Lymphocyte response to virus infection.

and administration of T cell peptides into TcR-transgenic mice can ultimately eliminate the T cells expressing the transgene [Kyburz et al., 1993; Mamalaki et al., 1993]. When mice are infected at high doses with strains of LCMV that disseminate and grow very well in macrophages and visceral organs, there is initially an antigen-driven expansion and then a selective deletion of the virus-specific CTL effector cells and their precursors [Moskophidis et al., 1993]. The consequence of this is the establishment of a persistent infection without T cell memory [Zinkernagel et al., 1993]. Hence, there appears to be a race between the antigen and the T cell response. Under normal conditions the T cells clear the antigen and give rise to immunological memory, but if high concentrations of antigen remain by the time the T cells become highly activated and thereby sensitive to apoptosis, the antigen will "clear" the T cells, leading to a persistent infection without T cell memory. It is noteworthy that during the later stages of human immunodeficiency virus (HIV) infection, when there is a global decrease in the number of $CD4^+$ T cells and a major increase in viral antigen load, there is next a selective elimination of HIV-specific $CD8^+$ T cell precursors [Carmichael et al., 1993].

IMMUNE DEFICIENCY UNDER CONDITIONS OF VIRUS-INDUCED T CELL ACTIVATION

Immune deficiencies under conditions of viral infections have been noted for over 80 years, ever since von Pirquet [1908] first showed that patients suffering from acute measles virus infection failed to develop a delayed-type hypersensitivity recall response to tuberculin, to which they had been previously immunized. This failure to mount a recall response to non-viral antigens has subsequently been observed in many other viral infections, including HIV and cytomegalovirus, and in the LCMV mouse model [Rouse and Horohov, 1986]. A second criterion for immune deficiency observed in many viral infections has been the failure of T cells isolated from the infected individual to productively respond to stimulation through the TcR by lectin mitogens and by anti-CD3 antibody [Jacobs and Cole, 1976; Saron et al., 1990; Groux et al., 1992; Meyaard, et al., 1992]. Although this non-responsiveness to TcR stimulation could be influenced by many suppressive factors, it is now clear that a major mechanism is simply the sensitivity of activated T lymphocytes to AICD [Groux et al., 1992; Meyaard et al., 1992; Razvi and Welsh, 1993]. T cells isolated from acutely LCMV-infected mice or from HIV-infected individuals undergo apoptosis when stimulated thusly. The kinetics of apoptosis in LCMV-induced T cells can be accelerated by further incubation in vitro in IL-2, confirming the work with T cell clones showing that cells rapidly cycling in IL-2 become highly sensitive to AICD [Razvi and Welsh, 1993; Lenardo, 1991]. Many memory T cells express receptors for IL-2 [Kos and Müllbacher, 1993; Razvi et al., 1995b], and we have proposed that exposure of the memory cells to IL-2 and to other cytokines liberated during the massive T cell response to viral infections would poise them to undergo AICD instead of proliferation when exposed to their respective antigen [Razvi and Welsh, 1993] (Fig. 2). This could result in a temporary deficiency in the recall response during acute viral infections that later resolve. Because not all of these memory cells would see antigen and undergo apoptosis, those that did not would return to a resting state and respond in a productive manner upon subsequent exposure to antigen.

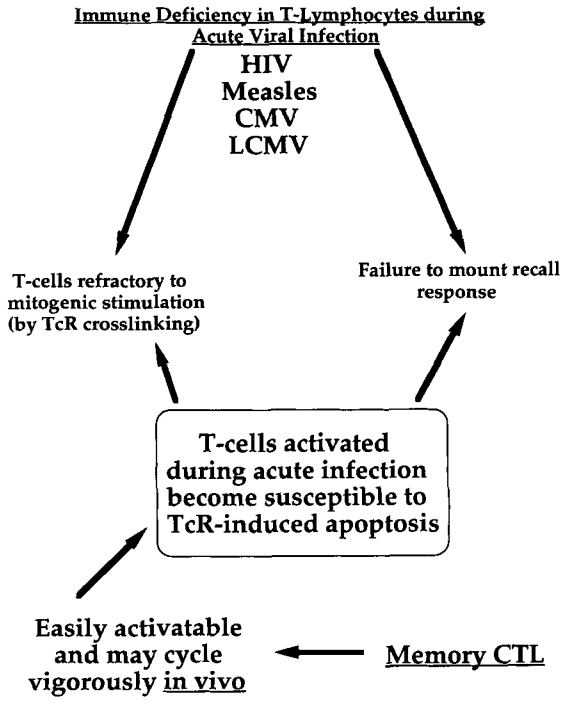

Fig. 2. Immunodeficiency in T-lymphocytes during acute viral infection.

APOPTOSIS AND THE MAINTENANCE OF THE MEMORY CTL RESPONSE

The apoptotic event that occurs at later stages in viral infections that are cleared by the immune response is likely to leave one with an immune system different from that which existed prior to infection. It is not clear what are the specificities of all the T cells stimulated during infection, as a relatively low frequency (usually much less than 10%) can be shown to react with viral antigens at high affinity [Nahill and Welsh, 1993]. Many of the rest of these T cells could be the product of low affinity stimulations with viral antigens, stimulation with virus-induced self antigens, or simply nonspecific bystander stimulations of T cells by virus-induced cytokines. Whatever their makeup, it is likely that not all cells are stimulated comparably, and the immune system at the peak of the virus-induced immune response reflects a different lymphocyte distribution than that which existed prior to infection. The antigen-non-specific cross-the-board apoptosis that silences this response at the end of infection does not eliminate all of these stimulated cells but instead leaves the host with a virus-modified immune system containing memory cell populations reflecting their frequencies at the peak of the immune response.

The frequency of virus-specific memory CTL remains remarkably stable over a period of *months as long as the immune system does not* experience a major antigenic insult [Lau et al., 1994; Moskophidis et al., 1993; Selin and Welsh, 1994], but infection with another virus can disrupt this homeostasis. Upon infection with a heterologous virus, virus-specific CTL precursors must now be obtained from the lymphoid population that was heavily influenced by the original virus infection, and recent data have shown that certain T cells responding to a second virus infection will crossreact at some level of affinity with antigens of the first virus [Selin et al., 1994]. As the T cell response to the second virus continues, those T cells with high affinity for the second virus will be gradually selected over the crossreactive T cells and over the T cells reactive only with the first virus. Thus, when the antigen-non-specific apoptotic events occur at the conclusion of the immune response to the second virus infection, there has been a decline in the frequency of T cells with high affinity for the first virus. The consequence of this is a significant reduction in memory to the first virus [Selin et al., 1994; Selin and Welsh, 1994]. We have in fact found that a series of heterologous viral infections, such as LCMV, Pichinde virus, vaccinia virus, and MCMV can with each infection drive down the memory response to the viruses from earlier infections [Selin and Welsh, 1994], (unpublished data). Hence, apoptosis contributes to the homeostasis in the immune system subsequent to acute viral infections and thereby shapes the quality and quantity of T cell memory.

CONCLUSION

Apoptosis is therefore a major regulator of lymphocyte homeostasis and plays a particularly important role in constraining the very vigorous T cell response to acute viral infections and the makeup of the memory T cell pool thereafter. Although certain specific genes and molecular pathways have been proposed to account for some forms of lymphocyte apoptosis, it is clear that much needs to be learned concerning the relative roles of different apoptosis-inducing factors and the mitigating elements that influence the course of T cell responses in vivo. Elucidating these events should shed light on the mechanisms behind the development of T cell memory and homeostasis, as well as the immune deficiencies that are commonly induced by viral infections.

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