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MINIREVIEW

Inhibition of Antiviral CTL Responses by Virus-Infected Cells: Line Item Veto (Cells) Revisited

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It is widely acknowledged that the key immune responses providing protection against viral diseases are neutralizing antibodies (Abs) and antiviral T cells, especially cytolytic T lymphocytes (CTL). Neutralizing Abs are effective against free virions, particularly in reinfections where preexisting Abs may protect against initial infection and/or memory Ab responses may be elicited with sufficiently rapid kinetics to limit the infection after the first few rounds of viral replication. In a complementary fashion, antiviral CTL, via their ability to lyse virus-infected cells and to secrete antiviral cytokines such as IFN-γ, are particularly important in defending against viruses that are transmitted by infected cells and cell:cell contact. CTL responses may also be critical in resolving a primary encounter with virus before sufficient cycles of infection have occurred to spread the virus systemically.

Because antiviral CTL are generally of the $CD8^+$ T cell phenotype, the endogenous class I MHC antigen (Ag) processing and presentation pathway, which monitors the intracellular compartment for foreign insults and/or for "danger," is essential to recognition of virus-infected cells by specific T cell receptors (TcR) of the CTL. Although a thorough discussion of the molecular players of the endogenous class I MHC pathway is beyond the scope of this minireview, suffice it to summarize here that the process begins with proteolytic cleavage of viral proteins, typically in the multicatalytic proteasome complex, followed by transport of peptides into the endoplasmic reticulum (ER) by a dedicated member of the ABC-transporter family, the TAP-1/-2 heterodimer. In the ER additional peptide trimming may occur. With the aid of both general chaperonin molecules and specialized proteins, such as tapasin, the ultimate trimolecular species of β_2-microglobulin, class I MHC heavy chain, and a short (8–11 amino acid) minimal peptide epitope is formed for export from the ER to the cell surface. The cell surface presentation of the viral epitope in the polymorphic binding groove of the α_1 and α_2 domains of class I heavy chain allows for identification of virus-infected cells and accounts for the class I MHC-restricted nature of CTL TcR recognition. Although the focus here will thus be on classical $CD8^+$ CTL, it is important to emphasize that antiviral $CD4^+$ T cells can also be crucial in the resolution of viral infections: (1) some antiviral CTL are class II MHC-restricted $CD4^+$ T cells; (2) Th1 $CD4^+$ T cells can also have direct antiviral effects via cytokine production and/or activation of effector macrophages; (3) Th2 T cells provide help to B cell responses, particularly for immunoglobulin class switching and affinity maturation to produce high-affinity, neutralizing Abs; and (4) most relevant to the present discussion, many antiviral $CD8^+$ CTL responses are dependent on $CD4^+$ Th, principally to "condition" or "mature" professional antigen-presenting cells (APC) so that they will more efficiently stimulate/costimulate naive $CD8^+$ antiviral CTL and/or to produce cytokines such as IL-2 to maximally drive $CD8^+$ CTL expansion. Thus, to the extent that antiviral $CD4^+$ Th are also involved, the efficient functioning of the alternative exogenous pathway of viral antigen processing and presentation by class II MHC may also be critical.

To combat recognition of virus-infected cells by $CD8^+$ CTL, viruses have developed a number of clever evasion strategies. These escape mechanisms fall into two main classical categories: (1) variation in viral amino acid sequences responsible for epitope production and (2) viral genome encoding of proteins that actively interfere with the production and presentation of unmutated viral epitopes. Both of these general evasion strategies have been discussed in detail in a number of recent review articles. Here we simply emphasize that for variations directly affecting viral epitopes per se, evidence has accumulated not only for changes within the epitopes to inhibit binding to MHC class I alleles or by the TcR, but

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also for alterations in the amino acid flanking sequences, resulting in impaired processing of the epitopes from their precursor proteins/larger peptides. On the other hand, for viral proteins that inhibit epitope processing or presentation, it is conceptually possible that every step of the endogenous class I pathway may provide an opportunity for a virus countermeasure and escape from antiviral T cells. Indeed, several reports demonstrate that an increasing number of the host cell molecular players have apparently been targeted by viral proteins. These studies range from the early identification of the adenovirus E3/19K protein that retains certain class I alleles in the ER to more recent descriptions of a variety of viral proteins encoded particularly by the large-genome DNA viruses, such as the Herpes family, that interfere with not only transport of MHC/peptide complexes from the ER, but also other steps of the presentation pathway, such as the TAP-1/-2 transporter. In addition, in those cases where $CD4^+$ T cells are also crucial for antiviral immunity, it seems likely that viral evasion strategies will be uncovered that target various points within the exogenous class II MHC processing and presentation pathway. Collectively, the importance of T cell-mediated immunity to the successful resolution of viral infections is underscored by these examples of T cell epitope variation and viral molecular inhibitors of host cell antigen processing and presentation.

In this minireview we explore an alternative general mechanism by which viruses, specifically as virus-infected cells, may escape clearance by antiviral T cell-mediated immunity—the ability of virus-infected cells to serve as "veto cells" that inactivate activated antiviral T cells. The principal differences between the virus escape mechanisms discussed above and the veto cell strategy are that in veto cell inhibition (1) viral epitopes recognized by the T cells are not modified, (2) viral epitope processing and presentation are not disturbed, and (3) TcR recognition and the initiation of the T cell response, and perhaps initial clonal expansion of antiviral T cells, are not inhibited. Rather, the veto cell mechanism embraces and takes advantage of normal antigen processing and presentation and MHC-restricted TcR recognition. In short, the virus-infected veto cell is a bona fide APC, but one which, subsequent to its specific recognition and binding by the antiviral T cell, functionally inactivates, or causes the apoptotic lysis of, that antiviral T cell. In the discussion that follows we will first reexamine the roots of veto cell regulation of nonviral specific CTL responses as first reported more than two decades ago and then merge and extend these concepts of inhibitory APC into the context of evidence for veto cells as a virus escape mechanism.

In 1979 R. G. Miller and collaborators first coined the term "veto cells" to explain the specific inhibition or vetoing of allo-MHC-specific CTL responses by cells bearing the allogeneic MHC. A series of studies by this group and other investigators over the next several years confirmed the concept of veto cells and distinguished them from T suppressor (Ts) cells and other inhibitory immune phenomena (see the review by Fink *et al.,* 1998). Thus, veto cells were highly specific, importantly at the effector phase in contrast to many then current reports of Ts cells, which were induced in an antigen-specific way but whose effector suppression was delivered in an antigen-nonspecific manner. Similarly, veto cell action depended on cell:cell contact between the veto cells and the responder T cells that were inactivated, and soluble inhibitory factors could not be identified that could account for veto cell function. Indeed, the hallmark characteristic of a veto cell was the exquisite specificity of its interaction with T cells—a degree of specificity that could be reconciled only with TcR recognition of the veto cell by the antigen-specific T cells about to be inactivated. Thus, veto function was extended from the early allo-MHC CTL systems to inhibition of both MHC-restricted anti-minor histocompatibility (H) antigen and anti-hapten CTL responses.

With respect to the phenotype of the veto cell in these early studies, the focus was on $CD8^+$ T cells/CTL. Depending on the specific system, $CD8^+$ CTL were frequently either the predominant or only veto cell type identified, or they were at least the most efficient kind of veto cell. Although a complete mechanism for inactivation by veto cells could not be proposed at this early stage, it appeared that the final result of veto cell action was the loss of the antigen-specific CTL. Thus, using the only method then available to enumerate antigen-specific T cells, limiting dilution analysis (LDA) which scores precursor CTL (pCTL), greatly diminished frequencies of pCTL were observed when veto cells were present. However, despite the predominant $CD8^+$ CTL phenotype of veto cells and this apparent lysis of antigen-specific pCTL, there was no experimental support for the obvious possibility that veto cells lysed pCTL by using the standard CTL lytic mechanism used to kill target cells. To be sure, engagement of the $CD8^+$ veto cell TcR, which, except in certain contrived circumstances, is a prerequisite for activation of the effector cell lytic mechanism of CTL, was not required for veto cell function. That the CD8 molecule was not just a marker of the most efficient veto cell population, but was relevant to veto cell function, however, was shown in a series of papers by Miller and colleagues and other investigators (see below). In fact, the emphasis on CD8 positivity of veto cells led some early investigators to consider $CD8^+$ T cells as the only veto cells or, as is more common currently, as the "classic veto cells."

As these early studies were extended through the mid 1980s, it became clear that various cells of phenotypes other than $CD8^+$ T cells possessed such APC inhibitory activity on antigen-specific T cells. These cell types included bone marrow and spleen cells from nude (athy-

mic) mice. From normal euthymic mice evidence for veto cell activity was reported for bone marrow cells—including both non-T and non-B cells as well as derived T cell colonies, thymus cells (both lymphoid colonies and thymic epithelium), fetal liver cells and lymphoid colonies grown from fetal liver, and activated spleen cells. More recently, veto-type activity has also been reported for an even wider range of cell types. Given that many of these cells are CD8-negative, and/or may inactivate more mature T effector cells instead of, or in addition to, pCTL, one could debate whether these cell types should be considered (classic) veto cells. In many cases T cell/CTL lysis by this expanded list of cells with veto-like activity has been shown to be due to veto cell expression of FasL (CD95L), in addition to the specific MHC/peptide complexes recognized by the T cells, with triggering of apoptotic lysis of Fas (CD95)-positive activated T cells. Here, we will use the term "veto cell" in the broadest context of any cell that lyses or irreversibly inactivates an antigen-specific T cell in a contact-dependent manner driven by TcR recognition of the veto cell, regardless of the phenotype of the veto cell (including CD8 status) or the molecular mechanism of veto cell-induced lysis/inactivation. In this light, there is evidence for veto cell activity and/or FasL expression (implicating potential veto activity) by various tumor types associated with melanoma, hepatocellular carcinoma, esophageal carcinoma, astrocytoma, colon cancer, T cell acute lymphocytic leukemia, chronic myeloid leukemia, acute myeloblastic leukemia, multiple myeloma, and natural killer cell lymphoma/large granular lymphocytic leukemia.

In our own recent studies of veto cell inhibition of CTL responses to "AKR/Gross" endogenous murine leukemia virus (MuLV)-encoded epitopes, CTL-nonresponder AKR.H-2^b congenic mice contained highly specific veto cells of the $CD8^+$ T cell, $CD4^+$ T cell, and B cell subsets. No evidence could be obtained for veto cell activity in the splenic macrophage population, however, despite their viral antigen positivity, like that of these three lymphoid veto cell populations. The same phenotypic subsets of inhibitory cells were found whether veto activity was measured by *in vivo* adoptive transfer experiments at the level of CTL priming or by *in vitro* coculture at the level of secondary antigenic restimulation. Thus, our data indicate that some (B cell) but not necessarily other (macrophage) members of the APC populations referred to as "professional APC" may have functional veto activity in this MuLV system. Of particular interest—given the current consensus that the third member of the professional APC group, dendritic cells (DC), is the most efficient stimulatory APC—recent studies by other laboratories have indicated that a DEC-205^+ DC subpopulation can function as veto cells of allo-MHC responses. Implicit in these studies of MHC class II-positive veto cells, which can therefore display both cell surface class I/ and class II/foreign peptide complexes, is at least the potential to also veto $CD4^+$ antigen-specific T cells. For example, in our MuLV system, in which optimal antiviral CTL production requires $CD4^+$ T cells, veto activity restricted against either only the $CD4^+$ or the $CD8^+$ responder T cell compartment was sufficient to similarly and dramatically inhibit antiviral CTL generation. Similarly, in the allo-MHC veto cell system, $CD4^+$ T cell responses were directly shown to be inhibited by the DEC-205^+ DC subpopulation.

In the context of the present broad definition of veto cells encompassing several different virus-infected cell types that may inactivate antiviral $CD4^+$ Th and/or $CD8^+$ pCTL/CTL, it is not unreasonable to speculate that there may be different veto molecular mechanisms. At least conceptually, these mechanisms may range from irreversible functional inactivation to lysis per se of the antiviral T cell. As for lytic veto mechanisms, both our anti-MuLV system and the DEC-205^+ DC system have been shown to be essentially totally dependent on FasL/Fas interactions, with veto cell FasL triggering the apoptotic lysis of Fas^+ T cells that recognize the veto cell. In these murine settings, this has been convincingly shown by comparing antigen-specific responding T cells from wild-type vs *lpr*- (Fas^-) or *gld*- ($FasL^-$) congenic mice for susceptibility to veto cell inhibition. The results implicating a FasL/Fas-mediated mechanism (veto insusceptibility of only Fas^- responder T cells) were confirmed with blocking reagents directed at one or both members of this interacting molecular pair—i.e., Fas–Ig fusion protein in our system—to inhibit the lysis of antiviral T cells mediated by veto cells and restore T cell responsiveness. We have further utilized *in vitro* reconstitution of isolated, antigen-primed $CD4^+$ Th vs $CD8^+$ pCTL populations from wild-type vs Fas^- congenic strains to limit Fas expression to one or the other T cell subpopulation. By these means, the substantial (25- to 70-fold) reductions in polyclonal antiviral CTL activity and/or pCTL expansion (by LDA determinations) were achieved by veto targeting of either $CD4^+$ or $CD8^+$ antiviral T cells, or both. The ability of $CD4^+$ Th cells to be vetoed was consistent with our identification of class II retroviral antigen-expressing veto B cells and with recent evidence that activated murine B cells and other B-lineage cells express FasL.

Taking all these findings into consideration, we propose an updated set of related models of veto cell action to explain the inactivation of antigen-specific $CD4^+$ and $CD8^+$ T cells, both generally and specifically in the case of antiviral T cells (Fig. 1). Thus, $CD8^+$ pCTL/CTL can be vetoed by any virus-infected class I MHC-positive cell that also expresses, or can be induced to express, the inhibitory machinery [here depicted as simply cell surface FasL (CD95L) expression], with or without CD8 (Fig. 1A). In contrast, antigen-specific $CD4^+$ Th can be vetoed only by virus-infected class II-positive APC, such as DC that may express CD8 (Fig. 1B) or B cells, which are

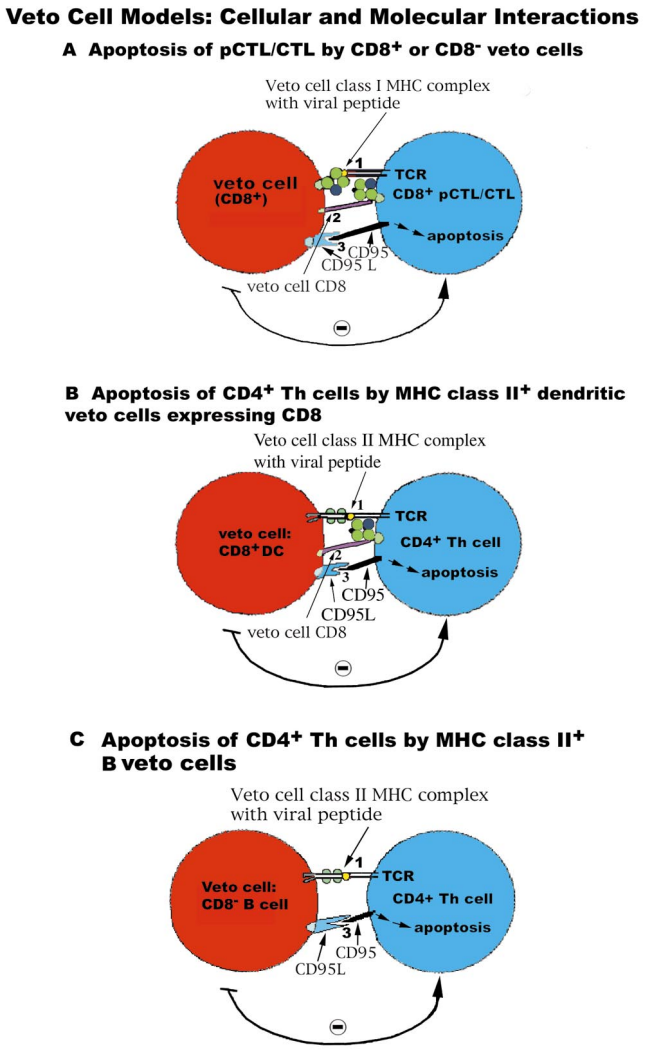

FIG. 1. A schematic depiction of veto cells with three different basic phenotypes and a representation of the sequential kinetic events leading to the vetoing of "targeted" T cells. Shortly following (or coincident with) the initial Ag/TCR interaction, veto cells that express CD8 molecules may ligate CD8 to α_3 domains of MHC class I complexes on the targeted T cell and contribute to the initiation of a signaling cascade leading to functional inactivation (or here, apoptotic lysis) of the targeted T cell. One molecular lytic mechanism is depicted here: the interaction of veto cell FasL with the Fas death receptor of the targeted T cell, initiating a cascade of caspases and eventual apoptosis. In other cases where veto cells are $CD8^-$, veto activity is by definition CD8 independent, thus indicating that signaling through class I of the targeted T cell is not essential.

$CD8^-$ (Fig. 1C). In each case the first step in the cellular interaction is governed by recognition of veto cell class I or II MHC/viral peptide complexes by the TcR of the activated antiviral T cell. It is the exquisite specificity of TcR recognition that accounts for the precision of the apparent veto cell "backward recognition" and veto cell-mediated inactivation. Indeed, in our murine retrovirus model, spleen cells from AKR.H-2^b mice that spontaneously express endogenous MuLV inhibit the secondary *in vitro* stimulation of the anti-MuLV CTL response that is dominated by a K^b-restricted p15E viral envelope peptide (KSPWFTTL) specificity, but not a "third-party" minor H specific CTL response. This is the case even when the same responder T cell population, primed against both sets of antigens, is cultured in the same well with AKR.H-2^b veto cells and a given positively stimulating, irradiated tumor cell that expresses both viral and minor H antigens.

In the case of veto cells that express CD8, the second step is engagement of nonpolymorphic residues of the α_3 domain of class I MHC of the TcR-bound antiviral T cell, whether this is a $CD8^+$ pCTL/CTL (Fig. 1A) or a $CD4^+$ Th (Fig. 1B). As alluded to above, early studies that focused on inactivation of pCTL by classic $CD8^+$ veto

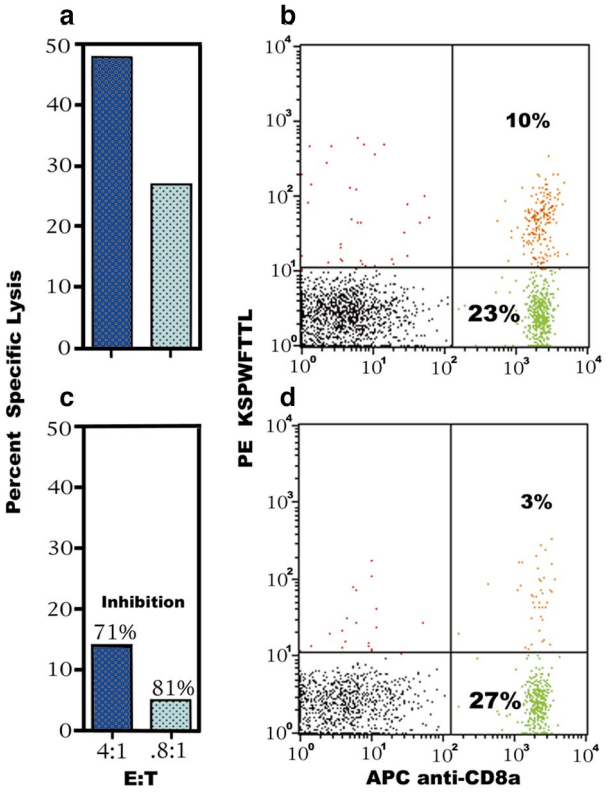

FIG. 2. Concurrent inhibition of antiviral CTL activity and reduction in the frequency of tetrameric MHC class I/peptide complex binding by specific $CD8^+$ CTL. B6 mice were inoculated with AKR/Gross $MuLV^+$ tumor cells. Eleven days later, responder lymphocytes were cultured in mixed lymphocyte tumor cell cultures (MLTC) for 6 days with irradiated, viral Ag^+ tumor cells, without (a, b) or with (c, d) AKR.H-2^b veto cells. At the end time point of the MLTC, responder cells were tested for their ability to lyse ^{51}Cr-labeled viral Ag^+ tumor target cells and were concurrently stained via flow cytometric analysis to identify and enumerate K^b/KSPWFTTL tetramer [phycoerythrin (PE)-labeled] and anti-CD8a mAb [allophycocyanin (APC)-labeled] binding to CTL. Responder T cells which were $CD8a^+$ but did not bind the tetramer are shown as green data points in the lower right quadrants (b, 23%; d, 27%). $CD8a^+$/$tetramer^+$ (double positive) T cells are shown as orange data points in the upper right quadrants (b, 10%; d, 3%). Thus, the percentage of $CD8^+$ T cells that are $tetramer^+$ is 30% (b) versus 10% (d).

cells suggested that recognition of responder T cell class I α_3 domains by CD8, in conjunction with simultaneous T cell TcR engagement, is necessary and sufficient for veto cell function. Consistent with this hypothesis, mAbs to the α_3 domain of responder T cell class I could inhibit the development of allogeneic CTL responses, and this effect was extended to Th responses in mixed lymphocyte responses, apparently in both cases due to responder T cell apoptosis. Furthermore, there was evidence from peripheral T cell deletion studies that CD8 with an intact cytoplasmic tail was required for veto cell function. These studies suggested that perhaps cell surface-initiated signaling cascades within both the veto cell and the target T cell might be crucial, but to our knowledge there were no additional data to advance a specific molecular model that connected these putative signals to a triggering of T cell apoptosis.

We suspect that activation of the veto cell is required and that in many cases the end result of such induced signaling pathways is the induction of expression, or the upregulated expression, of veto cell surface FasL. The signals culminating in increased FasL expression may emanate from veto cell class I upon its ligation by the TcR and/or from veto cell CD8, in the case of $CD8^+$ veto cells, as just discussed. However, we believe it unlikely that signaling from the cytoplasmic domain of CD8 is absolutely required: (1) clearly some veto cells are $CD8^-$ (Fig. 1C), and (2) in other cases it has been shown that $CD8^+$ veto cells may not necessarily employ CD8 in the veto cell mechanism (Figs. 1A and 1B). Thus, in the case of the DEC-205^+ veto cell DC population, this subset was initially thought to be functionally defined by the expression of CD8α. Subsequently, using CD8 knockout mice, it was discovered that CD8 expression was irrelevant to the veto function of the CD8α^+ DC and that the coexpressed DEC-205 marker also defined this negative regulatory DC subpopulation. Alternatively, or in addition, it is possible that veto cells, perhaps particularly virus-infected veto cells, have some constitutive expression of FasL, but at a level that is insufficient to trigger the apoptosis of Fas^+-activated T cells. Given recent evidence that reciprocal signaling can occur through FasL following its ligation of Fas, there could be a positive feedback signaling loop through which veto cell FasL expression is increased to levels that allow delivery of a death signal through Fas (step 3, Fig. 1) to cause the apoptotic lysis of the activated T cell.

Whatever the exact pathways of signal transmission in the veto cell, the concept of veto cell activation and induction of signal transduction is consistent with two widely observed characteristics of veto cell function. First, in studies to date, functional veto cells must be viable and metabolically active—veto cells that have been irradiated, or treated with the irreversible protein synthesis inhibitor emetine (our unpublished experiments), are not able to inhibit specific T cell responses. Second, the delayed kinetics of veto cell action in *in vitro* restimulation systems may, at least in part, be due to a requirement for veto cell activation. In our antiendogenous MuLV CTL system, where AKR.H-2^b veto cells are added to 6-day *in vitro* CTL restimulation cultures, kinetics experiments have suggested that veto cell-induced T cell inactivation is occurring primarily on days 2 and 3. This time frame is very consistent with other studies, including the $CD4^+$ Th and $CD8^+$ CTL allo-MHC responses studied in MLR cultures by Miller and collaborators. In these early studies, these delayed kinetics were interpreted as indicative that the T cell target susceptible to veto inactivation was neither an unactivated, naive pCTL or pTh nor a fully differentiated effector T cell, but rather a precursor T cell that was at the early stages of activation or clonal expansion. In the context of FasL/Fas-dependent veto cells, our view is that while responder T cell activation is necessary, if nothing else to induce or augment the expression of Fas on the responder T cell surface, it seems likely that the kinetics of veto cell-mediated T cell apoptosis also depend on the time frame of veto cell activation. Our preliminary experiments have shown a gradual increase in Fas expression by the responder T cell population over the course of the 6-day restimulation cultures. In contrast, FasL is known to be a very transiently expressed activation molecule. Thus, it would follow that the optimal time for veto cell inhibition is a complex phenomenon that may depend on both the time needed for the T cell and the veto/APC cell to reciprocally activate each other and the kinetics and duration of expression of those activation molecules on both cell types required for full veto function. Although the focus here has been on FasL/Fas, certainly the expression of the required veto cell MHC class I/II–peptide complexes and T cell TcR will play determining roles, as well as potentially other molecules, such as adhesion molecule receptors/coreceptors.

A limitation in the studies of veto cell inhibition of antigen-specific, polyclonal T cell responses to date has been the inability to enumerate and identify all the antigen-specific T cells that would potentially be susceptible to veto cell inactivation. LDA determination of pCTL frequencies has been employed, but LDA requires that an antigen-specific T cell be able to undergo several rounds of division upon activation to be scored and thus will not detect terminally differentiated effector T cells. Indeed, the more recently introduced techniques of ELISPOT analysis for cytokine (usually IFN-γ for $CD8^+$ CTL) secretion and the use of tetrameric class I MHC/peptide complexes in flow cytometric analyses have yielded frequencies of antigen-specific T cells that are generally at least 10-fold, and sometimes approaching 100-fold, higher than those obtained by LDA. This is logical since at the height of an immune response one would expect that the overwhelming majority of the clonally expanded T cells would be terminally differentiated effector cells.

Use of class I tetramers loaded with a given immunodominant epitope has proven to be a particularly effective approach that is readily amendable to further analysis of the antigen-specific T cells on an individual basis by taking advantage of the power of multicolor flow cytometry. In our antiendogenous MuLV CTL system we have conducted a set of initial experiments with K^b class I tetramers loaded with the immunodominant peptide epitope KSPWFTTL that is encoded by the p15E portion of the retroviral *env* gene. By integrating this technique of Ag-specific pCTL/CTL identification into the context of a functional veto cell inhibition experiment, we have been able to show corresponding veto cell-dependent decreases in the generation of polyclonal antiviral CTL activity and the frequency of these effector $CD8^+$ T cells (Fig. 2). Thus, at day 6 of the CTL restimulation cultures the observed 70–80% inhibition of the development of specific antiviral CTL activity caused by inclusion of endogenous viral antigen-positive AKR.H-2^b veto cells (compare Figs. 2a and 2c) was matched by the approximate 70% reduction in the percentage of $CD8^+$ T cells that specifically bound the K^b tetrameric complexes displaying the immunodominant KSPWFTTL epitope (compare the two-color analyses of Figs. 2b and 2d). Further analyses incorporating staining for CTL activation markers, such as Fas, and indicators of apoptosis such as annexin V staining or the flow cytometric modification of the TUNEL assay are ongoing and should permit us to examine the CTL restimulation cultures at various times to directly visualize and enumerate the veto cell-dependent apoptosis of antiviral CTL specific for the immunodominant or other subdominant epitopes. By these means and others our intent is to fully characterize the mechanism of FasL/Fas-mediated veto cell lysis of antiviral $CD4^+$ Th and $CD8^+$ CTL in the endogenous MuLV system. A particularly important issue to examine is the tantalizing possibility that retrovirus infection and expression either may cause the upregulation of FasL expression so that it is constitutive on virus-infected cells or may alter the signal transduction pathways that regulate FasL expression such that activation of veto cells leads to increased cell surface FasL.

In summary, we propose veto cell inactivation of antigen-specific antiviral T cells as another mechanism of virus escape from T cell-mediated immunity. Although there was early speculation that viruses might employ the veto strategy, this has remained an understudied area. Among the reports on viral veto cells in addition to our MuLV system there have been suggestions in the context of retrovirus-caused immunodeficiency, including evidence that $CD4^+$ T cells infected with SIV show a *nef*-dependent induction of FasL expression which correlated with the death of SIV-specific CTL. Whether FasL/Fas-mediated apoptosis of $CD4^+$ T cells is responsible for the decline of $CD4^+$ T cells in HIV/AIDS has been the subject of much debate and controversy, let alone the possible role of HIV-infected veto cell FasL-dependent apoptosis of antiviral T cells. However, there are several indications that veto cells can have significant inhibitory functions *in vivo*. These include both experimental and clinical systems such as peripheral tolerance and bone marrow transplantation-induced tolerance, as well as spontaneous examples of T cell nonresponsiveness. In the latter category we would offer our system of the natural CTL nonresponsiveness against endogenous MuLV in MHC responder strains which express these endogenous MuLV and have virus-infected veto populations, as assessed by both *in vivo* adoptive transfer and *in vitro* cell-mixing experiments, as discussed above. In addition and beyond the scope of virus infections, there have been several reports over the past few years indicating that, for at least some immunologically privileged tissues, veto cell inhibition may help to maintain this reduced immunological status. These tissues include the anterior chamber of the eye and the testes, where FasL-bearing stroma cells and Serotoli cells, respectively, may serve as veto cells to cause the apoptotic lysis of activated T cells entering these confined spaces before inflammatory damage is caused to these sensitive sites.

Finally, it is perhaps instructive to consider that virus-infected veto cells may represent an example of a "fail-safe" ultimate escape mechanism obtained by coopting a normal immunological process. Thus, the needed down-regulation of the large number of effector T cells, once an infection has been successfully cleared, is generally considered to be accomplished by a process referred to as activation-induced cell death (AICD). In AICD activated T effector cells expressing FasL and/or Fas undergo apoptotic lysis by either "suicide" or "fratricide" upon engagement of FasL and Fas in an antigen-nonspecific manner, although the involvement of other TNF/TNFR family members has also been described. AICD has been most readily demonstrated in situations in which there are very high frequencies of activated T cells, such as by superantigen-induced expansion of TcR V_β classes or by use of TcR-transgenic mouse models. We speculate that in cases where the frequency of antigen-specific T cells is more normal during the development of typical polyclonal responses, cell:cell interactions initiated only by FasL/Fas ligation may be relatively inefficient. However, virus infection leading to presentation of viral peptides by MHC class I and/or II would overlay TcR recognition onto the system to substantially increase the efficiency of cell:cell interactions and render them antigenically specific. Providing that the infected cells have the inherent ability to express FasL, or via the possibility the viral expression enhances FasL expression, a veto cell would be formed that could serve as a back-up escape device if the various viral strategies to mutate T cell epitopes or interfere with epitope processing have failed. If the virus-infected veto cells are present before substantial polyclonal T cell expansion and differ-

entiation to effector cells has occurred, the T cell response could thus essentially be "nipped in the bud." Further experimentation will obviously be required to determine whether virus-infected veto cells are physiological mediators of T cell low/nonresponsiveness in the viral systems analyzed so far and whether this concept can be extended to other viruses as a mechanism to escape T cell immunity.

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