MINIREVIEW

Polyclonality of the Cytotoxic T Lymphocyte Response to Virus Infection (43454)

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For many years, it has been known that certain viral infections induce a polyclonal B cell activation that can lead to the generation of antibody against self-antigens and nonviral foreign antigens (1–8). More recently, it has been shown that viruses can also be potent polyclonal cytotoxic T lymphocyte (CTL) inducers, spontaneously stimulating the generation of high levels of allospecific CTL (9, 10) and reactivating memory CTL specific for unrelated viruses from previous infections (11). Virus-induced stimulation of self-reactive or "autoimmune" CTL has also been reported in some systems (12–14). Stimulation of allospecific CTL has been observed in humans during episodes of acute infectious mononucleosis (Epstein-Barr virus [EBV] infection) (10, 15, 16) and in the mouse during acute infections with lymphocytic choriomeningitis virus (LCMV), vaccinia virus (VV), Pichinde virus, and murine cytomegalovirus (11). One might expect that acute viral infections would, therefore, be detrimental to allograft survival, and clinical studies have correlated allograft rejections with influenza and cytomegalovirus infections (17–19). Because the primary function of the immune system is to protect the host from infection, it is interesting to speculate whether the allospecific CTL contribute to the control of viral infection or whether they are an incidental byproduct of the antigen-specific response to virus.

In the case of polyclonal B cell stimulation, proliferation is often due to stimulatory or transforming properties of the viral proteins and may not contribute to viral clearance. EBV infection causes a true transformation of B cells (1), whereas the glycoproteins of Sindbis, influenza, and vesicular stomatitis viruses can be mitogenic to B cells, at least *in vitro* (3–5). Activated T cells and the cell growth and differentiation factors they secrete cause *in vitro* bystander stimulation of resting B cells that are not specific for the initial inducing antigen (20). Although polyclonally stimulated B cells do not appear to be involved in clearance of viral infection via antigen-specific antibody production, they have been associated with autoimmune phenomena. For example, EBV-transformed B cells produce antinuclear and rheumatoid factor antibodies (21).

The mechanisms of the polyclonal stimulation of CTL are not as well understood as those involved in polyclonal B cell stimulation. The polyclonal activation of allospecific T cells could be due to either a lymphokine-mediated, T cell receptor (TcR)-independent effect on T cells, akin to the bystander activation seen *in vitro* with B cells, or to an antigen-driven activation of T cells via cross-linking of the TcR. Recent developments in the characterization and mechanisms of induction of virus-induced polyclonal CTL suggest a role for these cells in the specific immunity to virus infection and in the evolution of CTL specificities during the immune response to virus.

Phenotypes of Cytotoxic Cells Induced during Virus Infection

In vivo polyclonal allospecific CTL activation was first seen in the mouse during acute LCMV infection (9). When analyzing the cytotoxic cell response to LCMV, two peaks of cytotoxic activity were observed (Fig. 1). The early peak appeared between Days 2 and 5 after infection and consisted of interferon (IFN)-activated natural killer (NK) cells. The second peak, which appeared between Days 7 and 9 after infection, consisted of virus-specific, major histocompatibility complex (MHC)-restricted CTL, as well as CTL that lysed allogeneic targets. This observation of allospecific CTL being generated concomitantly with virus-specific

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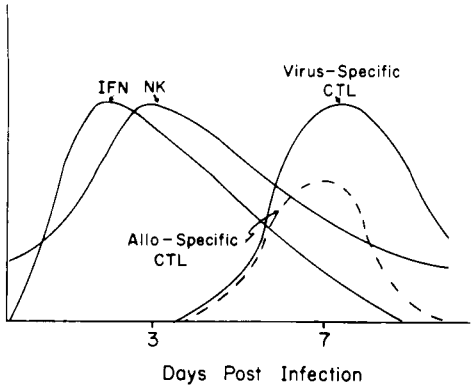

Figure 1. Kinetics of cytotoxic cells generated during LCMV infection. NK cells peak 3 days after infection, whereas virus-specific and allospecific CTL are generated approximately 7 days after infection. Reprinted from *BioEssays* (**8**:10–13, 1988) with permission.

CTL was also observed during infections with three other mouse viruses and in the EBV-infected human (10, 11). These virus-induced allospecific CTL had probably not been observed previously due to the high levels of NK cell activity induced during infection, because the allogeneic targets are sensitive to lysis by activated NK cells (22). Depletion of NK cells *in vivo* or *in vitro* by antibodies (and complement) eliminated lysis of syngeneic and xenogeneic targets, but did not abrogate the lysis of allogeneic targets (9, 10). In contrast, antibodies specific for T cells (anti-CD8 and anti-CD3) plus complement treatment of the mouse and human effector cell populations depleted the allospecific lysis (9, 10). These experiments as well as cell-sorting experiments, formally proved that the virus-induced allospecific lysis was due to $CD8^+$ T cells expressing the TcR β-protein, indicating that α/β T cells mediated this phenomenon (10, 23).

Identification of the Class I Molecule as the Target of Virus-Induced Allospecific CTL

LCMV-induced spleen CTL were able to lyse every tested allogeneic target, but spared syngeneic targets. The range of haplotypes on the allogeneic targets for LCMV-induced CTL from the C57BL/6 ($H\text{-}2^b$) mouse included cells expressing *d*, *f*, *k*, *p*, *q*, and *s* alleles (Fig. 2). Studies with MHC congenic mice showed that, as expected, the target structure of LCMV-induced allospecific CTL mapped to the Class I region of the MHC locus (11). Furthermore, (i) the lysis of target cells coated with antibody to the allogeneic Class I molecule was inhibited; (ii) IFN-β-induced up-regulation of Class I molecules on the surface of the target cell augmented lysis; and (iii) transfection of the human Class I molecule, HLA-A2, into the Class I-negative cell line, K562, conferred sensitivity to EBV-induced allospecific CTL (10, 12).

Mapping the Induction of Allospecific CTL within the MHC but Outside the Class II Region

The ability to generate CTL to a given alloantigen in response to LCMV depends upon the strain of mouse

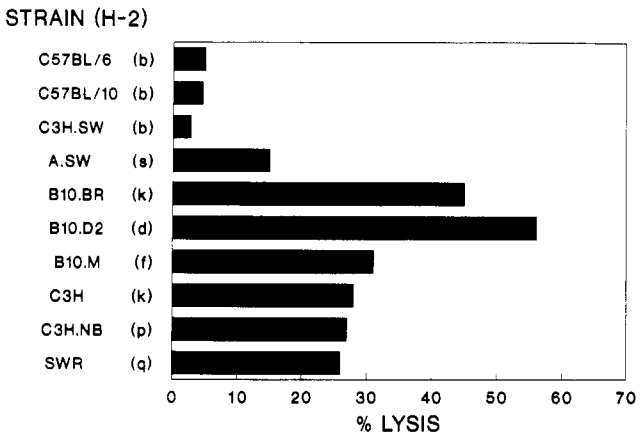

Figure 2. LCMV-induced, C57BL/6 ($H\text{-}2^b$) effector cells preferentially lyse allogeneic targets. Adherent peritoneal macrophages from strains of mice with different haplotypes were used as targets in an overnight chromium-51 release assay. Effector to target ratios are not given because of the uncertainty in the number of target cells remaining after the nonadherent cells have been removed (from Ref. 11).

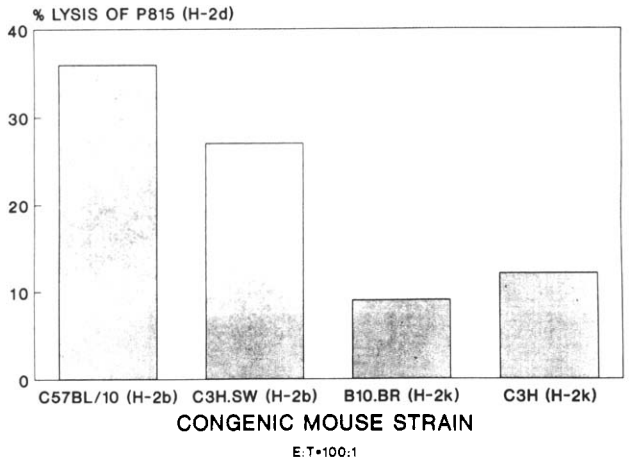

Figure 3. The ability to generate LCMV-induced allospecific CTL varies among strains. Spleen cells from NK cell-depleted mice 7 or 8 days after infection were tested for cytotoxicity against P815 cells ($H\text{-}2^d$). The assay length was 8 hr. The effector to target ratio was 100:1.

(23) with most mice generating high levels. Studies with congenic strains of mice showed that the ability to induce high levels of $H\text{-}2^d$-specific CTL mapped within the MHC locus (Fig. 3). $H\text{-}2^k$ and $H\text{-}2^r$ mice generated low levels, whereas mice expressing $H\text{-}2^{b,f,p,\text{ or }s}$ generated high levels (23). It is not known whether the induction of allospecific CTL maps to the MHC locus in humans.

To better understand the mechanism of induction, a more detailed mapping was performed using mice that were recombinant at the Class I and Class II MHC loci. Mapping the induction of allospecific CTL to the Class II region would argue that CD4 cells and their lymphokines are involved, whereas mapping to the Class I region would suggest that direct Class I antigen recognition is involved. The induction did not map to the Class II region of the MHC locus, because recom-

binant mice with low responder (k) alleles at I-E and I-A but high responder alleles (s and b) at K, D, and L were able to generate high levels of allospecific CTL (23). This result is supported by the finding that depletion of $CD4^+$ cells *in vivo* by administration of anti-CD4 monoclonal antibody did not inhibit the antiviral CTL response or the allospecific CTL response (23). This argues that the ability to induce allospecific CTL is not dependent upon $CD4^+$ T cell-produced lymphokines. Recently, Rahemtulla *et al.* (24) showed that transgenic mice lacking CD4 due to disruption of its gene are capable of mounting a normal virus-specific CTL response to LCMV and vaccinia virus. Although the LCMV-infected, $CD4^-$, transgenic spleen cells were not tested against allogeneic targets, our CD4 depletion data suggest that allospecific CTL induction would not be affected in the CD4-deficient mice.

Mechanisms of Allospecific CTL Induction

The virus-induced polyclonal allospecific CTL could be the result of specific or nonspecific mechanisms. The nonspecific mechanisms would entail bystander stimulation without TcR occupancy, whereas the specific mechanisms would involve a selective stimulation with TcR occupancy, perhaps due to either cross-reactivity with virus-modified self or cross-reactivity with virus-induced cellular neoantigens.

The abundance of viral antigen synthesized during the maturation phase of viral replication might effectively compete with endogenous peptides for binding to the nascent MHC Class I molecules, whose synthesis *in vivo* is up-regulated by IFN (25). As a result, a large proportion of Class I molecules on the surface of the cell may present viral peptides. Under these conditions of efficient antigen presentation, T cells with a wide range of affinities for virus-modified self would be selected to proliferate. Some of these same CTL might also cross-react with foreign or allogeneic MHC Class I molecules. If the viral peptide presented in the context of self looks to the CTL more like one alloantigen than another, then selection of virus-specific CTL cross-reactive to that alloantigen would occur.

Studies indicated that selected clones of allospecific CTL were stimulated during infection. The first line of evidence was that relative CTL precursor frequencies to allogeneic targets changed as the infection progressed, arguing that some specificities are selected over others. The second line of evidence used an analysis of short-term (8 days) CTL clones derived from LCMV-infected mice and stimulated against virus-infected syngeneic macrophages. The clones that killed virus-infected cells were then analyzed for their ability to kill allogeneic cells. The surprising result was that 26% (18 of 70) of the virus-specific clones cross-reacted with a single allogeneic L929 ($H\text{-}2^k$) target. Two other virus-specific clones cross-reacted with the allogeneic P815 ($H\text{-}2^d$) target (23). CTL clones that cross-react with virus-infected syngeneic cells and allogeneic cells of a particular MHC haplotype have also been isolated from EBV, influenza, and vesicular stomatitis virus infection (26–28).

An intriguing observation was that allospecific CTL clones were generated at a much greater frequency when stimulated by virus-infected macrophages than when stimulated by allogeneic macrophages. For example, in our assays, the precursor frequency for $H\text{-}2^k$ specific CTL in the 8-day LCMV-infected C57BL/6 mouse was only about 1 in 4000 spleen cells, yet when virus-infected macrophages were used to stimulate CTL clones, $H\text{-}2^k$-specific CTL precursors could be found in wells plated with as few as 75 spleen cells from LCMV-infected mice (23). This suggests that allospecific CTL induced during viral infection and selectively detected in these clonal analyses may have a greater affinity for viral antigen than for allogeneic Class I antigen.

Allospecific CTL that are crossreactive with viral antigen may play a role in the lysis of virus-infected cells early in infection, when viral titers are highest and before effective numbers of high affinity, virus-specific CTL have been generated. Previous studies using cold target competion assays showed that the lysis of ^{51}Cr-labeled allogeneic targets could not be competed with unlabeled virus-infected syngeneic targets (9, 10). Yet, clonal analyses indicated that a large component of the LCMV-specific CTL response was cross-reactive for allogeneic targets (23). This apparent discrepancy can be explained if many of the cross-reactive CTL observed in bulk assays have a higher affinity for allogeneic MHC than for virus-modified self-MHC. Eight days after LCMV infection, approximately 50% of the lysis of virus-infected targets was sensitive to inhibition by anti-CD8 antibody, which indicates that many of the virus-specific CTL are of low affinity and depend upon the accessory interaction of the CD8 molecule on the CTL with the MHC antigen on the target (23). These low affinity cells may constitute part of the allospecific CTL population.

Stimulation of Autoimmune CTL

Viral infections have long been associated with autoimmune antibody production, but more recently, autoimmune T cells have also been reported. For example, Coxsackievirus B3 elicits autoreactive $CD8^+$ T cells which kill uninfected myocytes and are associated with tissue necrosis during myocarditis (13). Coxsackievirus B3 also induces autoreactive $CD4^+$ CTL which lyse myocardiocytes "stressed" by a nonhomologous virus infection (encephalomyocarditis virus) or drug treatment (14). Recently, Kilham rat virus was shown to induce a $CD8^+$ cell-dependent diabetes in the Worcester BB diabetic-resistant rats (29; A. A. Like, personal communication, 1991). Some of the allospecific CTL elicited during acute virus infection may

cross-react with self-antigens and, therefore, be CTL with autoimmune potential.

Split well analyses of Day 8 LCMV-infected spleen cells revealed allospecific clones that were stimulated to proliferate in culture by virus-infected peritoneal macrophages, yet were unable to kill virus-infected, syngeneic fibroblast tumor cells (23). It would seem that these CTL are stimulated by either a macrophage-specific, self-antigen to which they are not tolerized or by a viral peptide that is processed and presented on virus-infected peritoneal macrophages but not on virus-infected fibroblasts. Qualitative tissue-specific differences in antigen presentation of viral peptides have not been reported previously. Of greater significance is the possibility that these allospecific CTL recognize self-antigens and could, therefore, potentially be autoimmune CTL. Novel endogenous antigens could be elicited by the effects of viral proteins on cellular functions or by the action of virus-induced IFN and other cytokines which alter the expression of many cellular proteins, including the MHC antigens.

Reactivation of Memory CTL during Acute Virus Infection

Another parameter of virus-induced polyclonal CTL stimulation is the reactivation of CTL specific for viruses from previous infections. Infection of LCMV-immune mice with MCMV, VV, or Pichinde virus restimulated LCMV-specific CTL, and no cross-reactivity could be shown among the viruses at the CTL level by cold target competition assays. In contrast, VV-immune mice challenged with LCMV did not generate VV-specific CTL (11). This discrepancy may be because the LCMV-immune C57BL/6 mouse has a high precursor frequency of CTL specific for LCMV as compared with other viruses (30), or the observation may be due to the fact that, unlike VV, LCMV, though difficult to detect, is never completely cleared from the mouse (31). The level of residual virus in the LCMV-immune mouse is controlled by CTL, and depletion with anti-T cell serum results in detectable viral titers. The mechanism involved in the virus-induced reactivation of LCMV-specific memory CTL is unclear, but may require small amounts of lymphocytic choriomeningitis viral antigen to be present. One theory to explain the function of memory CTL suggests that they are constantly proliferating at a slow rate either due to trace amounts of antigen or due to cross-reactivities with endogenous antigen (32). Memory CTL have an activated phenotype in that they express elevated levels of the lymphocyte adhesion molecules and the T cell activation antigens: interleukin 2 receptor, ICAM-1, and MAC-1 (33, 34). As a result, memory CTL are more responsive to lymphokines than resting CTL. Subsequent virus infection could provide the lymphokines necessary to up-regulate the antigen-dependent proliferation of these cells to detectable levels. Because EBV establishes lifelong persistence in the infected individual, it will be interesting to determine whether challenge of EBV-immune individuals with a different virus will yield EBV-specific memory CTL.

Evolution of CTL Specificities during Acute Virus Infection

The theory that many of the virus-induced allospecific CTL are responding to abundant viral antigen present during acute infection and have a low affinity for virus-modified self suggests that these cells would not be generated in a high affinity memory response, nor would they be stimulated in the immune-spleen when viral antigen is largely cleared. This was shown to be the case, as negligible levels of allospecific CTL were generated during secondary infection with LCMV *in vivo* (23) or EBV *in vitro* (10), and our preliminary results show that the number of cross-reactive, short-term clones decreases significantly in LCMV-immune spleen as compared with the acutely infected spleen. These results suggest that early in infection, virus-specific CTL with a broad range of reactivities are stimulated, but, after these clear the bulk of the viral burden, less cross-reactive, possibly higher affinity CTL are selected to proliferate, because they can be stimulated with limiting amounts of viral antigen. It is these CTL which comprise the memory response during a secondary infection.

Concluding Remarks

In addition to the control of virus infections and the potential for autoimmunity, virus-induced polyclonal CTL stimulation may have other consequences. Allograft rejection in viral infections might be mediated by effector T lymphocytes whose primary specificity is for viral peptide presented in the context of self but which also recognize or cross-react with allogeneic Class I MHC antigens (35). Virus-induced IFN would activate allospecific CTL as well as up-regulate the level of Class I expression on the allograft (25). This combination of events might prove lethal for the graft in individuals able to generate allospecific CTL, and graft rejection has been known to occur after human cytomegalovirus and influenza infections (17–19).

The polyclonal CTL response induced during infection may also play a role in the control of tumor growth. Viral infections inhibit the growth of implanted tumor cells in animal models (36), and tumor remission is sometimes seen after virus infection in humans (37). Other studies have shown that vaccination of humans with mixtures of virus and tumor cells (viral oncolysates) can induce tumor-specific immunity (38). The regulation of tumor growth is known to involve NK cells which are activated during virus infection, but virus-induced CTL may also play a role. Some of the virus-specific CTL could be cross-reactive for tumor antigens as well as alloantigens, or a component of the

allospecific CTL may recognize virus-induced self-antigens which happen to be expressed on the tumor.

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