Induction of Fas Ligand Expression by an Acutely Lethal Simian Immunodeficiency Virus, SIV<sub>smmPBj14</sub>

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Simian immunodeficiency virus strain PBj14, SIV<sub>smmPBj14</sub>, is unique among primate lentiviruses in its ability to trigger the proliferation of resting simian lymphocytes and to cause the rapid death of experimentally inoculated pigtailed macaques. Severe enteropathy, immune activation, and extensive apoptosis, particularly within gut-associated lymphoid tissue, characterize the acute disease syndrome associated with SIV<sub>smmPBj14</sub> infection. In the present study, we examined whether the ability of this virus to cause widespread apoptosis might be linked to the up-regulation of Fas ligand (CD95L) expression in virally infected cells. In vitro studies revealed that expression of the viral Nef protein, in the absence of any other viral gene product, was sufficient to up-regulate the transcriptional activity of the CD95L promoter and to cause cell surface expression of Fas ligand. This up-regulation was NFAT dependent (inhibited by cyclosporin A) and did not occur in cells that expressed a mutated derivative of the viral Nef protein, lacking a previously defined immunoreceptor tyrosine-based activation motif. These findings were corroborated by analysis of tissue sections from virally infected macaques. Immunohistochemical staining revealed that Fas ligand expression was efficiently up-regulated in the GALT of animals that had been experimentally infected with wild-type SIV<sub>smmPBj14</sub> but not in animals that were infected with a nonacutely pathogenic viral mutant lacking the Nef ITAM. Taken together, these results suggest that the ability of SIV<sub>smmPBj14</sub> to cause acutely lethal disease and to up-regulate FasL expression may be linked. Additional studies will be required to determine whether the induction of FasL expression is in itself important for acute disease pathogenesis.

INTRODUCTION

The acute phase of infection by human immunodeficiency virus-1 (HIV-1) is marked by high levels of virus replication and widespread viral dissemination in lymphoid organs. This initial stage of infection is believed to strongly influence the progression of subsequent disease (Lifson et al., 1997; Mellors et al., 1995; Wong et al., 1996), and it therefore is important to better understand the viral and host factors that determine the outcome of primary HIV-1 infection. The analysis of animal models for HIV/AIDS represents one approach to this problem.

The experimental inoculation of nonhuman primates with pathogenic strains of simian immunodeficiency virus (SIV) is a well-defined model for human AIDS and is characterized by wasting, inversion of the CD4/CD8 ratio, opportunistic infections, and death within 5±18 months postinoculation (Desrosiers, 1990). One strain of SIV, SIV<sub>smmPBj14</sub>, induces an unusually severe acute disease syndrome in experimentally inoculated pigtailed macaques (Dewhurst et al., 1990; Fultz et al., 1989). This syndrome can be characterized as an exaggerated form of acute HIV-1 infection (Cooper et al., 1985; Cossarizza et al., 1995; Henrard et al., 1995) and is marked by enteropathy and by extensive T cell activation, particularly within the gut-associated lymphoid tissues (GALT) (Fultz and Zack, 1994; Gummulluru et al., 1996; Schwiebert and Fultz, 1994).

Immune activation has been suggested to be an important factor in the progression of immunodeficiency disease (Andrieu et al., 1995), both through its effects on virus replication (Bukrinsky et al., 1991; Zack et al., 1990) and through the process of activation-induced T cell death. Consistent with this, immune activation has been correlated with apoptosis in SIV-infected macaques (Gummulluru et al., 1996), and the analysis of molecular mechanisms regulating programmed cell death has become a central theme in HIV/AIDS research (Ameisen et al., 1994; Gougeon, 1995; Kaplan and Sieg, 1998).

The CD95 (Fas) receptor/ligand system has been implicated in the process of activation-induced cell death (AICD) of T cells and may play an important role in the pathogenesis of AIDS (Nagata and Golstein, 1995). It has been appreciated for some time that T cell activation leads to increased levels of Fas ligand (CD95L), and recent results have shown that Fas ligand expression is regulated by a number of cellular transcription factors, including NF-κB, AP-1, Egr-3, and nuclear factor of activated T cells (NFAT) (Holtz-Heppelmann et al., 1998;
Kasibhatla et al., 1998; Latinis et al., 1997; Mittelstadt and Ashwell, 1998). NFAT binding sites have been identified in the CD95L promoter and shown to participate in the upregulation of Fas ligand expression on T-cell activation (Holtz-Heppelmann et al., 1998; Latinis et al., 1997). Furthermore, specific blockade of NFAT activation using cyclosporin A prevents up-regulation of Fas ligand after the exposure of T cells to a variety of stimuli (Holtz-Heppelmann et al., 1998; Latinis et al., 1997).

Previous studies have shown that among known primate lentiviruses, SIVsmmPBj14 has the unique ability to trigger the in vitro proliferation of unstimulated T cells (Fultz, 1991). This property was found to be dependent on an immunoreceptor tyrosine-based activation motif (ITAM) located within SIVsmmPBj14 Nef (Du et al., 1995; Saucier et al., 1998), and this ITAM was also found to be necessary for Nef-mediated activation of NFAT in Jurkat T cells (Luo and Peterlin, 1997). In light of the link between NFAT activation and Fas ligand expression, we hypothesized that expression of the SIVsmmPBj14 Nef protein in T-lymphocytes might be sufficient to trigger the up-regulation of Fas ligand. To investigate this hypothesis, experiments were conducted to examine whether expression of the SIVsmmPBj14 Nef in cultured T cells could elicit (1) an increase in the transcriptional activity of the CD95L promoter, and (2) up-regulation of cell surface expression of Fas ligand. In addition, we evaluated Fas ligand expression in intestinal tissue sections from pigtailed macaques that developed lethal enteropathy after infection with SIVsmmPBj14. The results presented here demonstrate that in the absence of any other viral gene product, the SIVsmmPBj14 Nef protein was sufficient to increase transcription from the CD95L promoter, in an NFAT-dependent manner. Cell surface levels of Fas ligand were also up-regulated by the wild-type Nef protein, whereas a mutant derivative of SIVsmmPBj14 Nef lacking the ITAM domain failed to increase CD95L levels. Finally, immunohistochemical analysis of tissue samples from the gut of virally infected macaques confirmed that Fas ligand expression was increased during acute viral infection in vivo and that this effect was dependent on the Nef ITAM domain.

RESULTS

Up-regulation of cell surface Fas ligand expression has been previously shown to occur in response to T cell activation (Anel et al., 1995). More recently, the activity of the CD95L promoter element was also found to be regulated by the transcription factor NFAT (Holtz-Heppelmann et al., 1998; Latinis et al., 1997) and to depend on the activation of the T cell-specific tyrosine kinase, ZAP-70 (Eischen et al., 1997). Because SIVsmmPBj14 Nef has been shown to increase the activity of NFAT and to associate directly with ZAP-70 (Luo and Peterlin, 1997), we sought to evaluate whether SIVsmmPBj14 Nef could up-regulate CD95L promoter activity. To test this hypothesis, plasmid expression vectors encoding either the wild-type SIVsmmPBj14 Nef protein or an ITAM-deleted version of this protein were constructed (plasmid inserts were derived from clone PBj6.6 and from its mutated derivative, clone PBj6.6YERO, respectively; Novembre et al., 1993; Saucier et al., 1998). Protein expression from these plasmids was verified by radioimmunoprecipitation of Nef from transfected COS cells, using a specific polyclonal antiserum (data not shown).

Jurkat cells then were cotransfected with a reporter plasmid containing the luciferase gene under the transcriptional control of the CD95L promoter (CD95L-486; Latinis et al., 1997) together with expression plasmids encoding either the wild-type or the ITAM-deleted SIVsmmPBj14 Nef proteins. In control experiments, cells were transfected with the CD95L reporter plasmid and then exposed either to medium alone or to the T cell-activating agent ionomycin. The results of these studies, which are presented in Fig. 1, show that expression of wild-type SIVsmmPBj14 Nef, but not of the ITAM-deleted mutant, was sufficient to cause a substantial (2-fold) increase in the transcriptional activity of the CD95L promoter in Jurkat T cells. The extent of promoter activation was roughly equivalent to that attained using ionomycin (also 2-fold) and was consistent with previously reported data (Holtz-Heppelmann et al., 1998). Furthermore, the combination of the wild-type SIVsmmPBj14 Nef plus ionomycin showed no synergistic effect on FasL expression compared with the effect of Nef alone (Fig. 1A). This suggested that SIVsmmPBj14 Nef and ionomycin might act through a common pathway to up-regulate FasL.

This prediction was experimentally confirmed, by analyzing FasL expression in cells treated with cyclosporin A. These studies showed that the up-regulation of CD95L expression by wild-type SIVsmmPBj14 Nef was inhibited on incubation of the transfected cells in the presence of cyclosporin A (Fig. 1B). This finding is consistent with the notion that the viral Nef protein may be acting through the transcription factor NFAT, as is the case for well-defined T cell-activating agents such as ionomycin (Holtz-Heppelmann et al., 1998) and engagement of the T cell receptor (Latinis et al., 1997).

Because we were able to demonstrate that SIVsmmPBj14 Nef had a positive effect on the transcriptional activity of the CD95L promoter element, we decided to examine whether the viral Nef protein could also up-regulate cell surface expression of the Fas ligand protein. We therefore transfected Jurkat T cells with Nef expression plasmids (wild type or ITAM deleted), together with a vector encoding the murine Thy-1.2 antigen. Flow cytometric analysis of Thy-1.2 expression was then used to identify successfully transfected cells, and Fas ligand levels were evaluated in this subpopulation of cells. These experiments revealed that SIVsmmPBj14 Nef caused an...
Having demonstrated that expression of SIV<sub>smmPBj14</sub> Nef was sufficient to trigger an increase in Fas ligand expression in cultured human T cells, we wanted to examine whether this antigen was also up-regulated in macaques that had been infected with either wild-type or ITAM-deleted molecular clones of SIV<sub>smmPBj14</sub>. For these experiments, we availed ourselves of cryopreserved tissue samples from pigtailed macaques that were killed after acute infection with the PBj6.6 molecular clone of SIV<sub>smmPBj14</sub> or after infection with the molecular clone PBj6.6 Y-R, which is isogenic to PBj6.6, with the exception of a single point mutation within the Nef ITAM (Saucier et al., 1998). In addition, specimens from SIV-seronegative pigtailed macaques were examined (Gummuluru et al., 1996).

Intestinal tissue specimens, corresponding to gut-associated lymphoid tissue, were subjected to immunohistochemical staining for Fas ligand using a specific monoclonal antibody (Nok1; Pharmingen). Representative results are shown in Fig. 3. It can be readily appreciated that the number of cells that exhibit dark cytoplasmic staining (indicative of Fas ligand expression) is markedly higher in Fig. 3A (intestinal tissue from a macaque infected with PBj6.6) than in the remainder of the figure (respectively, tissue from a macaque infected with PBj6.6 Y-R or from an SIV-seronegative animal). Thus at a qualitative level, it was apparent that there was an increase in the expression of Fas ligand in GALT from macaques that had died as a result of acute infection with wild-type SIV<sub>smmPBj14</sub> compared with animals infected with the ITAM-deleted mutant or SIV-negative macaques. To confirm this interpretation, multiple tissue sections were subjected to immunohistochemical staining for Fas ligand. Intestinal tissues from macaques that were killed at 6 days after infection with the PBj6.6 molecular clone (Gummuluru et al., 1996) were analyzed, as well as tissues from macaques that were killed at 6 or 10 days after infection with the PBj6.6 Y-R clone (Saucier et al., 1998) and specimens from SIV-seronegative animals (Gummuluru et al., 1996). In each case, tissues from two animals were examined; this sample size was dictated by the limited availability of tissues, notably, only two macaques were killed at acute time points after infection with the PBj6.6 Y-R clone (Saucier et al., 1998).

Quantitative analysis of immunohistochemical staining from these specimens is presented in Fig. 4, which shows that Fas ligand expression was increased by ~2-fold in the GALT of macaques that were infected with wild-type SIV<sub>smmPBj14</sub> (clone PBj6.6) compared with uninfected animals (no SIV). This difference was statistically significant (P < 0.00001). It also is apparent that there was a modest up-regulation of FasL expression in GALT from animals infected with clone PBj6.6 Y-R compared with SIV-negative controls (P < 0.05).

In light of the increase in both FasL expression (Figs. 3 and 4) and apoptosis (Gummuluru et al., 1996) in intestinal tissues from SIV<sub>smmPBj14</sub>-infected macaques,
we performed dual labeling experiments so as to colocalize apoptotic cells and FasL-positive cells within the same tissue section. The results, shown in Fig. 5, revealed that most of the apoptotic (TUNEL-positive) cells within GALT were also positive for Fas ligand. However, occasional cells were found to be TUNEL positive but Fas ligand negative. These cells were often located in close proximity to Fas ligand-expressing cells (e.g., the cell denoted by the arrow in Fig. 5).

**DISCUSSION**

Studies in rhesus macaques infected with nef-deleted strains of SIV have demonstrated that this gene is necessary for high levels of viral replication and for the consistent induction of immunodeficiency disease in mature animals (Kestler et al., 1991; Novembre et al., 1996). Evidence for the involvement of nef in HIV-1 pathogenesis is less direct but includes the fact that nef-deleted strains of HIV-1 exhibit attenuated pathogenicity in SCID-hu mice (Aldrovandi and Zack, 1996), as well as the observation that some HIV-1-positive individuals with nonprogressive disease harbor nef-deleted viral strains (Deacon et al., 1995; Kirchhoff et al., 1995). These findings have heightened interest in the biochemical and biological properties of Nef, which include the ability to down-regulate CD4 expression, to perturb T cell signaling, to associate with cellular kinases, and to enhance the infectivity of viral particles (Cullen, 1994; Mangasarian and Trono, 1997).

The nef allele from SIVsmmPBj14 is unique in that it contains an immunoreceptor tyrosine-based activation motif ([YxxL]xxx[YxxL]) close to its N-terminus (Du et al., 1995). When inserted into certain other strains of SIV, this element is sufficient to confer an acutely pathogenic and lymphoproliferation-inducing phenotype on the resulting recombinant virus (Du et al., 1996, 1995; Sasseville et al., 1996). Furthermore, the elimination of the Nef ITAM from an acutely lethal molecular clone of SIVsmmPBj14 resulted in the attenuation of viral pathogenicity and the loss of the ability to induce lymphoproliferation (Saucier et al., 1998). These data provide compelling evidence for the role of the SIVsmmPBj14 Nef ITAM in the induction of acute disease and T cell activation.

The discovery that SIVsmmPBj14 Nef associates directly with the T cell-specific tyrosine kinase ZAP-70 and the observation that intracellular expression of SIVsmmPBj14 Nef leads to the activation of the transcription factor NFAT (Luo and Peterlin, 1997) provoked us to consider whether this virally encoded protein might influence the expression of ZAP-70-dependent and NFAT-regulated genes, such as Fas ligand (Eischen et al., 1997; Holtz-Heppelmann et al., 1998; Latinis et al., 1997). We con-
FIG. 3. Immunocytochemical localization of Fas ligand-expressing cells in gut-associated lymphoid tissue from SIV-infected and uninfected macaques. (A) GALT from a pigtailed macaque that was killed after acute infection with wild-type SIV<sub>swrPBj14</sub> (clone PBj6.6). (B) GALT from a pigtailed macaque that was killed after acute infection with an ITAM-deleted derivative of SIV<sub>swrPBj14</sub> (clone PBj6.6 Y-R). (C) Matched tissue from an SIV-seronegative pigtailed macaque. Note the increase in the number of FasL-positive cells (dark cytoplasmic stain) in A, relative to the other tissues. Magnification, 120× (A–C).

FIG. 5. Colocalization of Fas ligand-positive cells and apoptotic cells within intestinal lymphoid tissue from SIV-infected and uninfected macaques. Apoptotic nuclei were detected using the TUNEL method (dark nuclear stain), and Fas ligand-positive cells (intense red cytoplasmic stain) were identified using immunohistochemistry with a FasL-specific monoclonal antibody (see Materials and Methods). Note the presence of large numbers of doubly stained cells (e.g., the cells denoted by the asterisk), as well as occasional TUNEL-positive cells that lack detectable Fas ligand (e.g., the cell indicated by the arrow). Magnification, 120×.
duct our initial studies using SIV\textsubscript{smmPBj14} Nef expression vectors, which were introduced into Jurkat T cells via transient transfection. This approach permitted us to examine the effects of Nef on FasL expression in the absence of other viral gene products such as gp120 and Tat, which have (at least in the case of HIV-1) been reported to up-regulate Fas ligand expression (Westendorp et al., 1995).

Our experiments revealed that SIV\textsubscript{smmPBj14} Nef up-regulated the transcription of the CD95L promoter and elicited an increase in cell surface expression of Fas ligand (Figs. 1 and 2). As expected, the effects of the viral Nef protein were dependent on activation of NFAT (i.e., they were inhibited by cyclosporin A; Fig. 1B). In addition, we were able to show that the effects of Nef on FasL expression were dependent on the presence of an intact ITAM because an ITAM-deleted Nef mutant was incapable of up-regulating Fas ligand expression (Figs. 1 and 2).

Analysis of tissue specimens from the GALT of pigtailed macaques that were killed within 6±10 days of infection with wild-type SIV\textsubscript{smmPBj14} (PBj6.6) or with an ITAM-negative viral mutant (PBj6.6 Y-R). In addition, tissues from SIV-seronegative pigtailed macaques were also analyzed (no SIV). In all cases, a total of eight microscope fields, for each of two animals (per condition), were examined. Data represent the mean number of positive cells per high-power field; bars represent the S.E.M. values.

FIG. 4. Quantitative analysis of Fas ligand expression in simian tissues. The mean number of FasL-positive cells per high-power microscope field is shown for GALT sections collected from pigtailed macaques that were killed within 6±10 days of infection with wild-type SIV\textsubscript{smmPBj14} (PBj6.6) or with an ITAM-negative viral mutant (PBj6.6 Y-R). In addition, tissues from SIV-seronegative pigtailed macaques were also analyzed (no SIV). In all cases, a total of eight microscope fields, for each of two animals (per condition), were examined. Data represent the mean number of positive cells per high-power field; bars represent the S.E.M. values.

The presence of an intact Nef ITAM is a requirement not only for the efficient up-regulation of Fas ligand (Figs. 3 and 4) but also for the immune-activating and apoptosis-inducing effects of SIV\textsubscript{smmPBj14} (Saucier et al., 1998). In light of the severe enteropathy that occurs during acute viral infection, it is interesting to consider the fact that FasL expression by intestinal intraepithelial lymphocytes has been shown to lead to apoptosis of intestinal epithelial cells (Lin et al., 1998; Strater et al., 1997). FasL expression may also contribute to the profound depletion of intestinal CD4\textsuperscript{+} T cells that occurs during the acute phase of SIV infection (Smit-McBride et al., 1998; Veazey et al., 1998) because T cells from GALT are known to be hypersensitive to Fas/FasL-mediated apoptosis (De Maria et al., 1996). Additional studies will be required to examine whether viral induction of FasL expression within GALT may contribute directly to the pathogenesis of acute SIV\textsubscript{smmPBj14} infection in pigtailed macaques.

In a broader sense, the relationship between apoptosis and infection by primate lentiviruses remains a con-
troversial and intensively studied topic (Kaplan and Sieg, 1998). Both HIV-1 and SIV have been shown to up-regulate the expression of FasL in cultured cells (Badley et al., 1996; Dockrell et al., 1998; Mitra et al., 1996; Xu et al., 1997). Furthermore, increased levels of FasL have been detected in tissue macrophages from HIV-1-infected persons (Dockrell et al., 1998), as well as on T-lymphocytes isolated directly from the blood of individuals with chronic HIV-1 infection (Mitra et al., 1996; Sloand et al., 1997) or macaques with chronic SIV infection (Xu et al., 1997). Our findings differ from these previous reports in at least two respects. First, we focused exclusively on events that occur during the very early stages of a severe immunodeficiency virus infection. Second, we have shown that the unique nef allele from SIV<sub>smmPBj14</sub> is both necessary and sufficient for the induction of Fas ligand expression. In contrast, Xu et al. (1997) showed that Nef was necessary for up-regulation of FasL by SIV<sub>mac239</sub> but could not demonstrate that Nef alone was sufficient to mediate this effect.

In summary, this study provides strong evidence that the unique nef allele from SIV<sub>smmPBj14</sub> is both necessary and sufficient to trigger the up-regulation of FasL expression in T cells and that this property depends on the presence of a previously defined ITAM motif within Nef. This finding may have important implications for the immunopathogenesis of acute SIV<sub>smmPBj14</sub> infection.

MATERIALS AND METHODS

Plasmid constructs

For the present study, the following oligonucleotide primers were used to PCR amplify and clone SIV<sub>smmPBj14</sub> nef sequences: (1) NefA, 5'-agcggatccAGTATGGGTGGCGTTACCTCCTCA; and (2) NefC, 5'-agcggatccGCGTTACCTCCA; and (2) NefC, 5'-agcggatccAGTATGGGTGGCGTTACCTCCTCA. Primer NefA includes the authentic nef start codon (shown in boldface lettering) and a T7 epitope tag (MASMTGGQQMG) fused to the C-terminus of the nef open reading frame (the final amino acid residues 17 and 18. The resulting virus fails to induce lymphoproliferation in vitro and does not cause acutely lethal disease in vivo (Saucier et al., 1998).

Cell lines and transfections

The human T cell line Jurkat was maintained in RPMI 1640 medium (Life Technologies Inc., Bethesda, MD) supplemented with 10% fetal calf serum (Sigma Chemical, St. Louis, MO), 0.2 mM glutamate, 50 U/ml penicillin, and 50 µg/ml streptomycin (Life Technologies). Jurkat cells (2 × 10<sup>6</sup> per condition) were transfected with plasmid DNAs using SuperFect transfection reagent (Qiagen, Santa Clarita, CA), using conditions recommended by the manufacturer; 3 µg of Nef-expressing plasmid was used per transfection, together with 5 µg of a CMV-βgal reporter (internal control) and 10 µg of the CD95L486 reporter plasmid (this construct contains a luciferase reporter gene, under the transcriptional control of the CD95L promoter, and was generously provided by Dr. Gary Koretzky; Latnis et al., 1997). After transfection, cells were washed in 1× PBS, resuspended in RPMI 1640 medium supplemented with fetal calf serum, and plated onto 60-mm dishes. Ionomycin (1 µg/ml; Sigma) and/or cyclosporin A (10 µg/ml; Sigma) was added as appropriate.

Luciferase assays

Jurkat cells were harvested 48 h after transfection with plasmid DNAs and resuspended in 1× Reporter Lysis Buffer (Promega Corp., Madison, WI) for 15 min. Luciferase activity was then determined by using a Lumicount luminometer (Packard Instrument Company, Meriden, CT). Each experimental condition was assayed in replicates of six, and transfection efficiencies were normalized by measuring expression of the internal control plasmid, using the β-galactosidase Enzyme Assay System (Promega).

Flow cytometry

For analysis of cell surface CD95L expression, Jurkat cells (2 × 10<sup>5</sup>) were transfected with plasmid constructs encoding SIV<sub>smmPBj14</sub> Nef, together with a vector encoding the murine Thy-1.2 antigen (gift of Dr. Vicente Planelles). Forty-eight hours later, CD95L and Thy-1.2 expression were analyzed as follows. First, cells were incubated with the anti-human CD95L monoclonal antibody Nok-1 (1:100; Pharmingen), cells treated with secondary antibody
-alone were used as negative controls. Flow analysis was performed using a Coulter Epics Elite ESP (Coulter Corp., Hialeah, FL).

Tissues and immunohistochemistry

Tissue specimens were obtained at necropsy from SIV-seronegative pigtailed macaques and from pigtailed macaques infected with molecular clones of SIV<sub>SMM-PBj14</sub> containing wild-type (clone PBj6.6) or mutant (clone PBj6.6YERQ) nef sequences. Tissues were frozen immediately at -80°C and then sectioned to 5-µm thickness onto Vectabond (Vector Labs., Burlingame, CA)-coated slides. FasL-expressing cells were detected using Nok-1 antibody, and visualization of positively stained cells was achieved via the avidin-biotin complex (ABC) method (Vector Labs.) using a peroxidase-avidin conjugate with diaminobenzidine (DAB) as the chromagen (this results in a dark-brown or black cytoplasmic stain in antigen-positive cells); counterstaining was performed with hematoxylin, as described previously (Dollard et al., 1995). Digitized images of immunohistochemically stained sections in eight 40× microscopic fields (per section) were then analyzed for numbers of antigen-positive cells using a densitometer (Imaging Research Inc., Ontario, Canada), as previously described (Gelbard et al., 1995; Gummuluru et al., 1996). Data were expressed as the mean number of positive cells per high-power microscopic field ± S.E.M. Tests of statistical significance between sample groups were determined by paired t tests.

Colocalization experiments, which involved the simultaneous detection of apoptotic cells and FasL-positive cells within the same tissue section, were performed using the TUNEL method (to identify apoptotic nuclei) with New Fuchsin as the chromagen. TUNEL-positive cells therefore anticrimeing the gift of the Th-12 expression plasmid, Dr. Sanjay Maggirwar for helpful discussions and advice on experiments, Dr. Harry Kestler for the gift of the anti-Nef antiserum, Dr. Gary Koretzky for the CD95L-486 reporter construct, and Ellen Lockwood and Selena Taylor for obtaining and shipping blood samples from macaques. This work was supported by National Institutes of Health Grants R01-AI39937 and KO4-A101240 (S.D.), R01-CA67364 (F.J.N.), F31-GM1782 (S.H.), and RR00165. Yerkes Regional Primate Research Center is fully accredited by AALAC, and all animals in this study were housed according to Animal Welfare Act guidelines.

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