Adoptive Immunotherapy of Feline Immunodeficiency Virus with Autologous Ex Vivo-Stimulated Lymphoid Cells Modulates Virus and T-Cell Subsets in Blood

J. Norman Flynn,1,2 Mauro Pistello,1 Patrizia Isola,1 Lucia Zaccaro,1 Barbara Del Santo,1 Enrica Ricci,1 Donatella Matteucci,1 and Mauro Bendinelli1*

Retrovirus Center, Department of Experimental Pathology, University of Pisa, Pisa, Italy,1 and Institute of Comparative Medicine, Retrovirus Research Laboratory, Department of Veterinary Pathology, University of Glasgow, Glasgow, United Kingdom1*

Received 2 December 2004/Returned for modification 22 February 2005/Accepted 16 March 2005

The potential of immunotherapy with autologous virus-specific T cells to affect the course of feline immunodeficiency virus (FIV) infection was explored in a group of specific-pathogen-free cats infected with FIV a minimum of 10 months earlier. Popliteal lymph node cells were stimulated by cocultivation with UV-inactivated autologous fibroblasts infected with recombinant vaccinia viruses expressing either FIV gag or env gene products, followed by expansion in interleukin-2. One or two infusions of both Gag- and Env-stimulated cells resulted in a slow increase in FIV-specific gamma interferon-secreting T cells in the circulation of cats. In the same animals, viral set points fluctuated widely during the first 2 to 3 weeks after adoptive transfer and then returned to pretreatment levels. The preexisting viral quasispecies was also found to be modulated, whereas no novel viral variants were detected. Circulating CD4+ counts underwent a dramatic decline early after treatment. CD4/CD8 ratios remained instead essentially unchanged and eventually improved in some animals. In contrast, a single infusion of Gag-stimulated cells alone produced no apparent modulations of infection.

In humans and in animal models, the induction and maintenance of virus-specific CD8+ T-cell responses are crucial for the elimination of acute viral infections, such as influenza (26), and in controlling viral replication in persistent infections such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV) infections (2, 27). Likewise, in persistent retroviral infections of humans and animals there is a close relationship between the presence of virus-specific cytotoxic T lymphocytes (CTL) and the control of retroviral replication (5, 7, 8, 18, 22, 24, 33, 35, 45). That virus-specific CTL are important in the control of human immunodeficiency virus (HIV) replication in people is supported by in vitro experiments (45) and by the frequent selection of viral mutants in vivo that are no longer recognized by CTL (11, 31) and consequently escape immune control. The use of histocompatibility complex tetramers presenting an immunodominant HIV peptide epitope has also shown that the numbers of HIV-specific T cells peak just after the level of viremia starts to decline after infection (44), implying that the CTL are responsible.

Recent improvements in antiretroviral chemotherapy have dramatically extended the life expectancy of HIV-infected individuals in the developed world. However, latent infection of memory CD4+ T cells and possibly other cells provides a mechanism for lifelong persistence of the virus in treated patients so that virus load rapidly increases again after the withdrawal of chemotherapy (28, 34).

Adoptive immunotherapy with virus-specific T-cell clones, in an attempt to achieve a meaningful augmentation of adaptive immune responses, has proven to be a useful adjunct to chemotherapy for certain persistent viral infections in humans, including EBV and CMV (16, 30, 42). However, the same approach has met with mixed success in HIV-infected patients: some studies have shown modulations in viral burdens (3), and others have shown rapid progression to clinical disease associated with the selection of CTL escape mutant viruses (23).

Feline immunodeficiency virus (FIV) represents the only naturally occurring disease model for HIV, with an abundance and diversity of strains isolated from cats worldwide. Further, FIV is an important pathogen of its natural host species, resembles HIV in pathogenesis and immunobiology, and is recognized as an important model for evaluating novel approaches to lentiviral therapy and prophylaxis (4, 10, 41). In the present study, we evaluated the safety and the clinical, virologic, and immunological efficacy of adoptive transfer of autologous lymphoid cells restimulated in vitro with viral antigens in chronically FIV-infected domestic cats. Our aim was to evaluate the potential therapeutic value of this approach and, possibly, obtain hints about the importance of FIV-specific T cells in containing FIV. Treatment-naive cats infected with either of two independent isolates of FIV received one or two infusions of mixed Gag-stimulated lymphoid cells (SLC) and Env-SLC or a single infusion of Gag-SLC alone. All treatments were well tolerated, but only the mixed infusions produced detectable effects on infection course. The changes were most evident for 2 to 3 weeks posttreatment and consisted of rapid wide fluctuations in viral set points and in a drastic decrease in circulating CD4+ counts. Subsequently, the virus stabilized again at prechallenge levels but CD4+ counts remained low. Initially, CD4/CD8 ratios were little modified but improved eventually in some cats.
MATERIALS AND METHODS

Animals and infection. Due to the exploratory intent of the study, the number of experimental animals was kept to a minimum. These were eight treatment-naive female outbred specific-pathogen-free (SPF) domestic cats who had been experimentally infected with either the prototype Petaluma (FIVPET) or the Glasgow isolate of FIV (FIVGlas) 10 to 55 months prior to initiation of the experiment (Table 1). They were housed individually in a climatized animal facility and had ad libitum access to fresh water and a proprietary brand of cat food in accordance with European Community guidelines.

Preparation of the SLC. A skin biopsy sample and a popliteal lymph node were aseptically removed from each individual cat under general anesthesia and used to produce the stimulator fibroblasts and the SLC, respectively (13). For stimulator cell production, 24-well flat-bottom tissue culture plates were seeded with 10^6 fibroblasts per well in 1 ml of MEM ALPHA medium containing 10% heat-inactivated fetal bovine serum (Sigma-Aldrich) containing 100 IU of penicillin per ml and 100 μg of streptomycin per ml and incubated at 37°C in 5% CO₂. When fibroblasts were confluent, the medium was aspirated and the cells were inoculated with 5 PFU per cell of recombinant vaccinia virus in a total volume of 200 μl of complete RPMI medium. Infection was allowed to proceed for 3 ha at 37°C to allow for optimal expression of FIV antigens (1). The fibroblasts were then irradiated for 300 s in a UV-cross-linker to inactivate the viral particles. For the production of Gag- and Env-SLC, single cell suspensions prepared from the popliteal lymph nodes by gentle manual homogenization were added to the respective autologous fibroblasts in a ratio of ca. 10:1 in 2 ml of RPMI 1640 medium (Sigma-Aldrich). The plates were then incubated for 7 to 10 days at 37°C in 5% CO₂. Prior to reinfusion into the experimental animals, the cultures were harvested and cultured for a further 7 to 10 days in complete RPMI 1640 medium for background of 1 μg of genomic DNA were used to produce a standard curve and determine the lowest limit of detection (10 copies).

Measurement of plasma viremia. Viral RNA was prepared from plasma samples by using the QIAamp Viral RNA kit (Qiagen). A total of 10 μl of RNA extracted from plasma was reverse transcribed and TM-PCR amplified (32). The sensitivity of the assay was 200 copies/ml of plasma, as evaluated by extracting and amplifying FIV-negative plasma spiked with serial 10-fold dilutions of FIVPET gag plasmid in a background of 1 μg of genomic DNA were used to produce a standard curve and determine the lowest limit of detection (10 copies).
nested PCR. The cDNA obtained with SUSC-I primer was amplified with sense primers V12 (GCAGGTAAGTTAGAAGAGCAAG; nucleotides 6503 to 6525) and SUSC-I (first-round PCR) and SUSC-II-S (CGCTCAGGTAGTATGGAGACTTCC; nucleotides 6784 to 6807) and SUSC-II-AS (GCTCCCGTTACTTCTCCATAAC; nucleotides 7559 to 7581) (second-round PCR). The cDNA obtained with SUT-I primer was amplified with sense primers V51 (AAATGTGGATGGTGGAATCAA; nucleotides 7322 to 7342) and SUT-I (first-round PCR) and SUT-II-S (GAGCAATCTCGTCATGGAAACAAAG; nucleotides 7431 to 7455) and SUT-II-AS (CCTATAGCAGTAGCCCCGTCCC TGC; nucleotides 8150 to 8175) (second-round PCR). The cDNA obtained with SUSC-I primer was amplified with sense primers V51 (AAATGTGGATGGTGGAATCAA; nucleotides 7322 to 7342) and SUT-I (first-round PCR) and SUT-II-S (GAGCAATCTCGTCATGGAAACAAAG; nucleotides 7431 to 7455) and SUT-II-AS (CCTATAGCAGTAGCCCCGTCCC TGC; nucleotides 8150 to 8175) (second-round PCR). Amplics were either directly sequenced for determining the predominant variant or cloned into pCRII Topo TA plasmid vector (Invitrogen Italia, San Giuliano Milanese, Italy), and individual clones were sequenced for determining virus quasispecies. Sequencing was performed by using the automated ALF ExpressII DNA sequencer (Amersham Pharmacia Biotech, Cologno Monzese, Italy) and the Cy-5-labeled primers A-S (ACAGACCCATTACAAATCCCAC; nucleotides 7226 to 7247), A-AS (GGGATTTGTGAATGGGTTCTGT; nucleotides 7226 to 7247), SEQII-AS (CTTTTGTTTCCATGACGAGATTGCTC); nucleotides 7431 to 7455, V4-S (AACCTTTGCAATGAGAAGTT; nucleotides 7534 to 7553), and V4-AS (TACAGACAAATITCCGAACA; nucleotides 7849 to 7868). Sequence data were edited and aligned by BioEdit (version 6.0.5, http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

**Flow cytometry.** Differential lymphocyte counts in the blood and in the SLC were performed by labeling with mouse monoclonal antibodies to feline CD4 (FEI.7B12), CD8a (FEI.10E9), and CD21 (CA2.1D6), all obtained from P. F. Moore, University of California at Davis. Bound primary antibodies were detected by using fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin G1 (Space-Serotec, Milan, Italy), and the samples were analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA).
TABLE 2. Phenotypic characterization of adoptively transferred SLC

| Cat ID     | % SLC with surface marker | FIV DNA copies/µg of SLC DNA |
|------------|---------------------------|------------------------------|
|            | CD4          | CD8          | CD21         | Gag stimulated | Env stimulated |
| Single infusion of Gag- and Env-SLC |               |               |               |                |                |
| BD         | 30           | 25           | 26           | 26,777         | 84             |
| BH         | 21           | 25           | 33           | 17,335         | 4,571          |
| BP         | 30           | 27           | 13           | 89,260         | 18,166         |
| Two infusions of Gag- and Env-SLC |               |               |               |                |                |
| BB         | 22           | 33           | 17           | 15,660         | 75             |
| BT         | 33           | 20           | 13           | 54,135         | 5,359          |
| BV         | 32           | 28           | 15           | 596,947        | 499,638        |
| Single infusion of Gag-SLC alone |               |               |               |                |                |
| AX         | 10           | 15           | 7            | 150,216        | ND*            |
| BE         | 26           | 47           | 16           | 108,440        | ND             |

* ND, not done.
numbers of T cells specific for Gag antigen, Env antigen, or both were substantially augmented. These increases did not appear to be dependent on the cats receiving a second infusion of SLC since they were also observed in cats who received only one infusion. In contrast, the increases appeared to be affected by the antigen used to stimulate the cells transfused since they were noted in the animals who had received both Gag-SLC and Env-SLC but not in the ones given Gag-SLC alone. In the former animals the effect was statistically highly significant ($P < 0.002$, by Friedman analysis).

**Effect of adoptive immunotherapy on FIV loads.** At selected intervals up to 80 days after the infusions, the study cats were assessed for plasma viral RNA and PBMC viral DNA copy numbers, as measures of the activity of FIV infection (Fig. 3). Consistent with the fact that the animals were in the steady-state phase of the infection, both parameters had stabilized at relatively low levels when the experiment was started. After adoptive transfer, the two cats who had received Gag-SLC alone underwent very little change in viral set points relative to pretreatment. In contrast, the posttreatment phase of all cats given one or two infusions of both Gag-SLC and Env-SLC was characterized by wide fluctuations of the viral parameters. The fluctuations were only partially in phase in individual cats; however, most lower values were concentrated in the very first days posttreatment and most peak values were concentrated at later times. This phase of perturbed infection balance lasted 2 to 3 weeks, after which plasma viremia levels and proviral copy numbers stabilized again around levels similar to the ones prior to treatment.

**FIG. 2.** Frequencies of FIV-specific IFN-γ-secreting cells in the blood of cats before and after adoptive immunotherapy. FIV Gag- and Env-specific IFN-γ-secreting T cells were enumerated directly in the PBMC by ELISpot assays prior to cell infusion and at intervals thereafter. Shaded areas are means ± the standard deviation of background spot-forming cells detected in the absence of FIV antigen in the assays. Arrowheads indicate the infusions.
Effect of adoptive immunotherapy on viral surface glycoprotein sequence. Since it was plausible that the viral load fluctuations seen after infusion of mixed Gag- and Env-SLC were associated with surface modifications in the infecting virus, we directly sequenced the entire surface glycoprotein of paired viral samples obtained from all of the recipients at day 0 and at the time postinfusion of maximum plasma viremia (Fig. 3, arrows). Direct sequencing showed identical pre- and posttreatment sequences in three cats, and 2, 7, and 10 irregularly dispersed amino acid differences between the paired samples of the other three (BH, BV, and BT, respectively, all infected with FIVPET). Because the observed interpair differences could reflect the emergence of novel viral variants or quantitative changes in the composition of the existent quasispecies, the paired samples of cats BT and BV were cloned, and 10 to 15 clones for each sample were sequenced. The results showed that, apart from one found in BV, all of the substitutions detected after treatment were already also present in the pretreatment samples (data not shown). However, the relative proportions of the sequences carrying one or the other residue were significantly different in the pre- and posttreatment samples, indicating that also in these animals adoptive immunotherapy had not led to the appearance of new variants but had simply modulated the preexisting viral quasispecies. It is, however, remarkable that of the amino acid positions involved five were shared between cats BT and BV (Fig. 4), suggesting the existence of at least some degree of consistency in such modulation.

Effect of adoptive immunotherapy on blood T-cell counts. At the start of the experiment, CD4⁺-cell counts were below the range of normality only in three of the eight cats, and these were all infected by long time with FIVGL8, known to be more virulent than FIVPET (19). After the infusions, CD4⁺-cell counts declined in all of the cats except the two given a single infusion of Gag-SLC, who already had very low pretreatment counts. The drop lasted for a couple of weeks, after which a certain recovery occurred in some cats. Similar fluctuations were also observed with CD8⁺-cell counts. As a result, CD4/CD8 ratios, which were generally low before treatment, did not change until, in four cats given Env-SLC, an improvement became evident starting from the second week posttreatment.
Cats who received a second infusion did not differ from cats receiving a single infusion.

**DISCUSSION**

This is the first report with the FIV model to explore the feasibility of immunotherapy of lentiviral infections with autologous lymphoid cells restimulated and expanded ex vivo. This kind of intervention has been considered as a possible therapeutic adjunct in the treatment of HIV infection (24) but has received little practical testing—and often under far-from-ideal conditions—in natural host systems. FIV is a well-characterized natural pathogen of domestic cats and can provide valuable insights about the usefulness of manipulating lentivirus-infected hosts immunologically (4, 10, 41). Adoptive immunotherapy has been exploited with some success to treat chronic viral infections in humans refractory to antiviral chemotherapy (38) or to prevent the development of virus-induced disease in immunosuppressed transplant recipients (30). Also, studies on feline leukemia virus (FeLV), a commonly occurring gamma retrovirus of domestic cats, have revealed that adoptive immunotherapy was associated with very significant declines in FeLV proviral loads (14). Here, adoptive immunotherapy of FIV infection was evaluated with regard to the number of SLC infusions and the viral antigen used to restimulate the infused SLC (gag versus env products) in terms of safety and modulation of viral burden, viral quasispecies, and lymphocyte subsets in the peripheral circulation.

Study animals were SPF cats who had been FIV infected for a minimum of 10 months and were therefore in the steady-state infection (21). The SLC to be infused were generated by expanding autologous lymph node cells ex vivo by using UV-inactivated autologous fibroblasts infected with rVV expressing FIV gag (Gag-SLC) or env gene products (Env-SLC) as pulsing stimulus. Cell permanence in vitro was kept relatively short (14 to 20 days) to avoid the selection of T-cell subpopulations conditioned for in vitro culture and to ensure that viral epitope specificity of the transfused cells was representative of that found in vivo. Furthermore, infused cells were not depleted of CD4+ T cells because a deficiency of the help provided by these cells may have been responsible for the failed long-term survival of infused CMV-specific CD8+ T cells (43), and its presence was associated with improved clinical benefit in EBV immunotherapy (38). As determined by ELISpot analysis of IFN-γ-secreting cells, in vitro restimulation expanded the HIV-specific functionally responsive T cells in all cats. This effect was more marked for Env-specific T cells compared to the Gag-specific counterpart, possibly due to the higher numbers of the former present in the starting lymph nodes. It should, however, be noted that analyses of cat T-cell responses to FIV with the use of a single-cell assays have thus far been very few (6). The findings thus far have differed somewhat from reports for HIV and SIV (9, 25, 29) with regard to both the frequencies (higher in primate lentiviral infections) and the preferential targeting of reactive T cells (in contrast to what was observed here, Gag appears to be targeted more frequently than Env in primate lentiviral infections). These discrepancies might reflect inherent differences in the antiviral cell-mediated immune responses mounted by different lentivirus-infected hosts. Traditional bulk tests of cell-mediated immunity, such as antigen-specific lymphoproliferation and cytotoxic assays, have also generally given weak readings in the course of FIV infection (14). The sensitivity of our ELISpot assay also needs to be better evaluated, for example, versus similar assays with peptide-loaded (6) or virus-pulsed dendritic cells that in HIV have been reported to perform better than the ones with rVV (39, 40).

The infusions contained Gag-SLC alone or equal numbers of Gag-SLC and Env-SLC, and the mixed ones were given either once or twice. All regimens were apparently well toler-
ated, since no evidence of pyrexia, anorexia, dullness, or lethargy was observed in the treated cats. Prior to treatment, the recipients had low numbers of FIV-specific effector cells as determined by ELISpot analysis of PBMC with Gag and Env antigens, and these numbers increased only marginally soon after infusion, possibly indicating that most infused cells were at least initially trapped in solid tissues (3). From day 21 onward a steady increase in the numbers of FIV-specific IFN-γ-secreting T cells was, however, noted in the PBMC of the cats who had received both Gag- and Env-SLC but not in those given Gag-SLC alone. This indicated that the former infusions had indeed ameliorated antiviral cell-mediated responses in the infected cats, although rather slowly.

The effects of treatment on the viral set points followed an interesting pattern. The first 2 to 3 weeks were characterized by rapid fluctuations of both PBMC viral DNA and plasma RNA copy numbers that were considerably greater than generally observed in steady-state infected cats than in the ones under study. The reasons for these fluctuations remained unresolved, but a clear possibility is that they were triggered by the coexistence in the adoptively transferred cats of opposing forces that reduced or stimulated virus replication. Adoptive transfer certainly introduced new and possibly very active FIV-specific T cells, but most likely also increased the availability of FIV-permissive cells in the hosts due to the actual proliferating cells inoculated or through the release by these cells of cytokines that enhanced lymphoproliferation in general. The latter effect may have greatly expanded the pool of productively infected cells, especially since the infused cats received no antiviral drugs that would have contained FIV replication. It may not be by chance that most dips in viral set points occurred soon after the first infusions, when the beneficial effects were likely to be predominant, and that most peaks occurred instead later on when the virus-favoring mechanisms had had the time to become operative.

CTL epitope mutation in the escape of retroviruses from immune surveillance is well recognized (31). Indeed, expansion of an HIV Nef CTL escape mutant virus has been associated with the failure of at least one HIV adoptive immunotherapy trial (23). Furthermore, correlative data from vaccine studies have previously suggested that both FIV Gag- and Env-specific immune responses are important for protection (20) and that FIV Env-specific CTL are important in the maintenance of long-term anti-FIV immunity (15). The possibility was therefore also considered that the observed fluctuations, which appeared to be mediated mainly if not solely by Env-SLC, might

---

**FIG. 5.** Changes in circulating T-cell subsets produced by adoptive immunotherapy. CD4⁺ and CD8⁺ T cells were enumerated in the blood by flow cytometry with monoclonal antibodies. Shaded areas are the range of normality as determined in a large number of historical uninfected age-matched control cats. Arrowheads indicate the infusions.
reflect the selective expansion of CTL escape mutant viruses. Sequencing studies performed in two cats at the time their plasma viral loads peaked posttransfer demonstrated no new amino acid residues throughout the entire viral surface glycoprotein relative to respective baseline viruses and showed that the treatment had only produced a perturbation of the preexisting viral quasispecies, as revealed by changes in dominant viral variants. Given the time frame used, the possible appearance of CTL escape mutants at later times cannot, however, be excluded.

The period of clearly unbalanced FIV infection was relatively brief and was followed by a return of viral set points to pretreatment levels. This implies that, whatever their nature, the mechanisms involved had been short lived. Nevertheless, the imbalance was not insignificant in terms of immunological status since most of the cats who went through it also showed a concomitant, often very dramatic, decrease in circulating CD4+ T cells, a well-recognized marker of increased FIV infection severity. This effect may have been contributed to by enhanced lymphocyte destruction due to increased viral replication or increased recognition and killing by FIV-specific T cells. However, it seems likely that altered recirculation of lymphocytes and possibly other mechanisms also concurred. Of note, CD8+ T cells also underwent a parallel decline, so even when CD4+ cell counts were lowest the CD4/CD8 ratios remained essentially unchanged. It is interesting that late during the follow-up this ratio actually showed a substantial improvement in some cats and that this occurred in the same animals who showed increased numbers of circulating FIV-specific IFN-γ-secreting cells as well.

In conclusion, the present study has shown that FIV-specific T-cell stimulation can be achieved ex vivo but that reimplantation of the in vitro-stimulated cells into their donors, in the absence of any concomitant antiviral drug treatment, produced no beneficial effects and was actually detrimental in ongoing FIV infections. However, the findings suggested that adoptive immunotherapy had exerted both favorable and harmful consequences on viral loads, although the latter had apparently been more important at least as judged from parallel effects on CD4+ T-cell counts. It seems therefore possible that, if the recipients were concomitantly given antiviral drugs to prevent the stimulation of virus replication produced by the cells infusions, the favorable effects would have outweighed the detrimental ones. Further studies are clearly warranted.

ACKNOWLEDGMENTS

J.N.F. is a BBSRC Advanced Fellow. This study was supported by the Ministero della Salute-Istituto Superiore di Sanità, the Programma per l’AIDS, and the Ministero dell’Istruzione, dell’Università e della Ricerca.

REFERENCES

1. Beatty, J. A., B. J. Willett, E. A. Gault, and O. Jarrett. 1996. A longitudinal study of feline immunodeficiency virus-specific cytotoxic T lymphocytes in experimentally infected cats, using antigen-specific induction. J. Virol. 70: 6199–6206.
2. Borysiewicz, L. K., S. Morris, J. D. Page, and J. G. Sissons. 1983. Human cytomegalovirus-specific cytotoxic T lymphocytes: requirements for in vitro generation and specificity. Eur. J. Immunol. 13:804–809.
3. Brodie, S. J., D. A. Lewinson, B. K. Patterson, D. Jiyama, J. Krieger, L. Corey, P. D. Greenberg, and S. R. Riddell. 1999. In vivo migration and function of transferred HIV-1-specific cytotoxic T cells. Nat. Med. 5:34–41.
4. Burkhard, M. J., and G. A. Dean. 2003. Transmission and immunopathogenesis of FIV in cats as a model for HIV. Curr. HIV Res. 1:15–29.
5. Daenke, S. A. G. Kermode, S. E. Hall, G. Taylor, J. Weber, S. Nightingale, and C. R. Bangham. 1996. High activated and memory cytotoxic T-cell responses to HTLV-1 in healthy carriers and patients with tropical spastic paraesthesia. Virology 217:139–146.
6. Dean, G. A., A. LaVoy, and M. J. Burkhard. 2004. Peptide mapping of feline immunodeficiency virus by IFN-gamma ELISPOT. Vet. Immunol. Immunopathol. 106:49–59.
7. Dittmer, U., B. Race, and K. J. Hasenkrug. 1999. Kinetics of the development of protective immunity in mice vaccinated with a live attenuated retrovirus. J. Virol. 73:8435–8440.
8. Dittmer, U., B. Race, K. E. Peterson, I. M. Strommes, R. J. Messer, and K. J. Hasenkrug. 2002. Essential roles for CD8+ T cells and gamma interferon in protection of mice against retrovirus-induced immunosuppression. J. Virol. 76:450–454.
9. Edwards, B. H., A. Bansal, S. Sabbaji, J. Bakari, M. J. Mulligan, and P. A. Goepfert. 2002. Magnitude of functional CD8+ T-cell responses to the gag protein of human immunodeficiency virus type 1 correlates inversely with viral load in plasma. J. Virol. 76:2298–2305.
10. Elder, J. H., G. A. Dean, E. A. Hoover, J. A. Hoxie, M. H. Malim, L. Mathes, J. C. Neil, T. W. North, E. Spargar, M. B. Tompkins, W. A. Tompkins, J. Yamamoto, N. Yuhki, N. C. Pedersen, and R. H. Miller. 1998. Lessons from the cat: feline immunodeficiency virus as a tool to develop intervention strategies against human immunodeficiency virus type 1. AIDS Res. Hum. Retr. 14:797–801.
11. Engels, D. T., D. H. O’Connor, P. Jing, J. L. Duriez, J. Sidney, J. da Silva, T. M. Hallett, J. H. Venham, R. A. Ruedersdorf, T. Vogel, C. D. Pauza, R. E. Bontrop, R. DeMars, A. Sette, A. L. Hughes, and D. L. Watkins. 1999. Virus-specific cytotoxic T-lymphocyte responses select for amino-acid variation in simian immunodeficiency virus Env and Nef. Nat. Med. 5:1270–1275.
12. Flynn, J. N., J. A. Beatty, C. A. Cannon, E. B. Stephens, M. J. Hosie, J. C. Neil, and O. Jarrett. 1995. Involvement of gag- and env-specific cytotoxic T lymphocytes in protective immunity to feline immunodeficiency virus. AIDS Res. Hum. Retr. 11:1107–1113.
13. Flynn, J. N., C. A. Cannon, J. A. Beatty, M. Mackett, M. A. Rigby, J. C. Neil, and C. Jarrett. 1994. Induction of feline immunodeficiency virus-specific cytotoxic T cells in vivo with carrier-free synthetic peptide. J. Virol. 68:5835–5841.
14. Flynn, J. N., S. Dunham, A. Mueller, C. Cannon, and O. Jarrett. 2002. Involvement of cytolytic and non-cytolytic T cells in the control of feline immunodeficiency virus infection. Vet. Immunol. Immunopathol. 85:159–169.
15. Flynn, J. N., P. Keating, M. J. Hosie, M. Mackett, E. B. Stephens, J. A. Beatty, J. C. Neil, and O. Jarrett. 1996. Env-specific CTL predominates in cats protected from feline immunodeficiency virus infection by vaccination. J. Gen. Virol. 77:657–665.
16. Gottschalk, S., O. L. Edwards, U. Sili, M. H. Hals, T. Goltsova, A. R. Davis, H. E. Heslop, and C. M. Rooney. 2003. Generating CTLs against the subdominant Epstein-Barr virus LMP1 antigen for the adoptive immunotherapy of EBV-associated malignancies. Blood 101:990–1912.
17. Graham, E. M., O. Jarrett, and J. N. Flynn. 2003. Development of antibodies to feline IFN-gamma as tools to elucidate the cellular immune responses to FeLV. J. Immunol. Methods 279:69–78.
18. Hislop, A. D., M. F. Good, L. Mateo, J. Gardner, M. H. Gatei, R. C. Daniel, B. V. Meyers, M. F. Lavin, and A. Subhrieb. 1998. Vaccine-induced cytotoxic T lymphocytes protect against retroviral challenge. Nat. Med. 4:1193–1196.
19. Hosie, M. J., T. Dunsford, D. Klein, B. J. Willet, C. Cannon, R. Osborne, J. Macdonald, N. Spibey, N. Mackay, O. Jarrett, and J. C. Neil. 2000. Vaccination with inactivated virus but not viral DNA reduces virus load following challenge with a heterologous and virulent isolate of feline immunodeficiency virus. J. Virol. 74:9403–9411.
20. Hosie, M. J., and J. N. Flynn. 1996. Feline immunodeficiency virus vaccination: characterization of the immune correlates of protection. J. Virol. 70:7561–7568.
21. Hosie, M. J., R. Osborne, J. K. Yamamoto, J. C. Neil, and O. Jarrett. 1995. Protection against homogeneous but not heterologous challenge induced by inactivated feline immunodeficiency virus vaccines. J. Virol. 69:1253–1255.
22. Kalamu, S. A. 2003. Cellular immunity for prevention and clearance of HIV infection. Curr. Mol. Med. 3:195–208.
23. Koenig, S. A., J. Conley, V. A. Brew, J. M. Jones, S. Leath, L. J. Boots, V. Davey, G. Pantaleo, J. F. Damarest, C. Carter, et al. 1995. Transfer of HIV-1-specific cytotoxic T lymphocytes to an AIDS patient leads to selection for mutant HIV variants and subsequent disease progression. Nat. Med. 1:330–336.
24. Lenti, N. L., and B. D. Walker. 2003. Immunopathogenesis and immunotherapy in AIDS viruses infections. Nat. Med. 9:861–866.
25. Masemola, A., T. Mashishi, G. Khoury, P. Mohube, P. Mokgotho, E. Vardas, M. Colvin, L. Zijenah, D. Katzenstein, R. Musonda, S. Allen, N. Kumwenda, T. Taha, G. Gray, J. McIntyre, S. A. Karim, H. W. Sheppard, and C. M. Gray. 2004. Hierarchical targeting of subtype C human immunodeficiency virus type 1 proteins by CD8+ T cells: correlation with viral load. J. Virol. 78:3233–3243.
26. McMichael, A. J., F. M. Gotch, G. R. Noble, and P. A. Beare. 1983. Cytotoxic T-cell immunity to influenza. N. Engl. J. Med. 309:13–17.
27. Moss, D. J., A. B. Rickinson, and J. H. Pope. 1978. Long-term T-cell-mediated immunity to Epstein-Barr virus in man. I. Complete regression of virus-induced transformation in cultures of seropositive donor leukocytes. Int. J. Cancer 22:662–668.
28. Nickle, D. C., D. Shriner, J. E. Mittler, L. M. Frenkel, and J. L. Mullins. 2003. Importance and detection of virus reservoirs and compartments of HIV infection. Curr. Opin. Microbiol. 6:410–416.
29. Nickle, D. C., D. Shriner, J. E. Mittler, L. M. Frenkel, and J. I. Mullins. 2003. Importance and detection of virus reservoirs and compartments of HIV infection. Curr. Opin. Microbiol. 6:410–416.
30. Peggs, K. S., and S. Mackinnon. 2002. Clinical trials with CMV-specific T cells. Cytotherapy 4:21–28.
31. Phillips, R. E., S. Rowland-Jones, D. F. Nixon, F. M. Gotch, J. P. Edwards, A. O. Ogunlesi, J. G. Elvin, J. A. Rothbard, C. R. Bangham, C. R. Rizza, et al. 1991. Human immunodeficiency virus genetic variation that can escape cytotoxic T-cell recognition. Nature 354:453–459.
32. Pistello, M., M. Moscardini, P. Mazzetti, F. Bonci, L. Zaccaro, P. Isola, G. Freer, S. Specter, D. Matteucci, and M. Bendinelli. 2002. Development of feline immunodeficiency virus ORF-A (tat) mutants: in vitro and in vivo characterization. Virology 298:84–95.
33. Regoes, R. R., R. Antia, D. A. Garber, G. Silvestri, M. B. Feinberg, and S. I. Staprans. 2004. Roles of target cells and virus-specific cellular immunity in primary simian immunodeficiency virus infection. J. Virol. 78:4866–4875.
34. Saksena, N. K., and S. J. Potter. 2003. Reservoirs of HIV-1 in vivo: implications for antiretroviral therapy. AIDS Rev. 5:3–18.
35. Sarzotti, M., D. S. Robbins, and P. M. Hoffman. 1996. Induction of protective CTL responses in newborn mice by a murine retrovirus. Science 271:1726–1728.
36. Stephens, E. B., E. J. Butfiloski, and E. Monck. 1992. Analysis of the amino terminal presequence of the feline immunodeficiency virus glycoprotein: effect of deletions on the intracellular transport of gp95. Virology 190:569–578.
37. Sun, Q., R. Burton, V. Reddy, and K. G. Lucas. 2002. Safety of allogeneic Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes for patients with refractory EBV-related lymphoma. Br. J. Haematol. 118:799–808.
38. Suni, M. A., H. S. Dunn, P. L. Orr, R. de Laat, E. Sinclair, S. A. Ghanekar, B. M. Bredt, J. F. Dunne, V. C. Maino, and H. T. Macek. 2003. Performance of plate-based cytokine flow cytometry with automated data analysis. BMC Immunol. 4:9.
39. Tanaka, Y., S. F. Dowdy, D. C. Linehan, T. J. Eberlein, and P. S. Goedebuure. 2003. Induction of antigen-specific CTL by recombinant HIV-transactivating fusion protein-pulsed human monocyte-derived dendritic cells. J. Immunol. 170:1291–1298.
40. Uhl, E. W., T. G. Heaton-Jones, R. Pu, and J. K. Yamamoto. 2002. FIV vaccine development and its importance to veterinary and human medicine: a review FIV vaccine 2002 update and review. Vet. Immunol. Immunopathol. 90:113–132.
41. van Rhe, F., and J. Barrett. 2002. Adoptive transfer of Ag-specific T cells to prevent CMV disease after allogeneic stem-cell transplantation. Cytotherapy 4:3–10.
42. Walter, E. A., P. D. Greenberg, M. J. Gilbert, R. J. Finch, K. S. Watanabe, E. D. Thomas, and S. R. Riddell. 1995. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N. Engl. J. Med. 333:1038–1044.
43. Wilson, J. D., G. S. Ogg, R. L. Allen, C. Davis, S. Shaunak, J. Downie, W. Dyer, C. Workman, S. Sullivan, A. J. McMichael, and S. L. Rowland-Jones. 2000. Direct visualization of HIV-1-specific cytotoxic T lymphocytes during primary infection. AIDS 14:225–233.
44. Yang, O. O., S. A. Kalams, A. Trocha, H. Cao, A. Luster, R. P. Johnson, and B. D. Walker. 1997. Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. J. Virol. 71:3120–3128.