NOTES

Relationship between Tumor Necrosis Factor Alpha and Feline Immunodeficiency Virus Expressions

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The presence of feline immunodeficiency virus (FIV) proviral DNA, expression of FIV p26 core protein, and production of tumor necrosis factor alpha (TNF- α) were assessed in sequential biopsies of spleen and lymph node sections, of mononuclear cells of the peripheral blood, and of the serum of specific-pathogen-free cats during the acute phase of FIV infection. A temporal relationship between TNF- α production and FIV p26 expression was noted. Two months following FIV infection, and preceding the detection of FIV viremia, levels of TNF- α in serum increased significantly ($P = 0.04$), and they remained elevated during FIV viremia in the third month postinfection. Immunoprecipitates representing expression of TNF- α and of FIV p26 were localized in common foci of lymph nodes of FIV-infected cats during this period of active viremia. With the advent of anti-FIV antibodies, circulating levels of TNF- α and p26 antigen and expression of TNF- α and p26 in the lymph nodes decreased during the fifth month postinfection, and p26 production became undetectable. With clearance of viremia, burden of proviral DNA in peripheral blood mononuclear cells became reduced ($P = 0.041$), with provirus remaining integrated principally within lymph nodes ($P = 0.046$). During aviremia, p26 expression was undetectable in any tissue but remained inducible in vitro. During acute FIV infection, TNF- α production and p26 expression are intimately linked.

In the weeks immediately following human immunodeficiency virus (HIV) infection, approximately two-thirds of HIV-infected individuals develop an acute mononucleosis-like illness, accompanied by elevated serum viral titers (36). During this acute viremia, HIV proviral DNA becomes principally seeded within lymphoid organs (28) and in other tissues within macrophages (1, 14, 16). A specific anti-HIV immune response develops, resulting in the reduction and eventual elimination of detectable viremia (6, 7, 13), but HIV continues to replicate locally within lymphoid organs (11, 29).

Our understanding of the interaction between host immunity and HIV pathogenesis prior to detectable viremia is particularly incomplete, since the moment at which HIV infection occurs is rarely known. In order to determine the nature of this relationship, sequential analyses of blood and lymphoid tissues are required soon after viral infection, but such analyses are logistically difficult (30).

Cats infected with the lentivirus, feline immunodeficiency virus (FIV), develop an immunodeficiency with many similarities to human AIDS, including the reversal of the T-lymphocyte CD4/CD8 ratio, polyclonal B-lymphocyte activation, hypergammaglobulinemia, neutropenia, reduced T-lymphocyte functions, and increased susceptibility to multiple intercurrent infections (2, 3, 31). Details of the relationship between lentiviral pathogenesis and host immunity could be described by

evaluating the acutely FIV-infected cat, even prior to detectable viremia.

Tumor necrosis factor alpha (TNF- α) is a cytokine secreted primarily by macrophages, with pleiotropic effects on biological and immunological processes (23). TNF- α contributes to the regulation of immune cell growth and differentiation (23) and has been paradoxically implicated in both the augmentation and the abrogation of lentiviral expression (32, 35). TNF- α contributes to monocyte-mediated cytotoxicity (9, 12) and may specifically contribute to the elimination of lentivirus-infected cells, since TNF- α induces apoptosis of cultured FIV-infected T lymphocytes (25) and kidney cells (24) and of HIV-infected T lymphocytes (21). In contrast, TNF- α has also been shown to augment HIV expression in vitro (20, 22, 26, 27, 38).

The relationship between TNF- α and lentiviral pathogenesis in vivo has been incompletely defined. Increased TNF- α expression has been detected in peripheral blood mononuclear cells (PBMC) (15, 37) and monocytes (34, 39) isolated from HIV-infected individuals, but levels of TNF- α in serum may (18) or may not (19) become elevated following experimental FIV infection in cats. Whether expression of lentivirus and that of TNF- α are temporally or quantitatively related during acute infection or whether expression of FIV and of TNF- α can be colocalized in lymphoid tissues during FIV pathogenesis has not been determined.

In the present study, in order to determine the nature of this relationship between expression of TNF- α and that of FIV, specific-pathogen-free cats were infected with FIV Petaluma. Expression of TNF- α and FIV core protein p26 expression were detected in FIV-infected and control cats by radioimmunoassay or enzyme-linked immunosorbent assay (ELISA),

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FIG. 1. (Left) PCR products specific for FIV LTR DNA from PBMC or lymph nodes (LN) of FIV-infected cats 3 and 9 months postinfection. Each lane represents an individual cat. (Right) Comparison of normalized optical densities (OD) of PCR bands produced from PBMC (closed circles) or lymph node (open circles) DNA of FIV-infected cats 3 and 9 months postinfection. —, mean normalized LTR PCR product. *, significant reduction in PBMC LTR PCR production from 3 to 9 months ($P = 0.041$). ‡, lymph node, 9 months postinfection. The LTR PCR product is greater than that of PBMC ($P = 0.046$).

and/or were localized by immunohistochemistry. FIV proviral DNA was detected and semiquantified by PCR. Viremia was confirmed by isolating infectious FIV from serum in vitro by culturing with stimulated donor leukocytes. During aviremia, persistence of FIV infection was confirmed by the induction of p26 expression in vitro.

Nine 6- to 8-month-old specific-pathogen-free cats (Harlan Sprague-Dawley, Indianapolis, Ind.) were administered 2 ml of supernatant intraperitoneally from the FL4 cell line ($>1,000$ tissue culture infective dose), an FIV Petaluma-infected feline T-lymphocyte line, provided by J. Yamamoto, University of Florida, Gainesville (17). As controls, nine age-matched cats were administered 2 ml of supernatant intraperitoneally from the 3201 cell line, an FIV-free feline T-lymphocyte line provided by W. Hardy, Bronx-Lebanon Hospital, New York. Samples of PBMC, serum, and bone marrow were collected from each of three FIV-infected and control cats monthly. Both submandibular lymph nodes were removed from each cat at separate intervals so that lymph node analyses for each experimental group could be made at monthly intervals. Splenic biopsies were performed once on each cat so that splenic analyses of three cats within each experimental group could be made at intervals of every four months.

For determination of proviral load, DNA was isolated from PBMC and from lymph node, spleen, and bone marrow cells by the addition of lysis buffer (12% DTAB [dodecyltrimethylammonium], 2.25 M NaCl, 150 mM Tris-HCl [pH 8.6], 75 mM EDTA); this was followed by chloroform extraction and washing with 75% ethanol. One microgram of DNA was amplified in a 100- μ l reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 300 ng of each primer, and 0.2 mM (each) dATP, dCTP, dGTP, and dTTP. *Thermus aquaticus* DNA polymerase (2.5 U) was added to each sample after the samples were heated to 95°C for 5 min and cooled to 80°C. PCR was performed for 30 cycles with TNF- α -specific primers and for 50 cycles with FIV Gag- or long terminal repeat (LTR)-specific primers as follows: 95°C for 1 min, 56°C for 1 min, 72°C for 2 min. PCR products were analyzed by electrophoresis through a 2% agarose gel containing 0.5 μ g of ethidium bromide per ml and visualized with a UV light source. Negatives were taken of each gel and scanned with the Millipore BioImage System (Millipore Corp., Ann Arbor, Mich.). The optical density of each band was determined by the 1D analysis protocol contained in the BioImage software package. The band densities for FIV from each sample were

then normalized according to the band density level obtained for the coinciding TNF- α PCR product.

The results showed that FIV-infected cats experienced the highest FIV proviral burden within PBMC and lymph node tissue at 3 months postinfection (Fig. 1). Splenic FIV proviral DNA was detected at 1 month postinfection (data not shown). The amount of FIV proviral DNA in PBMC, lymph nodes, and spleens declined by month 5 postinfection, with the most significant decrease occurring in PBMC ($P = 0.041$). By the ninth month postinfection, lymph node DNA contained significantly more FIV proviral DNA than that of the PBMC ($P = 0.046$), spleen, or bone marrow (Fig. 1). Uninfected cats were consistently negative for FIV proviral DNA.

In FIV infection, incorporation of proviral DNA does not always correlate with the expression of viral proteins or the occurrence of seroconversion to viral proteins (8). In the present study, viral expression was assessed by detection of the FIV p26^{gag} protein, a core protein of the virus. In serum, p26 antigenemia was detected by the PetChek FIV Antigen ELISA (IDEXX, Portland, Maine). Immunohistochemical analysis for FIV proteins was performed on lymph node and spleen sections. The sections were incubated with the 3B7 anti-FIV p26 monoclonal antibody (provided by Fernando de Noronha, Cornell University, New York). The presence of resulting immune complexes was detected with the VECTASTAIN Elite ABC Kit (Vector Laboratories, Burlingame, Calif.). Tissues were then stained with hematoxylin and examined for the presence of immunoprecipitates.

Our findings indicate that 1 month postinfection, FIV p26 was detected in 33% of evaluated FIV-infected cats and only within the lymph node and spleen tissues but not within the sera. Three months postinfection, p26 was detected in the lymph nodes of 66% (Fig. 2) and in the sera of 100% of evaluated FIV-infected cats. At this interval, the mean serum p26 level was significantly greater than that at prior intervals ($P = 0.003$) (Fig. 3). Serum from each of these individual cats was capable of establishing a productive infection in cultured normal, concanavalin A-stimulated PBMC obtained from donor, FIV-free cats (data not shown), indicating that the detected antigenemia represented a true viremia.

Anti-FIV antibody levels were greater in FIV-infected cats than levels measured in controls at ≥ 2 months postinfection (Fig. 4) (IDEXX). With the advent of anti-FIV immunity, expression of p26 became undetectable in both the sera and tissues of FIV-infected cats following the third month postinfection ($P = 0.007$) (Fig. 3). FIV p26 and anti-FIV antibodies were not detected in samples of uninfected cats at any time.

To ensure the presence of latent FIV proviral DNA with capacity for full viral expression in aviremic FIV-infected cats,

FIG. 2. Focal, granular, brown immunoprecipitates indicative of FIV p26 expression (left) and TNF- α expression (right) localized together in subadjacent lymph node sections of an FIV-infected cat 3 months postinfection. Magnification, $\times 1,000$.

FIG. 3. Serum TNF- α (●) and serum FIV p26 (---■) levels in negative control cats (above) and FIV-infected cats (below). *, significant increase in serum TNF- α levels at 2 to 3 months postinfection relative to controls ($P = 0.04$). ‡, significantly greater serum p26 levels 3 months postinfection relative to both prior and subsequent months ($P < 0.007$). OD, optical density.

PBMC from FIV-infected cats were cultured 12 months postinfection. The cultures were performed essentially as described by Dandekar et al. (8). Supernatants of concanavalin A-stimulated PBMC cultures from each of the FIV-infected cats were positive for p26 production (data not shown), confirming latency of FIV infection. Supernatants of PBMC cultures from uninfected cats were negative.

The inability to detect p26 expression in aviremic FIV-infected cats after the development of anti-FIV humoral immunity may be attributable to either the masking of low-level viral protein expression bound by antiviral antibodies or the complete absence of FIV mRNA transcription. To distinguish between these two potential states of latency, reverse transcription-PCR of lymph node, spleen, and PBMC RNA of FIV-infected aviremic cats was performed. Reverse transcription-PCR was also performed on RNA obtained from the FIV-infected FL4 cell line to serve as a positive control for amplification. RNA was isolated by a single-step method of purification (5). One to two micrograms of RNA was used for cDNA synthesis (Promega, Madison, Wis.). cDNA was amplified by PCR as described above. The primers used were as follows: for LTR, 5'-GGATGAGTATTGGAACCCTGA and 3'-GGATTCCGAGACCTCACGGTA; for *gag*, 5'-GAGATTCTACAGCAACATGGG and 3'-GATGGCCTAGTGTCTAATCCC; for *pol*, 5'-GGTGGGACCACATCAAATAAG and 3'-TTCCATCACTTGCCACTTTCC, for β -actin, 5'-GTGATGATGGGCATCGGTCA and 3'-TTAATGTCACGCACGATTCCC. FIV-specific PCR products were not produced at any interval of assessment at ≥ 5 months postinfection, although all gene products were consistently detected in the FL4 cell line. The lack of transcription of FIV mRNA indicates that the inability to detect p26 protein expression following the seroconversion to viral proteins is not due to the masking of p26 by the binding of antibodies. This down regulation of FIV mRNA expression maybe contributed to the inoculum

utilized, as FL4-adapted FIV Petaluma may be attenuated in virulence.

Production of TNF- α was determined to be temporally related to the production of FIV p26 (19, 24). Prior to the detection of FIV viremia, mean serum TNF- α levels increased significantly during the second month postinfection, and they remained elevated during detectable viremia in the third month postinfection in FIV-infected cats ($P = 0.04$) (Fig. 3). TNF- α levels in serum declined by the fifth month postinfection, concurrent with the decline in serum p26 levels, and approached TNF- α levels measured in control cats by the ninth month postinfection. The 2- and 3-month time points were the only intervals at which the mean values of the serum TNF- α levels differed between control and FIV-infected cats.

TNF- α expression in tissues was detected by immunohistochemistry performed with a monoclonal anti-human TNF- α antibody (Biosource International, Camarillo, Calif.). Immunoprecipitates indicative of concurrent TNF- α and FIV p26 expression were localized together in multiple consecutive sections of lymph nodes from FIV-infected cats 3 months postinfection (Fig. 2). Five separate fields were examined under the microscope and scored as 1 to 4; 1 represented 3 to 20 positive cells, 2 represented 21 to 40 positive cells, 3 represented 41 to 60 positive cells, and 4 represented 61 to 80 positive cells. TNF- α immunoprecipitate was most abundant in the lymph nodes of FIV-infected cats during this interval postinfection but was not detected at any interval in sections of spleens from FIV-infected or uninfected control cats. TNF- α immunoprecipitate was present in only scant or moderate amounts in lymph node sections of control cats. Early TNF- α expression within lymphoid tissues may have been induced by focally replicating FIV. HIV p24 content of rectal mucosal homogenates from AIDS patients is greatest in the subgroup of patients which coexpress TNF- α mRNA, and both HIV p24 and TNF- α RNA are colocalized in the submucosa of these patients (33).

Our observations extend those made recently regarding the pattern of FIV expression following infection, onset of antiviral humoral immunity, and reduction and clearance of virus from the peripheral blood (4, 10). Herein we describe the temporal

FIG. 4. Mean serum anti-FIV antibody levels of FIV-infected cats (closed circles) were significantly greater than those of controls (open circles) at every interval of assessment 2 months or more following FIV exposure. OD, optical density.

relationship between expression of TNF- α and that of FIV, which peak and subside together during clearance of acute viremia. These results demonstrate similarities in the pattern of acute FIV infection and that of HIV-infected patients (6, 7, 13) and support the appropriateness of the feline model for studies of lentivirus-associated immunopathology. Understanding the relationship between early lentiviral infection and evoked immunological responses will contribute to our knowledge of the manner in which slow viruses bypass host defenses and establish an insidious progression toward overt immunosuppression.

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REFERENCES

- Achim, C. L., M. P. Heyes, and C. A. Wiley. 1993. Quantitation of human immunodeficiency virus, immune activation factors, and quinolinic acid in AIDS brains. *J. Clin. Invest.* **91**:2769–2775.
- Ackley, C. D., J. K. Yamamoto, N. Levy, N. C. Pedersen, and M. D. Cooper. 1990. Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus. *J. Virol.* **64**:5652–5655.
- Barlough, J., C. Ackley, J. George, N. Levy, R. Acevedo, P. Moore, B. Rideout, M. Cooper, and N. Pedersen. 1991. Acquired immune dysfunction in cats with experimentally induced feline immunodeficiency virus infection: comparison of short-term and long-term infections. *J. Acquired Immune Defic. Syndr.* **4**:219–227.
- Beebe, A. M., N. Dua, T. G. Faith, P. F. Moore, N. C. Pedersen, and S. Dandekar. 1994. Primary stage of feline immunodeficiency virus infection: viral dissemination and cellular targets. *J. Virol.* **68**:3080–3091.
- Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156–159.
- Clark, S., M. Saag, D. Decker, S. Campbell-Hill, J. Roberson, P. Veldkamp, J. Kappes, B. Hahn, and G. Shaw. 1991. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *N. Engl. J. Med.* **324**:954–959.
- Daar, E., T. Moudgh, R. Meyer, and D. Ho. 1991. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N. Engl. J. Med.* **324**:961–964.
- Dandekar, S., A. M. Beebe, J. Barlough, T. Phillips, J. Elder, M. Torten, and N. C. Pedersen. 1992. Detection of feline immunodeficiency virus (FIV) nucleic acids in FIV-seronegative cats. *J. Virol.* **66**:4040–4049.
- Decker, T., M. L. Lohmann-Matthes, and G. Gifford. 1987. Cell-associated tumor necrosis factor (TNF) as a killing mechanism of activated cytotoxic macrophages. *J. Immunol.* **138**:957–962.
- Dua, N., G. Reubel, P. F. Moore, J. Higgins, and N. Pedersen. 1994. An experimental study of primary feline immunodeficiency virus infection in cats and a historical comparison to acute simian and human immunodeficiency diseases. *Vet. Immunol. Immunopathol.* **43**:337–355.
- Embreton, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Haase. 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature (London)* **362**:359–362.
- Feinman, R., D. Henriksen-DeStefano, M. Tsujimoto, and J. Vilcek. 1987. Tumor necrosis factor is an important mediator of tumor cell killing by human monocytes. *J. Immunol.* **138**:635–640.
- Graziosi, C., G. Pantaleo, L. Butini, J. F. Demarest, M. S. Saag, G. M. Shaw, and A. S. Fauci. 1993. Kinetics of human immunodeficiency virus type 1 (HIV-1) DNA and RNA synthesis during primary HIV-1 infection. *Proc. Natl. Acad. Sci. USA* **90**:6405–6409.
- Heng, M. C., M. Lambertus, and M. B. Goetz. 1990. Proliferation of HIV in lymphocyte-associated macrophages with cytopathic changes in AIDS erythroderma. *Australas. J. Dermatol.* **31**:37–46.
- Hober, D., A. Haque, P. Watre, G. Beaucaire, Y. Mouton, and A. Capron. 1989. Production of tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) in patients with AIDS. Enhanced level of TNF- α is related to a higher cytotoxic activity. *Clin. Exp. Immunol.* **78**:329–333.
- Jeffrey, A. A., D. Israel-Biet, J. M. Andrieu, P. Even, and A. Venet. 1991. HIV isolation from pulmonary cells derived from bronchoalveolar lavage. *Clin. Exp. Immunol.* **84**:488–492.
- Koup, R. A., and D. D. Ho. 1991. Isolation and quantitation of HIV in peripheral blood, p. 12.2.1. In J. Coligan, A. Kruisbeek, D. Margulies, E. Shevach, and W. Stober (ed.), *Current protocols in immunology*. Wiley & Sons, New York.
- Lawrence, C. E., J. J. Callanan, and O. Jarrett. 1992. Decreased mitogen responsiveness and elevated tumor necrosis factor production in cats shortly after feline immunodeficiency virus infection. *Vet. Immunol. Immunopathol.* **35**:51–59.
- Lehmann, R., H. Joller, B. L. Haagmans, and H. Lutz. 1992. Tumor necrosis factor alpha levels in cats experimentally infected with feline immunodeficiency virus: effects of immunization and feline leukemia virus infection. *Vet. Immunol. Immunopathol.* **35**:61–69.
- Locardi, C., C. Petrini, G. Boccoli, U. Testa, C. Dieffenbach, S. Buttò, and F. Belardelli. 1990. Increased human immunodeficiency virus (HIV) expression in chronically infected U937 cells upon in vitro differentiation by hydroxyvitamin D3: roles of interferon and tumor necrosis factor in regulation of HIV production. *J. Virol.* **64**:5874–5882.
- Matsuyama, T., Y. Hamamoto, G.-I. Soma, D. Mizuno, N. Yamamoto, and N. Kobayashi. 1989. Cytocidal effect of tumor necrosis factor on cells chronically infected with human immunodeficiency virus (HIV): enhancement of HIV replication. *J. Virol.* **63**:2504–2509.
- Michihiko, S., N. Yamamoto, F. Shinozaki, K. Shimada, G. I. Soma, and N. Kobayashi. 1989. Augmentation of in-vitro HIV replication in peripheral blood mononuclear cells of AIDS and Arc patients by tumor necrosis factor. *Lancet* **i**:1206–1207.
- Odeh, M. 1990. The role of tumor necrosis factor- α in acquired immunodeficiency syndrome. *J. Int. Med.* **228**:549–556.
- Ohno, K., T. Nakano, Y. Matsumoto, T. Watari, R. Goitsuka, H. Nakayama, H. Tsujimoto, and A. Hasegawa. 1993. Apoptosis induced by tumor necrosis factor in cells chronically infected with feline immunodeficiency virus. *J. Virol.* **67**:2429–2433.
- Ohno, K., Y. Okamoto, T. Miyazawa, T. Mikami, T. Watari, R. Goitsuka, H. Tsujimoto, and A. Hasegawa. 1994. Induction of apoptosis in a T lymphoblastoid cell line infected with feline immunodeficiency virus. *Arch. Virol.* **135**:153–158.
- Okamoto, T., T. Matsuyama, and S. Mori. 1989. Augmentation of human immunodeficiency virus type 1 gene expression by tumor necrosis factor- α . *J. Immunol.* **142**:702–707.
- Osborn, L., S. Kunkel, and G. Nabel. 1989. Tumor necrosis factor- α and interleukin 1 stimulate the human immunodeficiency virus enhancers by activation of the nuclear factor kappaB. *Proc. Natl. Acad. Sci. USA* **86**:2336–2340.
- Pantaleo, G., C. Graziosi, L. Butini, P. Pizzo, S. Schnittman, D. Kotler, and A. Fauci. 1991. Lymphoid organs function as major reservoirs for human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **88**:9838–9842.
- Pantaleo, G., C. Graziosi, J. F. Derarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci. 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature (London)* **362**:355–358.
- Pantaleo, G., C. Graziosi, and A. S. Fauci. 1993. The immunopathogenesis of human immunodeficiency virus infection. *N. Engl. J. Med.* **328**:327–335.
- Pedersen, N., E. W. Ho, M. Brown, and J. Yamamoto. 1987. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* **235**:790–793.
- Polli, G., and A. S. Fauci. 1992. The effect of cytokines and pharmacologic agents on chronic HIV infection. *AIDS Res. Hum. Retroviruses* **8**:191–197.
- Reka, S., and D. P. Kotler. 1993. Detection and localization of HIV RNA and TNF mRNA in rectal biopsies from patients with AIDS. *Cytokine* **5**:305–308.
- Roux-Lombard, P., C. Modoux, A. Cruchaud, and J. M. Dayer. 1989. Purified blood monocytes from HIV-1-infected patients produce high levels of TNF- α and IL-1. *Clin. Immunol. Immunopathol.* **50**:374–384.
- Sparger, E., B. Shackel, L. Renshaw-Gregg, P. Barry, J. Elder, and P. Luciw. 1992. Regulation of gene expression directed by the long terminal repeat of feline immunodeficiency virus. *Virology* **187**:165–177.
- Tindell, B., and D. A. Cooper. 1991. Primary HIV infection: host responses and intervention strategies. *AIDS* **5**:1–14.
- Voth, R., S. Rossol, K. Klein, G. Hess, K. Schutt, H. Schroder, K. H. Buschenfelde, and W. Muller. 1990. Differential gene expression of IFN- γ and tumor necrosis factor- α in peripheral blood mononuclear cells from patients with AIDS related complex and AIDS. *J. Immunol.* **144**:970–975.
- Vyakarnam, A., J. McKeating, A. Meager, and P. Beverley. 1990. Tumor necrosis factors (alpha, beta) induced by HIV-1 in peripheral blood mononuclear cells potentiate virus replication. *AIDS* **4**:21–27.
- Wright, S., A. Jewett, R. Mitsuyasu, and B. Bonavida. 1988. Spontaneous cytotoxicity and tumor necrosis factor production by peripheral blood monocytes from AIDS patients. *J. Immunol.* **141**:99–104.