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Kaposi's Sarcoma Cells of Different Etiologic Origins Respond to HIV-Tat through the Flk-1/KDR (VEGFR-2): Relevance in AIDS-KS Pathology

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Kaposi's sarcoma (KS) is an hyperplastic lesion whose main histological features are typical spindle shaped cells with a mixed endothelial-mesenchymal-macrophage phenotype, an intense vascularization and an inflammatory infiltrate. The etiology of KS appears to be linked to activation of a latent HHV8 infection. Sporadic and iatrogenic KS are slow progressing lesions that can undergo spontaneous regression. In contrast, KS, which is frequently associated with HIV infection, is found in a highly aggressive form in AIDS patients. The HIV-1 Tat has been shown to activate the VEGF receptor KDR in endothelial and KS spindle cells, suggesting this HIV protein could contribute to KS pathogenesis. We used primary 'reactive' KS cell culture from sporadic and epidemic KS, and an immortal KS-line (KS-Imm) isolated in our laboratory from a iatrogenic KS lesion, to verify if Tat-induced cell signaling is able to mediate cellular responses. We demonstrate that KS cells migrated in response to Tat and that VEGF is able to compete with the Tat chemotactic activity towards these cells. A function-blocking anti-KDR antibody was able to abrogate both VEGF and Tat-induced KS chemotactic response, indicating a direct involvement of this receptor. Our data show that HIV-Tat can also activate KS cells derived from sporadic or iatrogenic lesions, suggesting that in AIDS patients Tat could cooperate with VEGF in activation of KDS on KS precursor spindle and endothelial cells, and contribute to the aggressiveness of AIDS-KS lesions.

Key Words: **Tat; VEGF; flk-1/KDR; Kaposi's sarcoma; chemotaxis.**

Kaposi's sarcoma (KS) is a highly angiogenic lesion (1), whose development is linked to the concurrence of several factors. KSH/HHV-8 is a herpesvirus always present in KS, the onco-viral proteins of HHV8 seem to play a role in spindle cell transformation increasing cell survival (v-bcl-2), pro inflammatory status (v-IL-6), and angiogenesis (vMIPs) (2). However, HHV8 infection does not appear to be sufficient for KS development, a complex network of cytokine-mediated cellular activation may sustain and probably promote KS formation (3–11). Immunosuppression also plays an important role, as demonstrated by iatrogenic KS, which is associated with post-transplant immunosuppressive therapy. HIV infection is also an important co-factor for KS, providing not only strong immune suppression but probably other KS stimulatory cofactors, as AIDS patients that develop KS frequently have a very aggressive form involving epidermis, mucosae, lungs and lymph nodes (12). The use of anti-retroviral therapy against HIV has sensibly delayed the onset of KS in AIDS patients, but the incidence of KS has remained the same (13). Tat, the HIV transactivator protein, can be released extracellularly where it may exert activities mediated by cell surface receptors (14). Several studies suggest that Tat is a major cofactor in the aggressiveness of epidemic KS lesions. The Tat protein contains at least three domains which could promote KS development: a basic domain mediating KDR activation and $\alpha v\beta 5$ binding (15–17), a cysteine domain with chemokine-like activity mediating monocyte/macrophage recruitment (18, 19) and an RGD domain involved in $\alpha v\beta 3$ integrin binding (20, 21).

KS cell cultures express KDR and VEGF *in vitro*, showing clear autocrine activation (22, 23). In KS lesions the spindle cells are positive for KDR, while VEGF appears to be expressed at low levels (5, 24). HIV-Tat also binds and activates the KDR receptor on endothelial (16) and KS cells (22), this may explain the close association between HIV infection and the aggressiveness of epidemic KS. HIV-derived Tat could act, along with endogenous VEGF, as a KDR ligand in AIDS-KS lesions. Here we show that KS cells of differ-

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ent origins all express the KDR receptor for VEGF *in vitro,* and that the signal transduced by Tat-mediated activation of this receptor is functional, inducing migration of KS cells. In addition, the response of iatrogenic and sporadic KS cells to Tat indicates that KS cells show a general susceptibility to KDR mediated exacerbation/promotion of KS by HIV Tat.

METHODS

Cells. The epidemic KS (EKS) cell primary cultures KSist11 and KSist20, sporadic KS (SKS) cell primary culture KSist26 and the iatrogenic KS (IKS) cell line KS-Imm were obtained, cultured and characterized as previously described (25–27). Control, KDR-expressing, human umbilical vein endothelial cells (HUVEC) were grown in M199 containing 10% FCS, 10 ng/ml aFGF and 10 ng/ml bFGF (Peprotech), while negative KDR control Y-79 retinoblastoma cells were grown in RPMI 10% FCS.

Rt-PCR. Total RNA was extracted by the acid guanidinium thiocyanate-phenol-chlorophorm method (28). Reverse transcription of RNA was done using a commercial kit (Amersham) with 1× reaction buffer + $MgCl_2$ 5 mM, dNTPs 1 mM, Random Hexamers 2,5 mM, RNA 3 μg, RNase inhibitor 1U, Reverse Transcriptase 2.5 U in a final volume of 20 μl (42°C for 15 min, 99°C for 5 min, 5°C for 5 min). Ten microliters of cDNA were then amplified in 1× reaction buffer + $MgCl_2$ 2 mM, and 1 U AmpliTaq DNA Polymerase with either primers for KDR or control β-actin at 1 pm/ml in a final volume of 50 μl.

These primers amplify a 762 bp fragment (KDR forward: GGAAGACCAAGAAAAGACATTGCG, reverse: GGTTGACCACATTGAGATGGTGAC) at 94°C for 30 sec, 57°C for 30 sec, 72°C for 1 min each cycle, for 40 cycles. As an internal control, primers which amplify a 606 bp β-actin sequence were added to 10 μl of cDNA. The cycling conditions were: 94°C for 30 sec, 61°C for 30 sec, 72°C for 45 sec each cycle, for 30 cycles. (β-actin forward: GGCATCGTGATGGACTCCG, reverse: GCTGGAAGGTGGACAGCGA).

Biochemical studies. Quiescent KS cells were preincubated 15 min with 1 mM Na_3OV_4, and stimulated with recombinant Tat or VEGF. The cells were lysed in 50 mM Tris, Ph 7.4, containing 150 mM NaCl, 1% Triton X-100, ZnCl 100 μM, Na_3VO_4 1 mM and protease inhibitors (Pepstatin 50 μg/ml; Leupeptin 50 μg/ml; Aprotinin 10 μg/ml; Phenyl-methyl 4-sulfonyl fluoride 2 mM) at 4°C for 20 min. Lysates were incubated with a monoclonal antibody and the immunocomplex precipitated with Protein A. The immunoprecipitate was the electrophoresed on 6% SDS–PAGE and immunoblotted with monoclonal antibodies. Immunoreactive bands were detected by chemiluminescence using a kit (ECL-Amersham). The monoclonal antibodies used were an anti-phosphotyrosine (Upstate Biotechnology, Lake Placid, NY) and an anti-KDR (Santa Cruz Biotechnology, Santa Cruz, CA). A neutralizing anti-KDR chimeric antibody (c p1C11) was the kind gift of Dr. Zhenping Zhu (ImClone Systems Incorporated, NY).

VEGF quantification. VEGF protein released into conditioned media was measured using a commercial ELISA kit for VEGF (LISTARFISH, Cytimmune sciences, Inc., Collage Park, MD) following the manufacturer's instructions. Values obtained for the conditioned media samples were compared to serial dilutions of VEGF standards (0, 25, 50, 250, 500, 750, 1500 pg/ml). Assays were done in triplicate.

Migration assays. Chemotaxis assays on KS cells were performed in Boyden chambers using minor modifications of the assay described by Postlethwaite (29). 2×10^5 cells in serum free medium with 0.1% BSA (SFM) were placed in the upper compartment; in some experiment cell suspension was additioned with VEGF or anti-KDR as indicated. Tat (10 nM) or VEGF (2.5 nM) were added to the lower compartment as indicated. Heparin (0,1 U/ml), a cofactor, was added to the Tat chemoattractant as previously described (15). Collagen IV coated 12 μM pore-sized polycarbonate filters were placed between the two compartments. After incubation for 6 h at 37°C in 5% CO_2, the filters were recovered, the cells on the upper surface mechanically removed and those on the lower surface were fixed and stained. Five to ten fields per filter were counted under a microscope (1 field = 1/60 of the entire surface of migrated cells). In some experiments cells were quantified by densitometry. Experiments were performed in triplicate and repeated 3 times.

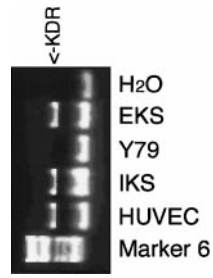

FIG. 1. Amplification of KDR mRNA by RT-PCR: the expected 762 bp fragment was amplified in KS cells (EKS, epidemic KS cell culture; IKS, KS-Imm iatrogenic KS cell line) and in the HUVEC control; Y79, a retinoblastoma cell line, was used as a negative control.

RESULTS

Expression of VEGF by KS Cells

It has been reported that EKS cells secrete high levels of VEGF in culture. Using a sensitive ELISA system, we observed that EKS released 259 pg/10^6 cells of VEGF into the culture medium over a 24 h of incubation. Similarly, IKS cells released 236 pg/10^6 cells and SKS cells 185 pg/10^6 cells of VEGF into the culture medium over a 24-h period of incubation.

Expression and Activation of KDR by VEGF and HIV Tat in KS Cells

We first examined the expression of the KDR mRNA in both reactive and immortal KS cells. RT-PCR indicated expression of the KDR mRNA in all the KS cells tested (Fig. 1, EKS and IKS shown). An identical band was amplified from HUVE cells that are known to synthesize functional KDR, while no amplification was observed with the negative control Y-79 retinoblastoma cells (Fig. 1).

To test whether the KDR expressed was functional, EKS, SKS and IKS cells were stimulated with VEGF or HIV-Tat for 3–5 min followed by lysis, immunoprecipitation with anti-KDR antibodies and western blotting with anti-phosphotyrosine antibodies. Clear tyrosine phosphorylation of a 205 kDa band which corresponds to KDR (Fig. 2) was observed after stimulation with either VEGF or Tat. Immunoprecipitation of the KS-Imm cell lysate using the anti-phosphotyrosine

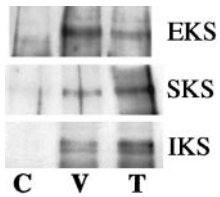

FIG. 2. HIV-Tat stimulates tyrosine phosphorylation of KDR in KS cells. Epidemic (EKS), sporadic (SKS), and iatrogenic (IKS) KS cells were stimulated with Tat (10 ng/ml) or VEGF (10 ng/ml) with heparin (0.1 U/ml), immunoprecipitated with an anti-KDR antibody and Western blotted with an anti-phosphotyrosine antibody. The expected 205 kDa band corresponding to KDR was observed in all treated samples (3 min of stimulation by Tat or VEGF shown).

antibody and immuno-blotting with the anti-KDR antibody confirmed that a single band corresponding to the 205 kDa KDR receptor after activation with either Tat or VEGF was present (Fig. 3).

Functional Activation of KDR in KS Cells

To establish a functional role for Tat activation of KDR, the ability of VEGF or HIV Tat to induce KS cell migration was tested in Boyden chemotaxis chambers. Primary KS cell cultures showed strong chemotaxis towards HIV-Tat (Fig. 4a, EKS shown). In a checkerboard-style assay, addition of VEGF in the upper chamber did not give a substantial increase in migration, indicating that VEGF is not chemokinetic for these cells. In contrast, addition of VEGF to the upper chamber strongly reduced migration to Tat in the lower chamber (Fig. 4a) further indicating that HIV-Tat uses the same receptors as VEGF in these cells.

Similar results were obtained for VEGF and HIV Tat induced IKS cell migration. KS-Imm cells responded strongly to both VEGF and Tat as a chemoattractants (Fig. 4b). Again, addition of VEGF in the upper chamber substantially inhibited migration to Tat in the lower chamber, suggesting that these two molecules share the same receptor on these cells.

Blocking of Tat and VEGF Induced Migration by Function-Blocking Anti-KDR Antibodies

A direct role for KDR in mediating the effects of Tat and VEGF on KS cells was demonstrated using a function-blocking anti-KDR chimeric antibody (c-p1C11) (30). The ability of KS cells to migrate in response to either of these stimuli in the presence of the function blocking anti-KDR antibody was evaluated. Addition of this anti-KDR antibody reduced both Tat and VEGF-induced KS cell migration (Fig. 4c) to that of background levels, indicating effective neutralization the activity of these chemoattractants. This result directly confirms that KDR mediates the effect of Tat on KS cells, acting as a Tat-responsive receptor.

DISCUSSION

Tat is a protein encoded by HIV that is critical for productive elongation of transcription of the HIV genome (31). Tat can also be released from HIV infected cells, and it appears to harbor diverse extracellular activities (14, 32). While there are several reports indicating that extracellular Tat can be internalized and translocated to the nucleus to activate host genes, many of the activities attributed to extracellular Tat appear to be mediated directly through activation of cell surface receptor(s) in the absence of internalization.

Previous studies have demonstrated that Tat acts as a heparin-binding angiogenic growth factor (15) and that flk-1/KDR is a high affinity Tat receptor on endothelial cells (16). Tat appears to mimic binding of the VEGF ligand to KDR, activating similar signal cascades and cellular responses in endothelial cells. This observation suggested that Tat could have a possible role as a pro-angiogenic factor that indirectly favors KS tumor growth. Subsequent studies demonstrated high levels of KDR in KS lesions, present both on vessels and on the spindle cells characteristic of KS (23, 24, 33). KDR expression may be induced by HHV8 infection (34). These observations show that the KS spindle cells themselves could respond to VEGF or Tat, suggesting that Tat could directly simulate KS expansion.

VEGF has been reported to be expressed at high levels by epidemic KS cells *in vitro* (23, 35). KS-Imm cells also have been found to express VEGF mRNA (M. Pratt in press). Our data demonstrate that IKS and SKS cells released levels of VEGF (approximately 200 pg/10^6 cells) similar to that of EKS cells. These data suggest that all forms of KS spindle cells in culture release similar levels of VEGF *in vitro*.

The observation that KS cells in culture release VEGF led Masood *et al.* (23) to hypothesize that an autocrine VEGF/KDR loop exists in KS lesions. However, using *in situ* hybridization Brown *et al.* observed VEGF expression by spindle cells in only in one of fifteen KS tumor biopsies tested (24). Further, the positive area of this biopsy was adjacent to an area of necrosis. Later studies using more sensitive *in situ*

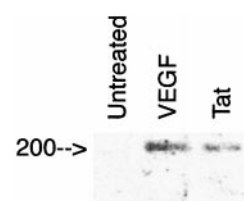

FIG 3. The IKS KS-Imm cells were treated with VEGF (10 ng/ml) or Tat (10 ng/ml) for 3 min, immunoprecipitated with an anti-KDR antibody and blotted with an anti-phosphotyrosine antibody. A unique band for corresponding to KDR was identified at the expected molecular weight.

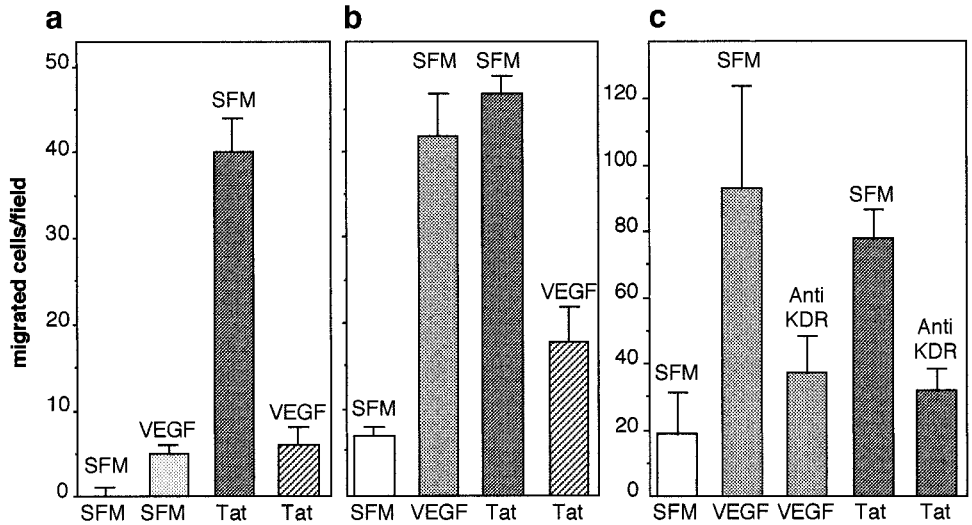

FIG. 4. Tat is chemotactic for KS cells. (a) Chemotaxis of EKS cells in response to Tat (10 nM). Addition of VEGF (2.5 nM) along with cells in the upper chamber showed little effect on cellular migration, but was able to block the ability of Tat to act as a chemoattractant. (b) Chemotaxis of IKS cells in response to Tat (10 nM) and VEGF (2.5 nM), again the addition of VEGF along with cells was able to abrogate the chemoattractant activity of Tat. (c) A neutralizing anti-KDR antibody strongly reduced the chemotactic response to either VEGF or Tat by IKS KS-Imm cells.

hybridization techniques showed that the spindle cells do express VEGF in KS lesions (5).

Endothelial cells in KS lesions, but not spindle cells, were found to express high levels of flt-1 (24), a VEGF receptor with a higher affinity for VEGF as compared to KDR (36). Although it has a higher affinity for VEGF, flt-1 appears to be principally involved in endothelial cell differentiation rather than in vessel formation (37). Through its higher affinity, flt-1 could sequester part or most of the VEGF produced within KS lesions. However, Tat has a limited ability to bind to or activate flt-1 (16). These observations suggest that VEGF available for activation of KDR in KS lesions *in vivo* may be limiting, while any Tat released within the lesion (38) in HIV infected patients would be free to bind and activate KDR on endothelial and KS spindle cells.

When compared to the sporadic and iatrogenic KS, the epidemic form is often more aggressive, involving not only the dermis but also gut, lungs and lymphonodes, and is often life-threatening (12). Like epidemic KS, iatrogenic KS also involves immune-suppression, this implies that an additional factor, linked to HIV infection, could explain the aggressiveness of epidemic KS. Our data demonstrate that functional KDR receptors are expressed by epidemic, sporadic and iatrogenic KS spindle cells. The endogenous ligand for this receptor, VEGF, appears to be present at limiting concentrations *in vivo*, while an exogenous ligand, Tat, would be free to bind and activate KDR in epidemic KS. The presence of Tat could explain the increased aggressiveness of epidemic KS when compared to other forms of KS.

Recently, Ganju *et al.* showed that the Tat-induced KDR signal activates several second messengers involved in cell adhesion, migration and gene transcription (22). Here we have shown that this signal evokes a specific chemotactic response in KS cells that is blocked by anti-KDR antibodies, indicating a functional and complete transduction of a Tat-induced, KDR-mediated signal. It is interesting to note that EKS, SKS and IKS cells are all responsive to Tat stimulation, this observation strongly suggests that Tat is not a primary cause of KS, but acts as an amplifier of KS.

These observations sustain a possible role of Tat in KS progression and indicate for the first time a general susceptibility of KS cells derived from different forms of this tumor to Tat activation.

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REFERENCES

1. Armes, J. (1989) *Adv. Cancer Res.* **53,** 73–87.
2. Boshoff, C., and Weiss, R. A. (1998) *Adv. Cancer Res.* **75,** 57–86.
3. Albini, A., Mitchell, C. D., Thompson, E. W., Seeman, R., Martin, G. R., Wittek, A. E., and Quinnan, G. V. (1988) *J. Cell. Biochem.* **36,** 369–376.
4. Bussolino, F., Arese, M., Montrucchio, G., Barra, L., Primo, L., Benelli, R., Sanavio, F., Aglietta, M., Ghigo, D., Pleszczynski,

M. R., Bosia, A., Albini, A., and Camussi, G. (1995) *J. Clin. Invest.* **96,** 940–952.

5. Cornali, E., Zietz, C., Benelli, R., Weninger, W., Masiello, L., Breier, G., Tschachler, E., Albini, A., and Stürzl, M. (1996) *Am. J. Pathol.* **149,** 1851–1869.
6. Ensoli, B., Nakamura, S., Salahuddin, S. Z., Biberfeld, P., Larsson, L., Beaver, B., Wong-Staal, F., and Gallo, R. C. (1989) *Science* **243,** 223–226.
7. Miles, S. A., Martinez-Maza, O., Rezai, A., Magpantay, L., Kishimoto, T., Nakamura, S., Radka, S. F., and Linsley, P. S. (1992) *Science* **255,** 1432–1434.
8. Naidu, Y. M., Rosen, E. M., Zitnick, R., Goldberg, I., Park, M., Naujokas, M., Polverini, P. J., and Nickoloff, B. J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5281–5285.
9. Roth, W. K. (1993) *Res. Virol.* **144,** 105–109.
10. Stürzl, M., Roth, W., Brockmeyer, N., Zietz, C., Speiser, B., and Hofschneider, P. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7046–7050.
11. Stürzl, M., Cornali, E., Gahlemann, C., Schreier, A., Benelli, R., Mantovani, A., Breier, G., and Zietz, C. (1996) *in* HIV and Cytokines (Guenounou, M., Ed.) INSERM Focus Serie, Paris.
12. Buchbinder, A., and Friedman-Kien, A. (1991) Clinical Aspects of Epidemic Kaposi's Sarcoma.
13. Bower, M., Fox, P., Fife, K., Gill, J., Nelson, M., and Gazzard, B. (1999) *AIDS* **13,** 2105–2111.
14. Noonan, D. M., and Albini, A. (2000) *Adv. Pharmacol.*, in press.
15. Albini, A., Benelli, R., Presta, M., Rusnati, M., Ziche, M., Rubartelli, A., Paglialunga, G., Bussolino, F., and Noonan, D. (1996) *Oncogene* **12,** 289–297.
16. Albini, A., Soldi, R., Giunciuglio, D., Giraudo, E., Benelli, R., Primo, L., Noonan, D., Salio, M., Camussi, G., Rockl, W., and Bussolino, F. (1996) *Nat. Med.* **2,** 1371–1375.
17. Vogel, B. E., Lee, S.-J., Hildebrand, A., Craig, W., Pierschbacher, M. D., Wong-Staal, F., and Ruoslahti, E. (1993) *J. Cell Biol.* **121,** 461–468.
18. Albini, A., Benelli, R., Giunciuglio, D., Cai, T., Mariani, G., Ferrini, S., and Noonan, D. M. (1998) *J. Biol. Chem.* **273,** 15895–15900.
19. Albini, A., Ferrini, S., Benelli, R., Sforzini, S., Giunciuglio, D., Aluigi, M. G., Proudfoot, A. E. I., Alouani, S., Wells, T. N. C., Mariani, G., Rabin, R. L., Farber, J. M., and Noonan, D. M. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 13153–13158.
20. Albini, A., Barillari, G., Benelli, R., Gallo, R. C., and Ensoli, B. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4838–4842.
21. Barillari, G., Gendelman, R., Gallo, R. C., and Ensoli, B. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 7941–7945.
22. Ganju, R. K., Munshi, N., Nair, B. C., Liu, Z. Y., Gill, P., and Groopman, J. E. (1998) *J. Virol.* **72,** 6131–6137.
23. Masood, R., Cai, J., Zheng, T., Smith, D. L., Naidu, Y., and Gill, P. S. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 979–984.
24. Brown, L. F., Tognazzi, K., Dvorak, H. F., and Harrist, T. J. (1996) *Am. J. Pathol.* **148,** 1065–1074.
25. Benelli, R., Albini, A., Parravicini, C., Carlone, S., Repetto, L., Tambussi, G., and Lazzarin, A. (1996) *Cancer Lett.* **100,** 125–132.
26. Benelli, R., Repetto, L., Carlone, S., Parravicini, C., and Albini, A. (1994) *Res. Virol.* **145,** 251–259.
27. Albini, A., Paglieri, I., Orengo, G., Carlone, S., Aluigi, M. G., DeMarchi, R., Matteucci, C., Mantovani, A., Carozzi, F., Donini, S., and Benelli, R. (1997) *AIDS* **11,** 713–721.
28. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
29. Postlethwaite, A. E., Snyderman, R., and Kang, A. H. (1976) *J. Exp. Med.* **144,** 1188.
30. Zhu, Z., Lu, D., Kotanides, H., Santiago, A., Jimenez, X., Simcox, T., Hicklin, D., Bohlen, P., and Witte, L. (1999) *Cancer Lett.* **136,** 203–213.
31. Jeang, K. T., Xiao, H., and Rich, E. A. (1999) *J. Biol. Chem.* **274,** 28837–28840.
32. Ensoli, B., Buonaguro, L., Barillari, G., Fiorelli, V., Gendelman, R., Morgan, R. A., Wingfield, P., and Gallo, R. C. (1993) *J. Virol.* **67,** 277–287.
33. Skobe, M., Brown, L. F., Tognazzi, K., Ganju, R. K., Dezube, B. J., Alitalo, K., and Detmar, M. (1999) *J. Invest. Dermatol.* **113,** 1047–1053.
34. Flore, O., Rafii, S., Ely, S., O'Leary, J. J., Hyjek, E. M., and Cesarman, E. (1998) *Nature* **394,** 588–592.
35. Samaniego, F., Markham, P. D., Gendelman, R., Watanabe, Y., Kao, V., Kowalski, K., Sonnabend, J. A., Pintus, A., Gallo, R. C., and Ensoli, B. (1998) *Am. J. Pathol.* **152,** 1433–1443.
36. Dvorak, H. F., Detmar, M., Claffey, K. P., Nagy, J. A., van de Water, L., and Senger, D. R. (1995) *Int. Arch. Allergy Immunol.* **107,** 233–235.
37. Fong, G.-H., Rossant, J., Gertsenstein, M., and Breitman, M. L. (1995) *Nature* **376,** 66–70.
38. Ensoli, B., Gendelman, R., Markham, P., Fiorelli, V., Colombini, S., Raffeld, M., Cafaro, A., Chang, H., Brady, J. N., and Gallo, R. C. (1994) *Nature* **371,** 674–680.