Regulation of Immune Activation/Retroviral Replication by CD8+ T Cells

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CD8+ T cells from humans infected with HIV and monkeys infected with simian immunodeficiency virus (SIV) have the ability to inhibit viral replication *in vitro*.[1,2] The exact mechanism of this inhibition remains to be elucidated. In light of the fact that viral replication is intimately linked to T cell activation, we hypothesized that CD8+ T cells may inhibit viral replication at the level of cellular activation. To test this hypothesis an EBV-transformed cell line from a naturally SIV-infected sooty mangabey monkey was transfected with a human CD4 gene (courtesy of Dr. R. Morgan NHLBI, NIH) and shown to be replication competent for SIV, HIV-1 and HIV-2. Autologous activated lymphocytes from the sooty mangabey monkey from which the cell line was derived were shown to have the ability to inhibit replication of all three of these lentiviruses as measured by reverse transcriptase activity (data not shown). Such findings suggest that the inhibition is not viral-type specific and is directed at a pathway common to all three of these diverse lentiviruses.

Next the EBV-transformed cell line was transiently transfected with the plasmid pU3RIII which contains an LTR-driven CAT reporter gene.[3] As seen in FIGURE 1 (lane a vs b), autologous activated lymphocytes are able to inhibit viral activity as measured by CAT activity. It should be noted that this inhibition does not appear to be secondary to the cytolysis of the transfected cells by the autologous lymphocytes. These data suggest that the inhibition of viral replication can occur at the level of transcription. Experiments utilizing a dual-chamber culture vessel separated by a semipermeable membrane were performed in order to determine whether this effect was mediated by a soluble factor(s). As seen in FIGURE 1 (lane a vs c) even when the lymphocytes are not in direct contact with the transfected cells the inhibition of viral replication as measured by CAT activity is apparent. However, it should be noted that in these experiments and in similar experiments measuring reverse transcriptase activity, the inhibition is quantita-

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tively less than when the effector cells are in direct contact with the transfected or infected targets.

It has been shown that cellularly derived nuclear binding factors have the ability to bind to the NFkB element of the LTR and activate viral replication.[4] It was of interest to determine whether such NFkB binding proteins were responsible for the activation of pU3RIII in our cell line. In an effort to address this issue, a pU3RIII plasmid with mutations in the NFkD binding motifs of the LTR (termed Kb-) was employed.[4] When the CD4 expressing EBV-transformed cell line was

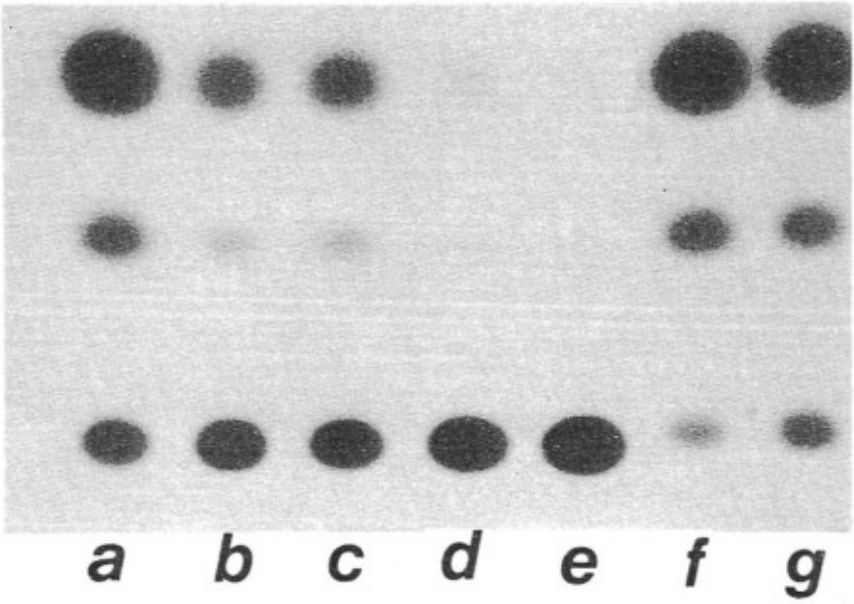

FIGURE 1. The CD4 expressing EBV-transformed cell line was transfected with the plasmid pU3RIII and aliquoted at 2×10^6 cells/well in a 6-well plate. The data represented in lane a show the CAT activity of the cells cultured alone. The data in *lane b* show the activity when 2×10^6 autologous activated lymphocytes were added to the well and *lane c* represents the CAT activity when 2×10^6 autologous activated lymphocytes were added to the well separated from the transfected cells by a semipermeable membrane. *Lanes d and e* represent CAT activity of cells transfected with the Kb- plasmid and cultured either alone (*lane d*) or with autologous activated lymphocytes (*lane e*). *Lanes f and g* represent CAT activity of cells co-transfected with the Kb- plasmid and a plasmid which constitutively expresses TAT. The transfected cells were cultured either alone (*lane f*) or with autologous activated lymphocytes (*lane g*).

transfected with the Kb- plasmid, no significant CAT activity was demonstrable (FIGURE 1, lanes d and e). On the other hand, when the cell line was co-transfected with the Kb- plasmid and a plasmid which constitutively expresses TAT (PIIIextatIII)—which can drive the Kb- plasmid in trans independently of the NFkB binding sites—there is marked CAT activity, thus demonstrating that the lack of activity seen in lanes d and e is not secondary to a defective plasmid. These data suggest that the majority of viral activation, as measured by CAT activity, in this model is dependent upon the binding of cellularly derived nuclear binding proteins to the NFkB elements of the LTR. As such, our data suggest that

CD8+ T cells have the ability to inhibit viral replication at the level of NFkB activation.

The model described herein involves EBV immortalized B cells and may not faithfully mimic HIV infection in T cells and monocytes. However, the mode of action of NFkB binding proteins in a variety of diverse cells is remarkably similar.[5] In T cells NFkB binding proteins have been implicated in the upregulation of the IL-2 receptor as well as the production of IL-2.[5] Since viral replication is so intimately linked to T cell activation, it is unclear as to whether CD8+ T cell inhibition of viral replication is primarily an antiviral or immunosuppressive response. Indeed it has been proposed that the downregulation of the immune response seen early on in HIV infection may represent active immunosuppression which plays a protective role by concomitantly inhibiting viral replication.[6] If such is the case, we hypothesize that this suppression may in fact be mediated by CD8+ T cells and may be directed at NFkB-mediated activation of infected cells.

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