have disclosed that nuclear factors from non-induced U937 cells bind to κB sequences in the HIV-LTR and stimulate transcription at least ten-fold[4]. In fact, the U937 cell line appears to be composed of at least two phenotypically different sublines, one of which expressed NF-κB in an inducible manner and a second in which NF-κB is constitutively expressed (anonymous referee, unpublished observations).

We suggest, therefore, that the correlation of NF-κB induction and transcriptional activation of the HIV-LTR with monocyte differentiation shown by Griffin *et al.* needs to be substantiated in normal promonocytes before any firm conclusions about monocyte differentiation, κB binding activity and induction of HIV gene expression can be drawn.

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GRIFFIN *ET AL.* REPLY—Hennighausen and Furth raise two issues regarding NF-κB binding in the monocyte lineage. We acknowledged that phenotypic differences among cell lines are of great importance; we carefully detailed the phenotype of our monocyte-like tumour cell lines. Our findings in the U937 line were also confirmed in the independent myelomonocytic cell line HL-60, representative of an earlier stem cell, which, when induced to differentiate to the monocyte lineage, also acquired NF-κB binding activity. So finding that NF-κB binding can be detected in some U937 sublines is thus not unexpected; it would be of interest to know the phenotypic characteristics of this cell line.

It is also important that we confirmed our findings in normal cells, showing that monocytes and macrophages of mouse and man contain NF-κB (see ref. 1, Fig. 2*b*). Thus there is no obvious discrepancy between mature U937, HL-60 or other macrophage leukaemia cells in tissue culture and normal monocytes and macrophages *in vivo*. We agree with Hennighausen and Furth that it would also be of interest to analyse NF-κB binding in promonocytic stem cells, but these are rare and not amenable at present to further study.

Several factors can affect the detection of NF-κB binding in cultured cells. Cells not maintained in log phase, or which are contaminated by mycoplasma, may acquire NF-κB binding activity, possibly by differentiation. Such conditions could contribute to the detection of NF-κB binding in the uninduced U937 cells of Lubon *et al.*[4] and could also explain their observation of NF-κB binding in uninduced HeLa cells, reported previously not to contain this factor[5,6]. Another study has reported findings similar to our own[3]. The degree of DNase protection seen for U937 by Wu *et al.* was low, and its NF-κB binding activity increased markedly on PMA stimulation, consistent with our observations.

Further studies of normal haematopoietic cells will undoubtedly help to validate our model based on studies of leukaemia lines and normal monocytes and macrophages. Several studies have indicated that HIV infects hematopoietic progenitors[7,8]. Further studies of the role of NF-κB in normal promonocyte differentiation and HIV infection will await more sensitive NF-κB probes and the ability to maintain such promonocytic stem cells in continuous culture.

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A chink in HIV's armour?

SIR—Elegant work has established that the human immunodeficiency virus (HIV) infects susceptible cells following interactions between the viral envelope glycoprotein (gp120) and a cell-surface receptor (CD4) (refs 1,2). However, other factors must play a role to explain, for example, why mouse cell lines expressing human CD4 on their cell surfaces are not susceptible to infection. In addition, neutralizing anti-bodies to the V3 loop of gp120 prevent infection even though they do not inhibit binding to CD4. A recent paper by Hattori *et al.*[3] and results emerging from our own laboratory may shed some light on these other factors.

During the development at Celltech of a process for the production of recombinant HIV-1 gp120 from Chinese hamster ovary cells for the MRC Aids Directed Programme, we have identified a specific proteolytic cleavage event of the gp120 molecule under certain cell culture conditions. Cleavage becomes apparent only after SDS-PAGE of the purified protein under reducing conditions, indicating that the two cleavage products, relative molecular mass 70,000 (70K) and 50K, are held together by a reducible disulphide bridge. Amino-terminal sequences analysis of the 50K fragment indicated that cleavage occurs between residues 315 and 316 of the intact protein. This corresponds to the arginine and alanine residues at the tip of the V3 loop, a region previously demonstrated to be one of the major sites on gp120 able to elicit neutralizing antibodies[4]. The cleaved gp120 is still able to bind CD4 with an affinity similar to that of uncleaved material, but neutralizing antibodies directed at the V3 loop are unable to bind cleaved gp120.

Hattori *et al.* have recently shown that trypstatin, a new Kunitz-type protease inhibitor purified from rat mast cells, inhibits the formation of syncytia in HIV-1 infected cultures of CD4 T cells[3]. As trypstatin has a similar sequence to the V3 loop of gp120 and as a synthetic peptide corresponding to the loop both inhibit protease activity and prevents syncytia formation, it may be that cleavage of this loop is essential for viral infection. Interestingly, it is predicted that the reactive site of trypstatin is the same as the arginine-alanine site that we have found is cleaved during gp120 production. Experiments are now in progress at Celltech to determine whether this specific cleavage of the V3 loop is a prerequisite for viral infection.

The requirement for cleavage in the V3 loop may explain the inhibitory effect of neutralizing antibodies which bind this region of the protein. Presumably, the cleavage is required to bring about a conformational change necessary for the fusion of viral and cell membranes that follows binding of virus to CD4. Neutralizing antibodies may either prevent cleavage of the protein or block the conformational change that occurs upon cleavage and in this way abolish infectivity. Should murine fibroblasts lack the necessary protease activity, it would explain why they are not susceptible to HIV infection even after transfection with human CD4.

Clearly these observations indicate another possible chink in the HIV-1 armour which could lead to therapeutics for the control of AIDS, but the significance of the gp120 cleavage we here observed still needs to be established.

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