HIV suppression by interleukin-16

SIR — In addition to their function as cytotoxic T cells, lymphocytes carrying the CD8 receptor ($CD8^+$) secrete one or more soluble factors which inhibit replication of HIV and SIV (human and simian immunodeficiency viruses, respectively) in primary $CD4^+$ cells[1,2]. This antiviral activity is positively correlated with the health of HIV-infected patients, and is high during the asymptomatic stages of infection. Old World monkeys such as the African green monkey *Cercopithecus aethiops*, which are naturally infected with SIV in the wild, possess high immunodeficiency virus-suppressing lymphokine (ISL) activity[2], a low viral load[3], and never develop simian AIDS[4].

Here we show that the previously identified lymphocyte chemoattractant, interleukin-16 (IL-16), which is secreted from activated $CD8^+$ cells and binds to T cells through the CD4 receptor[5,6], suppresses the replication of HIV and SIV. The IL-16 from African green monkeys is highly homologous to its human counterpart. It may therefore contribute to the low viral load in healthy HIV-infected patients and naturally infected African green monkeys.

The IL-16 molecules isolated from African green monkeys and humans (Fig. 1) are proteins of 130 amino acids and relative molecular mass around 13,500. The genes encoding human (HU) and African green monkey (AGM) IL-16 differ in 16 of their 390 coding nucleotides, resulting in 7 non-clustered amino-acid changes.

On analysis by gel filtration, recombinant soluble $IL\text{-}16_{HU}$ and $IL\text{-}16_{AGM}$ elute predominantly as homodimers (apparent M_r 28,000), with minor peaks corresponding to monomeric and tetrameric forms. The lymphocyte chemoattractant activity of IL-16 was previously linked to its tetramer formation[5], so it is possible that a proportion of the $IL\text{-}16_{HU}$ and $IL\text{-}16_{AGM}$ preparations contain incorrectly folded protein. Dimerization of the human IL-16 is partially due to disulphide bridging between the cysteine residues at position 31, which is replaced by tyrosine in $IL\text{-}16_{AGM}$. Neither thrombin treatment to remove the histidine tag of the fusion proteins nor changes in induction by isopropyl-β-D-thiogalactoside or bacterial growth temperature abolish the high degree of dimerization (data not shown). Higher concentrations of $IL\text{-}16_{HU}$ than of $IL\text{-}16_{AGM}$ are needed to achieve inhibition of HIV replication (Fig. 2). Preliminary data suggest that the apparent differences in activity between $IL\text{-}16_{HU}$ and $IL\text{-}16_{AGM}$ are due to different folding characteristics of the bacterially derived proteins.

Although IL-16 has been shown to induce signal transduction[5,7], the precise way in which it inhibits HIV replication is unknown. Within the concentration range tested, IL-16 does not impair cell viability as judged by trypan blue exclusion and cell number determination. It is known to bind to the CD4 receptor, and so may resemble certain anti-CD4 antibodies that inhibit transcription driven by the long terminal repeats of HIV without affecting initial binding by the virus[8]. Further investigation is needed to determine what fraction of the ISL activity is due to IL-16, but it has not escaped our attention that IL-16 may possess an antiviral therapeutic effect.

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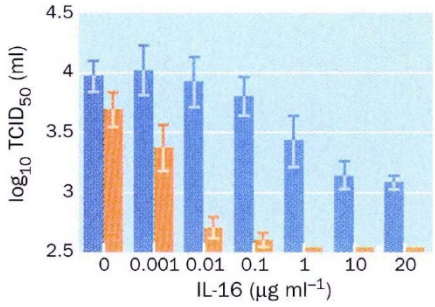

FIG. 2 Inhibition of HIV-1 replication by human (blue columns) and simian (pink columns) recombinant IL-16 (rIL-16) on $CD8^+$-depleted PBMCs. $CD8^+$-depleted PBMCs (1.5×10^6) stimulated with phytohaemagglutinin ($CD8^+$ cells <5%, by fluorescence-activated cell sorting) were plated in RPMI 1640 medium supplemented with 20% FCS, 2 mM glutamine and 180 U ml^{-1} IL-2 into 96-well plates and infected with 50 $TCID_{50}$ (the 50% tissue-culture infectious dose) of PBMC-grown HIV-1_{SF2} for 1 h. Unbound virus was removed, and cells were replated in culture medium containing IL-16. At 8, 10 and 12 days post-infection, virus content of cell-culture supernatants was determined by titration on the highly HIV-1_{SF2}-susceptible cell line MT-4 (NIH AIDS Research and Reference Reagent Program). $TCID_{50}$ was calculated according to Spearman/Kärber. The result of titrations of quadruplicates 10 days post-infection are shown.

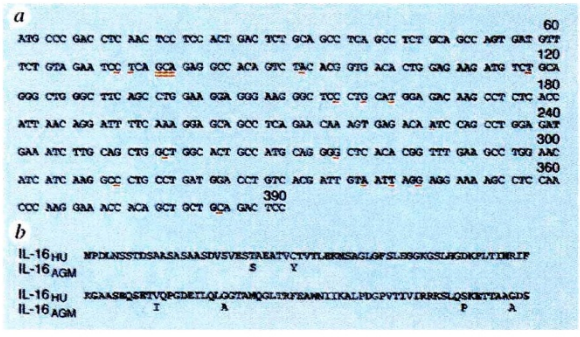

FIG. 1 *a*, Nucleotide sequence of $IL\text{-}16_{AGM}$. Differences from the human IL-16 sequence are underlined. Codon 26, which is deleted in some of the human and African green monkey IL-16 alleles, is marked by a double line. *b*, Alignment of the deduced amino-acid sequences of $IL\text{-}16_{HU}$ and $IL\text{-}16_{AGM}$. Peripheral blood mononuclear cells (PBMCs; 5×10^6) were cultivated with 10 μg ml^{-1} concanavalin A and IL-2 for 48 h. Total RNA was prepared and a cDNA synthesis was performed in a standard polymerase chain reaction. Purified products were cloned for nucleotide sequence confirmation and protein expression into the vector pET15b (Novagen). Expressed IL-16 was obtained by Ni^{2+}–NTA agarose (Qiagen) chromatography to >95% purity.

Tetrodotoxin as a pheromone

SIR — Tetrodotoxin (TTX) is one of the most potent marine toxins[1], and so one possible physiological role is as a defence agent[2,3]. Here I report another function of TTX, as a male-attracting pheromone at the time of spawning.

Measurement of attracting activity of the puffer fish *Fugu niphobles* against TTX was conducted by using a Y-maze

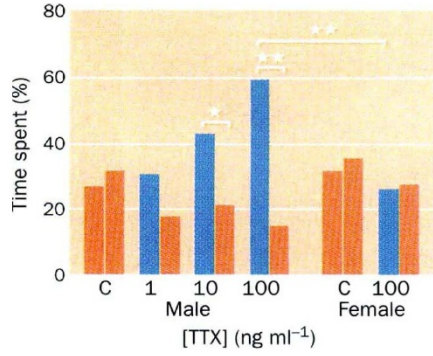

FIG. 1 Preference activities of *F. niphobles*, against TTX. Results represent medians of eight males and females in each concentration. $*P<0.05$; $**P<0.01$ when compared with control reactions. The preference reactions of spermating males and sexually mature females were tested by the Y-maze flow-through system[4] operated after a fish was placed in the starting point at the centre of Y maze. Artificial sea water was introduced into both arm inlets of the maze (dimensions $45 \times 8 \times 8$ cm) with a flow of 2,000 ml min^{-1}. The TTX solution and control distilled water were introduced into the arm channel in each inlet with a constant flow of 1.0 ml min^{-1}. The percentage of the spent period (5 min) for the test fish in each channel was analysed statistically with Friedman's two-way analysis of variance, followed by Wilcoxon's signed-rank test or the Mann–Whitney *U*-test. Blue columns, TTX; pink columns, distilled water.