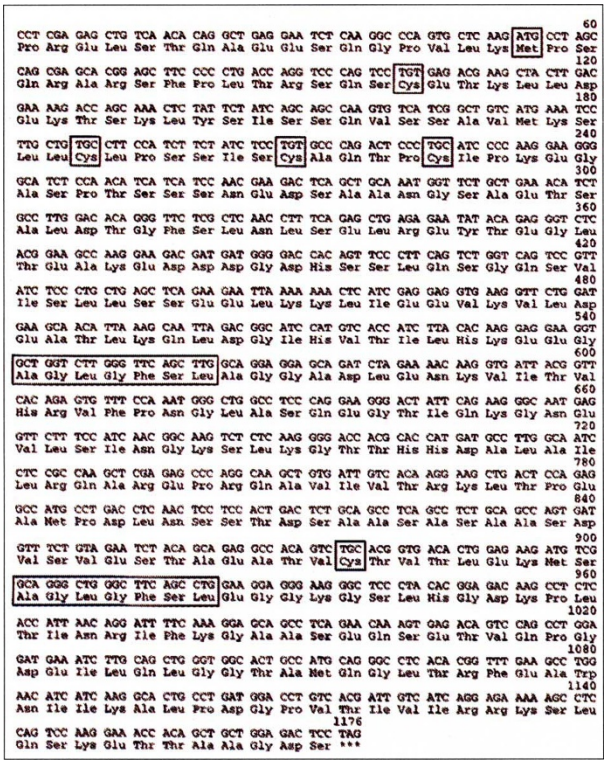

The 1,173 base pairs in the human IL-16 cDNA. The first Met residue, the five Cys and the GLCF-motif-like repeats are boxed. Dots above corresponding proteins indicate the three nucleotides not present in the previously published sequence[5]. Details of our sequence determination are available on request.

protein (encompassing the DHR3 module of the 40,000-M_r LCF) to CD4, causing some activating events and cytoskeletal reorganization. This could explain the upregulation of interleukin-2 receptors, and the general increase in $CD4^+$ cell motility[2–5,11,12]; antibody blocking of CD4 would diminish these effects. Moreover, blockade of CD4 by LCF/IL-16 binding (even if this were a nonspecific event) could inhibit HIV entry into these cells, perhaps explaining the recent observations of Baier *et al.*[1]. Although this does not necessarily detract from the potential therapeutic use of this polypeptide, the notion that it represents an endogenous cytokine[5] useful as a natural HIV inhibitor[1] must be scrutinized further.

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SIR — Cruikshank *et al.*[5] described interleukin-16 as a 130-residue protein encoded by one main open reading frame of a 2,150-base-pair-long complementary DNA. A biologically active protein is expressed by this DNA even when it has a truncated 5′ end (as originally cloned)[5], indicating that one functional domain of interleukin-16 is in a 390-base-pair coding region. A 130-amino-acid fragment derived from *Escherichia coli* based on this sequence is active as a lymphocyte chemoattractant[5] and as an inhibitor of HIV replication[1].

Nevertheless, our nucleotide sequence comparison of interleukin (IL)-16 cDNA clones from non-human primates reveals that the previously postulated ATG start codon[5] is mutated in two out of six species tested — saimiri and aotus monkeys. Because evolutionarily highly conserved genes probably use the same ATG, we decided to reanalyse the published 5′-untranslated region[5]. We found one large 1,173-bp open reading frame, which includes at its 3′ end the 390 bp of the original IL-16 sequence[5] (see figure).

The first ATG in this open reading frame at position 52 is probably where translation starts[14], and would therefore represent the most likely start codon of this putative IL-16 precursor (prIL-16), with a predicted relative molecular mass of 39,358. This protein is detectable in correspondingly transfected COS-7 cells with a molecular mass of about 42,000 (M.B. *et al.*, manuscript in preparation).

The prIL-16 sequence contains five cysteine residues and two repeats, which resemble GLGF motifs[15] (see figure). Sequence database searches reveal no significant homologies between the prIL-16 sequence and that of other proteins (except those that carry the GLGF motif). As for the originally published IL-16, there is no consensus signal sequence in prIL-16 (also missing, for example, in IL-1α and -β) and the secretion pathway for prIL-16 or its processed products is unknown.

Both the 130-amino-acid and the naturally purified versions of IL-16 have a relative molecular mass of 17,000 as measured by SDS gels[5]. This method would not exclude small size differences between the proteins. Although the primary structure of prIL-16 does not resemble a secreted protein, it is conceivable that processing of prIL-16 could lead to the 17,000 form of IL-16 that is detectable in cell culture supernatants of histamine- or mitogen-induced $CD8^+$ cells[16]. Knowledge of the precise amino terminus of naturally processed and secreted IL-16 is important for investigating its chemoattractant and anti-HIV activities, as altered amino termini can be critical for the biological properties of proteins. Processing of the native prIL-16 may well be essential for generating properly folded IL-16, which is difficult to obtain in *E. coli* expression systems[1]. We are now investigating the processing of prIL-16 and the properties of both prIL-16 and the carboxy-terminal domain of IL-16 from primary lymphocytes to elucidate the biological functions of this protein.

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Three-dimensionally preserved insects

SIR — The Tertiary limestones of Riversleigh (northwest Queensland) have yielded an assemblage of beetles (including larvae), flies and millipedes preserved in three dimensions, in addition to the already diverse range of vertebrates so far reported[1]. These arthropods were recovered from the Upper Site (Late Oligocene/ Early Miocene), which represents a small, shallow, lime-rich pool in a tropical rainforest[1]. Scavengers were inhibited by the high salinity, and the conditions for exceptional preservation were enhanced by microbial mats[1,2]. The arthropods are preserved by mineralization in calcium phosphate (carbonate fluorapatite) without collapse or compaction. They were recovered by acid digestion of the vertebrate-packed limestone; they are preserved in remarkable detail, particularly of the eyes. The only other well-known three-dimensionally phosphatized terrestrial arthropods are from the slightly older Quercy Phosphorites of France, but these specimens have been diagenetically recrystallized or encrusted[3].

Replication of soft tissues is more rapid in calcium phosphate than in any other authigenic mineral, and therefore preserves the highest fidelity of detail[4–6]. The

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