The findings of the mRNA analyses described above by Kovanen et al. (1) agree with our previous conclusion, based on phylogenetic analysis (2), that the reported M15530 cDNA (3) does not encode 12-kD BCGF. In their answer to our 1994 comment, Sharma et al. (4) proposed that the 12-kD BCGF was encoded by the originally reported cDNA, but in another reading frame starting from an AUG codon at residue 7 of the M15530 sequence. However, our phylogenetic indications indicated that the newly proposed ORF not only had different lengths in all primates analyzed (121, 135, 59, 53, 71, and 71 amino acids in human, chimpanzee, gorilla, gibbon, baboon, and macaque, respectively) but also, as a result of frame shifts found only in certain species, differed in the amino acid composition. For example, the chimpanzee ORF was identical with the human for the first 101 codons, while in gorilla, gibbon, baboon, and macaque, a single A insertion changed the ORF after the first 45 codons in gorilla and gibbon, and after the first 47 in baboon and macaque. In the haploid human genome, a 120-codon-long ORF following an ATG codon would occur more than 10^5 times by chance alone, assuming an equal probability of all codons. Although our findings (2) do not formally eliminate the M15530 as 12-kD BCGF coding sequence, they seriously call into question its functional nature, especially with no further evidence for the presence of transcriptional, translational, or RNA processing signals suggesting that the M15530 sequence was actively expressed.

Our criticism is supported by the work of Kovanen et al. (1). With the use of sensitive assays, they do not detect M15530-specific mRNA in cells known to express 12-kD BCGF. In agreement with these researchers, our search of the EST database (a total of 460,000 fragments of human mRNA sequences) using the BLASTN program resulted in no single relevant hit with their probe 2 sequence, and numerous (over 3000) hits with the Alu-containing probe 1 sequence. This is not unexpected because over 5% of human mRNAs contain an Alu sequence in their untranslated regions (5). Although Alu elements may in some cases provide the sequence for functional elements in proteins (6, 7), the finding of Alu-related sequences in the coding regions of cDNAs raises the possibility of artifact and should be treated with caution (8).

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Response: The comment by Kovanen et al. accurately describes both the biological and molecular characteristics of a cDNA previously reported to putatively represent low-molecular-weight BCGF (12-kD BCGF) (1). The apparent verification of sequence by Southern analysis and confirmation of biological activity of the translated protein (2) substantiates several of the original observations, yet particular conundrums remain unresolved. The apparent inability of the Alu-free cDNA to hybridize to mRNA from activated human peripheral blood mononuclear cells brings forth the question of whether this cDNA accurately encodes the 12kD B cell proliferative activity previously isolated from normal activated human T lymphocytes and detected by distinct B cell proliferative assays. The sequence underlying the translation of an apparently functional B cell proliferative protein warrants investigation. However, it is evident that the presence of the Alu-encoding sequences may have led to the data originally compiled. For these reasons, we concur with the observations reported by Kovanen et al. and believe that the cDNA appropriately associated with 12-kD BCGF activity remains to be determined.

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Surendra Sharma, Shashi Mehta, John Morgan and Abby Maizel

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