Streptomycin and self-splicing

SIR—The aminoglycoside antibiotic streptomycin, which interferes with prokaryotic protein synthesis by interacting with the ribosomal RNA, is also able to interact with the RNA of an autocatalytic group I intron. It is likely to do so by competing with the substrate guanosine via the guanidino group, which guanosine and streptomycin have in common.

Group I introns, like the *Tetrahymena thermophila* rRNA and the T4 phage *td* introns, have the ability to undergo self-splicing *in vitro* by a two-step reaction[1,2]. The first step requires binding of the substrate guanosine, which becomes covalently bound to the first nucleotide of the intron after cleavage of the 5′ splice site, and the second involves cleavage at the 3′ splice site and ligation of the exons. The cofactor guanosine binds to the ribozyme by hydrogen bonds using the guanidino group[3]. This guanosine-binding site has been partially assigned to the conserved G·C base pair in the P7 helix (G264–C311 in the *Tetrahymena* intron)[4].

Streptomycin contains two guanidino groups that could interfere with guanosine binding. We performed splicing reactions *in vitro* with increasing amounts of streptomycin and found that it can completely inhibit the splicing reaction at a concentration of 10 mM. Half-maximal inhibition was achieved at 5 mM, with guanosine at its K_m concentration. Increasing the guanosine concentration restored splicing, suggesting competitive inhibition. The same results were obtained with the *Tetrahymena* rRNA intron. Similar competition with guanosine has been described for arginine, which also contains a guanidino group[5].

Streptomycin directly interacts with the 16S rRNA of *Escherichia coli* [6] and interferes with translation initiation and proofreading in prokaryotic protein synthesis[7]. Both translation initiation and proofreading involve GTP binding and hydrolysis; it is not known if streptomycin interferes with GTP binding during these processes. Elucidating the mechanism of splicing inhibition by streptomycin may reveal how this antibiotic acts on the prokaryotic ribosome.

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Kaposi's sarcoma and AIDS

SIR—The role of human immunodeficiency virus type 1 (HIV-1) in the pathogenesis of Kaposi's sarcoma is thought to be indirect. HIV-1 sequences are absent in DNA from cultured Kaposi's sarcoma-derived cells[1], and transgenic mice carrying the *tat* gene and developing Kaposi's-like lesions express *tat* in their skin but not in the tumour cells[2]. In a recent Letter[3], Ensoli *et al.* reported that the *tat* protein of HIV-1 is released extracellularly from HIV-1-infected cells *in vitro* and stimulates the growth of cells derived from Kaposi's lesions of AIDS patients[3]. The same growth-promoting properties were observed with recombinant *tat*, and were inhibited by anti-*tat* antibodies, independent of the source of *tat*[3]. These findings indicate that *tat* released from HIV-1-infected cells may contribute to the induction of Kaposi's in HIV-1-infected individuals. We therefore compared the prevalence of anti-*tat* antibodies in patients with AIDS whose AIDS-defining diagnosis did or did not include Kaposi's.

We assayed sera obtained and stored from 297 HIV-1 seropositive patients at the time of or within one month of diagnosis of AIDS (according to Centers for Disease Control criteria) for anti-*tat* antibodies with a previously described enzyme immunoassay using *Escherichia coli*-produced *tat* as antigen[4]. The group with Kaposi's consisted of patients with a sole diagnosis of Kaposi's ($n = 67$), as well as of patients in whom AIDS was diagnosed because of the concurrent presence of Kaposi's and an AIDS-defining opportunistic infection ($n = 11$). The group without Kaposi's consisted of people presenting with an AIDS-defining opportunistic infection ($n = 203$, including two patients with concurrent non-Hodgkin's lymphoma), but also included patients who were diagnosed with AIDS solely on the basis of a non-Hodgkin's lymphoma ($n = 12$) or AIDS-dementia complex ($n = 4$).

TAT-SPECIFIC ANTIBODIES IN AIDS

AIDS-defining condition	Kaposi's sarcoma	
	present	absent
	No. of patients(%)	
Anti-*tat* positive	10 (13)	21 (10)
Anti-*tat* negative	68 (87)	198 (90)
Total	78 (100)	219 (100)

The *tat*-specific antibody response was determined in serum obtained within one month of diagnosis of AIDS

In both groups, the prevalence of anti-*tat* antibodies was low (13 versus 10 per cent), without significant difference between them (Fisher's exact test $P = 0.27$; see table). Evidently, HIV-1 infection in Kaposi's patients does not increase the antigenicity of *tat* relative to the infection in non-Kaposi's patients, pleading against overexpression of *tat* in Kaposi's patients. Also, the presence of anti-*tat* antibodies in 10 of the 78 Kaposi's patients clearly did not protect them from developing Kaposi's. Our data indicate that no evidence can be put forward for either high antigenicity of *tat* or a protective effect of anti-*tat* antibodies in patients with AIDS-related Kaposi's sarcoma.

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ENSOLI AND GALLO REPLY—Reiss *et al.* describe data on anti-*tat* antibodies in HIV-1-infected individuals with or without Kaposi's sarcoma which, in their opinion, are sufficient to indicate "that no evidence can be put forward for either high antigenicity of *tat* or a protective effect of anti-*tat* antibodies in patients with AIDS-related Kaposi's." These conclusions are based on the detection of anti-*tat* antibodies in 13 per cent of kaposi's patients with AIDS versus 10 per cent of AIDS patients without kaposi's. In our opinion, these data are not sufficient to support any conclusions about the role of *tat* in Kaposi's for the following reasons.

(1) Antibodies directed against HIV-1 regulatory proteins may change in titre and disappear during HIV-1 infection[5]. Therefore, longitudinal studies are necessary for evaluating their significance.

(2) Antibodies against HIV-1 regulatory proteins are relatively weak compared with other HIV-1 proteins, indicating that these proteins may be poor antigens *in vivo*[5] or that they are present in low amounts.

(3) Reiss *et al.* do not show whether anti-*tat* antibodies detected in AIDS patients or in earlier stages of the disease, are neutralizing the biological activities of *tat*[3,6]. Therefore, the conclusion that they cannot prevent the occurrence of Kaposi's in HIV-1 infected individuals is still open.

(4) Reiss *et al.* do not show data concerning the presence of *tat* in the sera of the same patients. The two phenomena (protein and antibodies to them) may not be parallel and both may depend on the stage of the disease, the rate of infection