

must be considered latent. However, as has been the case for most patients with mycosis fungoides, virus particles emerged from his PBMC after short-term culture. In contrast to the white blood cells obtained from patients with mycosis fungoides [2], this patient's PBMC did not become immortalized on three different occasions. This speaks against the existence of ongoing transformation in the lymphoid lineage.

The above considerations are offered in support of the hypothesis that, whatever the etiology of KS may be, overt or subtle immune dysregulation may be necessary for it to become clinically manifest. The patient reported here has changed his sex practices and has not had a recurrence of KS to date.

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False-Positive ELISA for Human Immunodeficiency Virus after Influenza Vaccination

Colleagues—In December 1991, federal health officials reported an apparent association between influenza vaccination and false-positive results of ELISA for serum human immunodeficiency virus (HIV) antibody [1]. To assess this putative relationship, 167 subjects received trivalent influenza vaccine for the 1991-1992 season (Wyeth, Philadelphia); serum samples were collected before and 3 and 6 weeks after vaccination. Specific serum antibody against influenza A/Beijing and B/Panama, two components of the vaccine, were quantified by ELISA, and sera were screened for HIV antibody using commercial ELISA kits.

Informed consent was obtained and human experimentation guidelines of the institutional review board were followed.

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With the RECOMBIGEN HIV-1 immunoassay (Cambridge BioScience, Worcester, MA), all samples were negative. With the Vironostika HIV-1 Microelisa System (Organon Teknika, Durham, NC), apparent HIV antibody levels in 6 (4%) of 167 subjects exceeded the manufacturer's cutoff value. The specificity reported in the package insert was estimated to be 99.96%. Two subjects were positive in sera collected 3 weeks after vacci-

Table 1. Relationship between levels of serum antibody against influenza virus and HIV.

	Interval (weeks) since vaccination	r_s	P
Influenza A/Beijing vs. HIV	3	.165	<.05
	6	.121	NS
Influenza B/Panama vs. HIV	3	.251	<.002
	6	.164	<.05

NOTE. r_s , Spearman rank correlation coefficient; $n = 167$ for 3-week samples and 165 for 6-week samples. NS, not significant.

nation, 1 subject at 6 weeks, 2 subjects at 3 and 6 weeks, and 1 subject at baseline and 3 and 6 weeks; all had negative Western blots.

The relationship between levels of serum antibody against influenza virus and HIV was assessed by Spearman rank correlation (table 1). There was only a modest relationship between influenza A/Beijing antibody levels and HIV antibody at 3 weeks. In contrast, a significant association between serum antibody against influenza B/Panama and HIV antibody was apparent at 3 weeks. This relationship was weaker but still significant at 6 weeks. These observations support the hypothesis that false-

positive HIV ELISA results during the 1991–1992 influenza season were related to postvaccination levels of serum antibody, particularly against influenza B/Panama.

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Localization of Priming Epitope to the C-Terminal Portion of Hepatitis A Virus VP1

Colleagues—The presence of only a single antigenic serotype makes hepatitis A virus (HAV) an attractive target for the development of a subunit vaccine. Of possible relevance to the development of such a vaccine are several reports [1–3] that, following immunization with HAV capsid proteins, animals were primed to respond rapidly with neutralizing antibodies upon challenge with a subimmunogenic dose of whole virus. These reports suggested there was a T cell response to the recombinant proteins. It is not known whether T cell immunization with recombinant capsid proteins might be protective in primates. Thus, the role of T cells in HAV immunity needs further study.

In previous studies, immunization of rabbits with VP1-containing proteins was shown to prime the animals for a rapid neutralizing antibody response. To localize the putative T cell epitope(s), we prepared recombinant HAV proteins representing either the N- or C-terminal halves of VP1 and tested each antigen separately for its ability to mimic the intact VP1 priming activity. pATH-VP1-N contained a segment of cDNA [1] from the *Hind*III site in VP3 (nucleotide [nt] 2065) to the *Bgl*II site in VP1 (nt 2625), resulting in an expression plasmid containing coding sequences for the C-terminal 45 amino acids of HAV VP3 and the N-terminal 144 amino acids (~48%) of VP1. pATH-VP1-C contained a segment of cDNA from the *Bgl*II site in VP1 (nt 2625) to a *Bsm*I site in 2A (nt 3112) and resulted in a construct containing HAV coding sequences for the C-terminal 156 amino acids of VP1 (~52%) and the N-terminal 4 amino acids of 2A. Both HAV sequences were fused to a portion of the *Escherichia coli* TrpE protein, driven by the *trp* promoter; pATH-VP1-C contained a deletion of most of the TrpE coding sequence to reduce its contribution to the immunogenicity of the fusion protein.

The proteins were expressed in *E. coli* as previously described [1], and HAV proteins were identified by analyzing bacterial lysates by SDS-PAGE. Unique proteins of the predicted molecular weight were observed by Coomassie blue staining and by immunoblot analyses of proteins transferred to nitrocellulose and probed with rabbit anti-TrpE/HAV VP1 antisera [1] and anti-HAV convalescent human sera known to contain HAV-specific antibody (data not shown). Rabbits were immunized with proteins in the pellet fraction after sonication and centrifugation of a bacterial culture. Each antigen was used to immunize 2 rabbits.

Antisera raised to each of the recombinant HAV partial VP1 proteins reacted similarly with denatured VP1 by immunoblot (not shown). None of these antisera, however, was able to bind to intact hepatitis A virions, measured by a commercial EIA (HAVAB; Abbott, Abbott Park, IL) (data not shown). To increase sensitivity, we used a modified HAVAB protocol in which the competitive ratio of test sample to standard HAV antiserum was reduced from the normal HAVAB ratio of 1:20 to 1:1.

To determine the priming activity of the recombinant partial VP1 proteins, rabbits immunized previously with the recombinant partial VP1 proteins were challenged with a single subimmunogenic dose of formalin-treated HAV vaccine (provided by L. Binn, Walter Reed Army Institute of Research, Washington, DC). The interval between the initial immunization and the challenge was 9–18 months. The challenge dose chosen (~1 ng) was shown not to elicit any detectable antiviral antibody response in control animals injected at the same time. All rabbits were bled every 3–4 days after challenge, and the resulting sera were analyzed for the presence of antibodies that either bound intact virus (by HAVAB EIA) or neutralized virus infectivity by radioimmunoassay inhibition test [4]. Figure 1A shows the results of a HAVAB test for the presence of anti-HAV antibody. Rabbits immunized with the C-terminal portion of VP1 developed anti-HAV antibody within 8 days after challenge, and remained HAVAB-positive for ≥37 days, during which samples were taken. Rabbits immunized with the N-terminal half of VP1 developed no anti-HAV antibody. Control rabbits received only the subimmunogenic challenge dose without prior immunization and developed no detectable antibody. For comparison, the results of a previous experiment that demonstrated priming activity of intact VP1 is plotted on the same graph. The immunogen used in this experiment was prepared from Sf9 cells in-

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