Jpn. J. Infect. Dis., 53, 2000

Laboratory and Epidemiology Communications

Confined Replication of a Chimeric Simian Immunodeficiency Virus

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Communicated by Hiroshi Yoshikura

(Accepted November 2, 2000)

During the acute phase of infection with lentiviruses such as human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus (SIV), virus-specific cellular immune responses play an important role in controlling virus replication (1-3). DNA vaccine is a promising candidate for AIDS vaccine strategies to induce the virus-specific cellular immune responses efficiently. Conventional DNA vaccine strategies, however, have failed to prevent the onset of disease (4).

We previously reported a novel DNA vaccine strategy to induce safer, restricted replication of an avirulent virus (5). We generated a chimeric SIV, FMSIV, by replacing SIV *env* with ecotropic Friend murine leukemia virus (FMLV) *env* to confine its replication to FMLV receptor (mCAT1)-expressing cells. In primate cells lacking mCAT1, FMSIV did not replicate unless mCAT1 was introduced exogenously. Vaccination to macaques with both the FMSIV DNA and the mCAT1-expression plasmid DNA induced mCAT1-dependent FMSIV replication. This strategy elicited SIV-specific cellular immune responses and resistance against pathogenic SIVmac239 challenge more efficiently than did a conventional replication-negative DNA vaccine. mCAT1-dependent FMSIV replication would be safer than live attenuated virus replication. In our previous in vitro study, SIV Gag p27 production in

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supernatant was observed not in the mock-transfected but in the mCAT1 DNA-transfected COS cells after FMSIV infection, indicating the occurrence of mCAT1-dependent FMSIV replication. This study was undertaken in order to confirm that the FMSIV replication was confined within the mCAT1-expressing cells.

For preparation of viral supernatants, COS cells were transfected with SHIV-MD14YE (6) or FMSIV DNA. Two days later, the supernatants were harvested and filtered through a 0.45-μm-pore-size filter to obtain the SHIV or FMSIV viral supernatant. For infection, COS cells were plated at a density of 2×10^5 per well in 6-well plates, incubated overnight, and transfected with 2 μg of mock plasmid vector (pCMVN [5]) or 2 μg of pmCAT1-GFP, a plasmid vector expressing mCAT1-GFP fusion protein (7). The mCAT1-GFP was previously shown to work as an FMLV receptor. One day after the transfection, the cells were cultured with the SHIV or FMSIV viral supernatant. The dose of the supernatant used for each infection contained 10 ng/ml of p27. Two days after the infection, cells were fixed, permeabilized, and immunostained using a monoclonal mouse anti-p27 antibody and a rhodamine-conjugated anti-mouse immunoglobulin G (IgG) antibody (Cappel, Aurora, Ohio, USA) as previously described (8). The mock-transfected COS cells were resistant to FMSIV infection (Fig. 1B), whereas the pmCAT1-GFP-transfected COS cells were sensitive to FMSIV infection (Fig. 1C). The p27-positive staining was localized within GFP-positive cells (Fig. 1C), indicating that FMSIV could infect only the cells expressing the FMLV receptor.

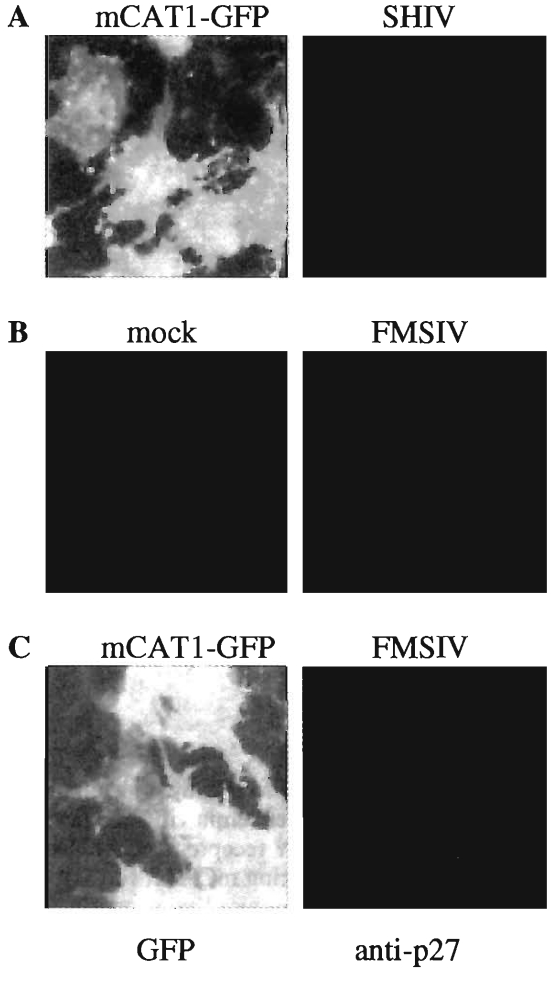

Fig. 1. Immunofluorescent analysis. Left panels show GFP expression and right panels show SIV Gag p27-expression stained by a mouse anti-p27 antibody with a rhodamine-conjugated anti-mouse IgG antibody. In each group, left and right panels show the same locus. (A) The pmCAT1-GFP-transfected COS cells cultured with SHIV. (B) The mock-transfected COS cells cultured with FMSIV. (C) The pmCAT1-GFP-transfected COS cells cultured with FMSIV.

The above results confirmed that the FMSIV infection was confined within the mCAT1-expressing cells. Thus, our strategy may be useful for development of a safe and effective AIDS vaccine, although its potential risk requires further evaluation.

We thank M. A. Martin for providing SHIV-MD14YE DNA, M. Masuda for providing pmCAT1-GFP DNA, and T. Sata for providing the anti-p27 antibody. This work was supported by a grant for AIDS Research from the Ministry of Health and Welfare in Japan.

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