RESEARCH NOTE

In Vitro Transfer of Cellular Immunity to Synthetic Peptides of HIV-1 to Human Lymphocytes with Exogenous RNA

Valéria SF Sales*, Fahim M Sawan, Maria AE Watanabe, João S Silva*, Júlio C Voltarelli , Fernando L De Lucca/+**

Departamento de Bioquímica *Departamento de Parasitologia, Microbiologia e Imunologia **Departamento de Clínica Médica, Faculdade de Medicina, Universidade de São Paulo, 14040-900 Ribeirão Preto, SP, Brasil

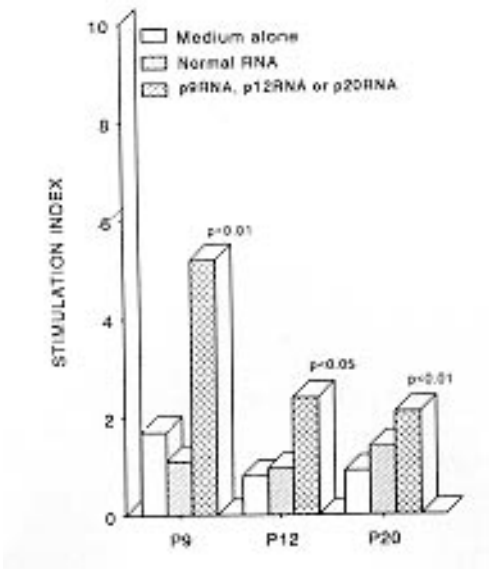
Key words: exogenous RNA - HIV peptides - cellular immunity - AIDS - Brazil

The cellular immunity plays an important role in host defense against viral infections. It is known that the cellular immune response to HIV antigens is impaired in HIV-infected individuals. Thus, there is a search for immunomodulators that are able to restore specifically the reactivity of T lymphocytes to HIV antigens. It is well established that exogenous RNA is incorporated into eukaryotic cells and is able to exert various biological responses (JA Wolff et al. 1990 *Science 247:* 1465-1468, RA Ribeiro et al. 1995 *Mol Cell Biochem 148:* 105-113). One of the most interesting biological properties of exogenous RNA is its ability to transfer cell-mediated immune responses to a variety of antigens. Here, we investigated the possibility of transferring cellular immunity to synthetic peptides of HIV-1 *in vitro* to normal human lymphocytes by means of exogenous RNA.

The following peptides were used to immunize BALB/c mice and sheep: p9 (pol: 476-484; Peptide and Protein Research, MRC AIDS Reagent Project), p12 (env gp41 :598-609; Escola Paulista de Medicina, Brazil) and p20 (env gp120 V3 region: 297-337; Peptide Products Ltd., MRC AIDS Reagent Project). The RNA was extracted from spleen and lymph nodes of immunized (p9 RNA, p12 RNA or p20 RNA) and nonimmunized (nRNA) animals by the cold phenol method (BN White & FL De Lucca 1977, p. 85-130. In RB Turner, *Analytical Biochemistry of Insects*, Elsevier Publishing Scientific Company, Amsterdam). The transfer of cellular immunity by means of exogenous RNA was assessed by the lymphocyte proliferative assay. Lymphocytes from seronegative individuals were obtained by centrifugation of peripheral blood leukocytes on Fycoll-Hypaque gradients. After incubation of lymphocytes with RPMI-1640 alone, nRNA, p9, p12 or p20 RNA (1mg of RNA/10^7cells) at 37°C for 30 min, triplicate cultures (5 x 10^5 cells/well) in RPMI-1640 medium supplemented with 10% fetal calf serum (Hyclone, Logan, UT), 5 x 10^{-5} M 2-mercaptoethanol, 2mM L-glutamine, and 50 µg/ml gentamicin, were maintained in 96-well flat bottom microtiter plates in a final volume of 0.2ml/well. The synthetic peptides p9, p12 and p20 were used at a final concentration of 25 µg/ml. To measure blastogenesis 1 µCi of [^{3}H]-thymidine (specific activity 65 Ci/mmol) was added after five days in culture for an 18 hr pulse. Cultures were harvested on glass fiber filters and radioactivity determined by liquid scintillation. The results are expressed as the "stimulation index" which is the ratio: c.p.m. in the presence of peptide/c.p.m. in the absence of peptide.

Figure shows that RNA preparations obtained from animals immunized with p9, p12 and p20 are active in converting lymphocytes from seronegative individuals to a state of immunological reactivity to these synthetic peptides of HIV-1. On the other hand, RNA extracted from nonimmunized animals (nRNA) is not effective in transferring immunoreactivity to p9, p12 and p20. We have already demonstrated that this phenomenon is antigen specific (FL De Lucca et al. 1982 *J Inf Dis 145:* 148-151) and that immunological activity of exogenous RNA is abolished by RNase and not by DNase and proteinase K

+Corresponding author. Fax: 55-16-633.6840
Received 7 December 1995
Accepted 10 January 1996



Transfer of immunoreactivity to the synthetic peptides p9, p12 and p20 *in vitro* to human lymphocytes with exogenous RNA. The lymphocytes were incubated with p9 RNA, p12 RNA and p20 RNA and the transfer of cellular immunity to these peptides was assessed by the lymphocyte proliferative assay. The RNA extracted from nonimmunized (nRNA) animals was used as control. Stimulation index is the ratio: c.p.m. in the presence of peptide/c.p.m. in the absence of peptide. The Student's t-test was used to calculate the statistical significance of differences between test and control results.

(GAS Passos Jr & FL De Lucca 1991 *Mol Cell Biochem 108:* 1-8).

It is now clear that some immune responses elicited by HIV may enhance infection and contribute to the development of immune deficiency (KB Cease & JA Berzofsky 1994 *Annu Rev Immunol 12:* 923-989). To overcome this problem, one possible approach is to select epitopes that are able to elicit helper T cells, CD8+ cytotoxic T cells and neutralizing antibodies. The present study shows that exogenous RNA is active in transferring immunoreactivity to different epitopes *in vitro* to human lymphocytes. In this regard, it is noteworthy that p12 corresponds to an immunodominant and highly conserved epitope of gp41 of HIV-1 and p9 is an epitope that elicits cytotoxic T lymphocytes. Thus, the findings reported here could be useful in designing clinical protocols of adoptive cellular immunotherapy for HIV infected individuals.

Acknowledgements: to the donations of the synthetic peptides p9 by Peptide and Protein Research and p20 by Peptides Products Ltd through the MRC AIDS Reagent Project. To Mrs Cacilda Dias Pereira Zanon for technical assistance. Valeria SF Sales was the recipient of a fellowship from CAPES.