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Plant based HIV-1 vaccine candidate: Tat protein produced in spinach

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Abstract

The HIV-1 Tat protein has been recently explored as a prospective vaccine candidate with broad, subtype non-specific action. We approached the problem of delivery of Tat through the mucosal route by expressing Tat in an edible plant. The *tat* gene was assembled from synthetic overlapping oligonucleotides, and was subsequently cloned into a plant virus-based vector tobacco mosaic virus (TMV). Spinach plants inoculated with the Tat-producing constructs were collected and fed to mice 7–14 days post inoculation. DNA vaccinations were performed using a gene gun. Codon optimization of the *Tat* gene expressed in spinach plants resulted in several-fold yield increase as detected in immunoblots, and did not cause severe symptoms in inoculated plants. Mice were fed with the Tat-producing or control vector-inoculated spinach. After three feedings, 1 week apart, 1 g per mice, no differences were detected in the growth rate or behavior of the animals fed with these three types of spinach. None of the animals developed measurable Tat antibodies. Following DNA vaccination, however, mice having previously received oral Tat developed higher antibody titers to Tat than did the controls, with the titers peaking at 4 weeks post-vaccination. Codon optimization allows production of up to 300–500 μg of Tat antigen per 1 g of leaf tissue in spinach using a plant virus-based expression system. The plant produced Tat does not seem to have any apparent adverse effect on mice growth or behavior, when fed with spinach for 4 weeks. ELISA data suggested that oral Tat primed for the development of Tat antibodies when mice were subsequently vaccinated with plasmid DNA designed for Tat expression.

Keywords: Tat protein; Plant-made pharmaceuticals; Vaccine

1. Introduction

There is a persistent and urgent need for a preventive AIDS vaccine. There are no consistent correlates of protection, however. Some vaccine studies in monkeys have shown correlations of CTL activity and protection. Protection has also been obtained with administration of neutralizing antibodies, suggesting that antibody responses could play a role in protection. In some cases, neither CTLs nor antibodies correlated with protection, and the only correlation was an increased production of β chemokines by lymphocytes activated in vitro, suggesting that other aspects of immunity may be important. Although infected people mount an immune response to HIV that includes CTLs and neutralizing antibodies, virus is not cleared, viral replication continues, and there is an almost universal progression to disease (although the time course can vary substantially). It is not clear why there is a failure to clear replicating virus. In part, the extensive genetic variability of HIV-1 contributes to this failure. There is considerable evidence for escape from neutralizing antibodies, which target gp120, the most variable viral protein. There is also evidence for escape from CTLs. In part, immune dysregulation by Tat may play a critical role.

Recent studies have suggested that HIV-1 Tat should be considered as an important component of potential HIV vaccines (cf. [1–5]). Tat is not only a key regulator of HIV-1 replication in infected cells, but is also an extracellular immunomodulator which increases efficiency of virus dissemination and promotes AIDS progression. Recently, Tat was

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demonstrated to be indispensable in a multicomponent AIDS vaccine composed of gp120, Nef and Tat proteins that prevented disease development in rhesus monkeys after challenge with a partially heterologous virus [5]. Various forms of recombinant Tat have demonstrated their vaccine potential in experiments on monkeys as well [2,4,6], and Tat thus may be considered a good target for plant expression.

In recent years, plants have been increasingly explored for production of biomedicines and vaccine components [7–18]. The two main advantages of plant systems are low cost and a greater potential for scalability as compared to microbial or animal systems. An additional advantage from the public health point of view is their high safety compared to animal systems, which is important for vaccine production; there are no known plant pathogens capable of replicating in animals, and in humans in particular. At the same time, edible plants may be used directly as delivery vehicles for different proteins (for recent reviews see [19,20]). Transient expression based on plant virus-based vectors results in high yield of expression, rapid accumulation of the products, and considerably less time-consuming design of expression constructs [21]. From a range of transient vector systems available now, two have shown the greatest progress: tobacco mosaic virus (TMV) based vectors and a capsid protein (CP) fusion system based on alfalfa mosaic virus (AlMV). The TMV system has been successfully used for expression of malaria [11] and hepatitis C [22] epitopes, single-chain Fv epitopes specific for mouse B cell lymphoma [23], and, when combined with the AlMV fusion system, rabies virus [24] and respiratory syncytial virus [25] protective epitopes. Previously, the TMV vector system was used for successful expression of the V3-loop segment of the HIV-1 envelope protein in plants as a fusion protein with the AlMV CP [24].

TMV has a single-stranded, positive-sense RNA genome of 6395 nucleotides and has been extensively used as a transient plant vector system (reviewed in [21]). A convenient line of TMV-based transient expression vectors was developed by Prof. W.O. Dawson (University of Florida) [26–28]. Here, we present data on production of the HIV-1 Tat protein in plants using the TMV-based transient expression system. Tat protein was successfully expressed in an experimental plant *Nicotiana benthamiana*, and in an edible plant, spinach, at levels of up to 300 μg of Tat per 1 g of leaf tissue. Feeding these Tat-producing spinach leaves to mice primed them for antibody production following a subsequent immunization with a plasmid expressing Tat and resulted in significantly higher anti-Tat antibody levels compared to control mice.

2. Methods

2.1. Antisera, conjugates and monoclonal antibodies

The Tat-specific monoclonal antibodies were obtained from ABI (Columbia, MD) or through the AIDS Research and Reference Reagent Program, AIDS Program, NIAID, NIH: HIV-1_{BH10} Tat monoclonal antibody (#15.1) [29] from the Division of AIDS, NIAID; and monoclonal antibodies #4138 [30], NT88D1.8, NT77D5.1 and NT74A4.8 from Dr. Jonathan Karn. The polyclonal rabbit antisera against tobacco mosaic virus was from Agdia (Elkhart, IN), and was used according to the manufacturer's instructions. Goat anti-rabbit globulins conjugated to alkaline phosphatase were from Sigma (St. Louis, MO), and were used according to the manufacturer's instructions. Tat protein for ELISAs, produced in bacteria and supplied as a LPS-free purified protein, was a generous gift from Dr. B.C. Nair (ABL, Kensington, MD).

2.2. Primers and gene assembly

The *tat* gene from the MN isolate of HIV-1 (GenBank accession number AR034234) was used as a source of the amino acid sequence for the Tat-protein. To synthesize the *tat* gene optimized to the codon usage in plants, the database of codon frequencies in the genome of TMV (http://www.kazusa.or.jp/codon/) was used. The corresponding synthetic sequence of the plant-optimized *tat* gene was deposited in the GenBank under the accession number AY573055; the nucleotide sequence identity to the native *tat* gene was 80%. This plant-optimized gene was assembled from four overlapping synthetic primers: tat1+ (5′-ATGGAACCAGTTGATCCTAGATTGGAGCCTTGGAAACATCCTGGTTCTCAACCT-AAAACTGCATGTACTACTTGTTACTGTAAAAAGTGT-TGTTTTCAT-3′), tat2− (5′-TTCAGGTGCTCTTCTTCTT-TGTC TTCTCTTTTTTCTACCGTAAGAAATACCCAAT-GCTTTTTTAGTAAAACAAACTTGACAATGAAAACA-ACACTT-3′), tat3− (5′-CTGAGGTGCAGGTTGCTTAG GTAAAGAAACTTGATGAGTTTGAGAATCTTCAGGT-GCTCTTCTTCTTTGT-3′) and tat4− (5′-ATCAACAGGA-TGAGTCTCAGTCTCTCTTTCAACCTTCTTTTTAGATT-CTTTAG GACCAGTAGGATCACCTCTAAACTGAGGT-GCAGGTTGCTT-3′). All four primers were mixed and subjected to 20 cycles of a regular PCR amplification. The resulting PCR products were separated in a low-melting point agarose gel, and a diffuse DNA band of about 300 bp was cut and used as a template for PCR with the following two primers: #151 (+) 5′-GGTTTAATTAAAATGGAACCAG-TTGATCCTAGA-3′ and #154 (−) 5′-CCACTCGAGATT-CAACCTTCTTTTTAGA-3′. The resulting product of ca. 280 bp was agarose-purified and cloned into the pTMV125C vector between *Pac*I and *Xho*I sites resulting in the construct pTMV125C/tatCP. DNA optimized for expression in humans, synthesized and cloned into the expression vector pcDNA3.1 (Invitrogen, Carlsbad, CA), was used for DNA vaccinations.

2.3. Vectors, recombinant constructs and plant inoculation

The TMV-based vector 125c [28] was kindly provided by Prof. W.O. Dawson, University of Florida (Lake Alfred)