Tat HIV-1 Primary and Tertiary Structures Critical to Immune Response Against Non-homologous Variants

Classtticlal studies show that in the absence of anti-retroviral therapy an immune response against the human immunodeficiency virus type 1 (HIV-1), transacting transcriptional activator (Tat) protein correlates with long term non-progression. The purpose of this study is to try to understand what can trigger an effective immune response against Tat. We used five Tat variants from HIV strains identified in different parts of the world and showed that mutations of as much as 38% exist without any change in activity. Rabbit sera were raised against Tat variants identified in rapid-progressor patients (Tat HXB2, a European variant and Tat Eli, an African variant) and a long term non-progressor patient (Tat Oyi, an inactive African variant). Enzyme-linked immunosorbent assay (ELISA) results showed that anti-Tat Oyi serum had the highest antibody titer and was the only one to have a broad antibody response against heterologous Tat variants. Surprisingly, Tat HXB2 was better recognized by anti-Tat Oyi serum compared with anti-Tat HXB2 serum. Western blots showed that non-homologous Tat variants were recognized by antibodies directed against conformational epitopes. This study suggests that the primary and tertiary structures of the Tat variant from the long term non-progressor patient are critical to the induction of a broad and effective antibody response against Tat.

Immunization with the HIV Tat protein represents a putative protocol for a vaccine against AIDS (1). The rationale for this is based not only on the ability of this HIV-encoded protein to cross membranes but also on its many extracellular functions. These are thought to play a major role in enabling HIV to escape immune surveillance and contribute to AIDS pathology (2, 3). Tat is a short viral protein that is essential for the activation and expression of HIV genes (4). This function, called transactivation, requires the binding of Tat to a nascent leader RNA hairpin (TAR), located at the 5'-end of all HIV type 1 (HIV-1) mRNAs (5-7). Tat from most field isolates has 101 residues and two functional regions, a cysteine-rich region and a basic region (4). The three-dimensional structure of Tat shows that the basic region and the cysteine-rich region are well exposed to solvent, whereas a part of the N-terminal region constitutes the core (8). A similar folding pattern, but with local structural variations, is observed between African and European variants (9).

Tat has a number of effects on both HIV-1-infected and uninfected cells and actively contributes to the pathology of AIDS (1). Kaposi’s sarcoma lesions are caused by the direct interaction of Tat with a basic fibroblast growth factor (bFGF) (10), and HIV-1-infected patients with Kaposi’s sarcoma do not have anti-Tat IgG antibodies (11). Tat inhibits the proliferation of uninfected T cells (12-14) possibly by the repression of major histocompatibility complex (MHC) class I transcription (15) and contributes to the dementia associated with AIDS caused by its effect on the transmigration of monocytes and astrocytes (16). Tat induces an immunosuppressive effect on uninfected macrophages by increasing the expression of Fas ligand (17). Extracellular Tat regulates the expression of the HIV-1 co-receptor CXC-chemokine receptor 4 on T-lymphocytes (CXCR4) (18). During HIV infection transcripts of Tat are found before integration and lead to increased T-lymphocyte activation and viral replication (19).

The rationale for a Tat vaccine against HIV was recently validated in vivo using monkeys challenged with a chimeric simian/human immunodeficiency virus (SHIV) following immunization with either active Tat (20), detoxified Tat (21), Tat peptides (22), or DNA containing tat coding sequences (23). Furthermore, clinical studies show that an immune response against Tat correlates with the low viral load in HIV-1 seropositive patients in the absence of antiretroviral therapy (3, 24, 25). Interestingly, an epidemiological study in Gabon showed that patients infected by the HIV-1 Oyi strain were long term non-progressors (26). No anomaly was found in the virus sequence except in the gene tat where the mutation of cysteine 22 to serine seemed to account for the loss of transactivation activity (26).
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RESULTS

Mutations in the Tat Variants Maintain Transactivation Activity —The AIDS epidemic is in constant evolution, and the geographical diversity of HIV-1 strains is reflected in the Tat sequences with as much as 38% mutations observed between Tat variants (Fig. 1). In this study, we used the subtype A Ug11RP (found in eastern/central Africa), a subtype B represented by HXB2 (found in Europe and North America), a subtype C represented by 92BR (common to southern Africa, southern Asia, and India), a subtype D represented by Tat Eli (found in eastern/central Africa) and a subtype AE represented by CM240 (found in southeast Asia) (Fig. 1). We tested the ability of these Tat variants to cross the membrane and transactivate the HIV-1 LTR in transfected HeLa cells (Fig. 2). With the exception of Tat Oyi, all the Tat variants tested had transactivation activity (Fig. 2). This result shows that Tat variability presents a problem for the development of a Tat vaccine, because many mutations can be tolerated without the loss of Tat activity. For example, there is only 62% identity between the sequences of HXB2 and CM240 (Fig. 1). Moreover, the different vaccine approaches targeting Tat (20–22) all use a

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similar sequence that is closely related to Tat Bru (or Laı́) (33), which is composed of 86 residues instead of the 101 residues found in most field isolates (4).

Tat Oyi Serum Has the Highest Antibody Response—Rabbits were separately immunized with one of the following three proteins: Tat Eli, Tat HXB2, or Tat Oyi. The pre-immunization sera show that the Tat variants can be recognized nonspecifically at low sera dilution (Fig. 3). Table I shows that the Tat Oyi serum had the highest antibody response against different Tat variants. Surprisingly, the titration curve for Tat HXB2 (Fig. 3B) shows that the anti-Tat Oyi serum recognizes Tat HXB2 better than the anti-Tat HXB2 serum. The antibody response against Tat Eli is the lowest compared with Tat HXB2 and Tat Oyi with a homologous titer of 256,000 at 60 days postinfection (Table I). The antibody response against Tat HXB2 and Tat Oyi was maximal at 60 days postinfection. Only the serum against Tat Eli has an anamnestic response, with an increase in titer between 60 and 90 days postinfection (data not shown).

![Fig. 3. Titration curves of the three rabbit sera against Tat variants.](image)

**Fig. 3.** Titration curves of the three rabbit sera against Tat variants. A pool of rabbit sera anti-Tat HXB2 (solid square), anti-Tat Eli (triangle) or anti-Tat Oyi (open square) was used at 60-days postimmunization. A pool of all the pre-immunization rabbit sera was used as a control (cross). Titration curves were measured by ELISA against Tat Ug11RP (panel A), Tat HXB2 (panel B), Tat 92Br (panel C), Tat Eli (panel D), Tat CM240 (panel E), and Tat Oyi (panel F). B/BO corresponds to the OD measured for each serum dilution divided by the maximal OD measured for each Tat variant. The vertical bars represent the S.D. measured between two independent experiments carried out in duplicate.

![Fig. 4. Western blot with Tat variants under denaturing conditions.](image)

**Fig. 4.** Western blot with Tat variants under denaturing conditions. A pool of rabbit sera anti-HXB2 (panel A), anti-Eli (panel B), or anti-Oyi (panel C) at 60-days postimmunization.

| Titre of Pooled Rabbit Sera Against the Different Variants of Tat (60 Days Postinfection) |
|---------------------------------|---|---|---|---|
| Anti HXB2 | Anti ELI | Anti Oyi | Preimmune |
| UG11RP   | 2,000 | 16,000 | 8,000 | 500 |
| HXB2      | 512,000 | 16,000 | 512,000 | 125 |
| OYI       | 32,000 | 8,000 | 32,000 | 125 |
| 92Br      | 32,000 | 32,000 | 32,000 | 250 |
| Eli       | 4,000 | 256,000 | 16,000 | 500 |
| CM240     | 8,000 | 4,000 | 128,000 | 250 |

**TABLE I**

Titre corresponds to the reciprocal of the last positive dilution obtained by ELISA (cut-off: mean of preimmun sera + 3 S.D.).
In this study we have tried to understand why only a minority of patients develop an effective antibody response against Tat and become long term non-progressors. Apart from human genetic factors, the result of this study suggests that the primary and tertiary structures of Tat variants can dramatically influence the magnitude and breadth of antibody response against Tat. The choice of Tat Oyi to represent Tat variants from long term non-progressor patients was motivated by a follow up of HIV infection during the eighties in a remote area of Gabon showing that a large majority of HIV-infected patients were long term non-progressors (26). The high proportion of long term non-progressors was difficult to explain by genetic factors, our hypothesis is that these individuals developed an immune response against Tat. This hypothesis was not mentioned by Huet et al. (26) because the role of extracellular Tat was unknown at that time. However, the authors mentioned that the tat gene was defective because of the mutation C22S, and reversion of this mutation could lead to an effective tat gene (26).

The mutation C22S certainly explains why Tat Oyi cannot transactivate in our cellular assay (Fig. 2), but it certainly does not explain why the Tat Oyi serum has the highest antibody response against non-homologous Tat variants in comparison to the other two sera (Fig. 3). Other mutations in Oyi compared with HXB2 certainly play a role in this antibody response (Fig. 1). These mutations are located mainly in the cysteine-rich region and the glutamine-rich region, which have been identified as the two main epitopes in Tat (34). However, the unique mutation E99P observed in the C terminus might also be important. Epitope mapping of Tat has been carried out only with a short Tat variant of 86 residues. Thus the immunological properties of residues 87–101 in the long Tat variants are unknown. The PIP sequence is common in the glutamine-rich region of Tat ELI and Tat Ug11RP (Fig. 1). This sequence could explain why the Tat ELI serum can still recognize Tat Ug11RP in Western blot, unlike the anti-Tat OYI and anti-Tat HXB2 sera (Fig. 4).

The ELISA showed that the anti-Tat antibodies were able to recognize all the Tat variants tested; however, this was not found to be the case in the Western blot. This implies the presence of conformational epitopes. This result confirms the presence of a similar folding among the Tat variants. This is in agreement with the NMR studies of Tat Bru (8) and Tat Mal (9). A circular dichroism (CD) study of Tat Oyi, Tat Bru, Tat Mal, and Tat Eli shows that these proteins have similar CD spectra and indicates that mutations in Tat Oyi preserve the regular folding of the Tat protein (31). Tat Mal and Tat Eli are both D-subtypes and have closely related sequences (29). The main difference between the three-dimensional structure of Tat Bru and Tat Mal is located in the glutamine-rich region that is a short α-helix in Tat Mal (9) and two β-turns in Tat Bru (8). The presence of a similar α-helix in Tat Eli and Tat Ug11RP ended by a similar β-turn constituted by the PIP sequence, also present in Tat Mal, might explain the high antibody response of Tat Eli serum against Tat Ug11RP.

Tat Oyi has a higher antibody response against non-homologous Tat variants compared with Tat HXB2 and Tat Eli. It is possible that this immune property induced a long term non-progressor phenotype for individuals infected with the HIV-1 Oyi strain. HIV-1 Oyi has a low virulence because the tat gene was defective, but patients infected with HIV-1 Oyi were protected against highly virulent D-subtypes (26). Although the HIV-1 Oyi strain was classified as a B-subtype, Tat Oyi has sequence homology with other subtypes of Tat that are not found in a regular B-subtype of Tat, such as HXB2 (Fig. 1). This study suggests also that the tertiary structure of Tat Oyi is critical to the induction of a broad and effective antibody response against Tat. It confirms that a similar folding exists among Tat variants, but the folding of Tat HXB2 and Tat Eli from rapid progressor patients did not induce a broad response against non-homologous Tat variants. The paradox is more striking between Tat HXB2 and Tat Oyi because despite 14 mutations Tat HXB2 was better recognized by anti-Tat Oyi serum compared with anti-Tat HXB2 serum. These mutations induce a better antibody response against Tat Oyi compared with Tat HXB2. Only six mutations (Ser-22, Leu-32, Thr-39, Asp-61, Ala-74, Glu-100) are not observed in the other Tat variants selected in Fig. 1. Tat Oyi mutants will allow the evaluation of the role of these residues in the antibody response.

A vaccine made with Tat Oyi could be efficient against a wide range of HIV-1 strains found in Africa, Asia, and South America where a vaccine remains the only therapeutic alternative against AIDS. It is interesting to note that the prevalence of AIDS in Gabon is low in comparison with other subequatorial African countries (35). What is certainly more interesting is that the woman identified as Oyi was still alive in 1995 and gave birth to three children who are HIV negative.3

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