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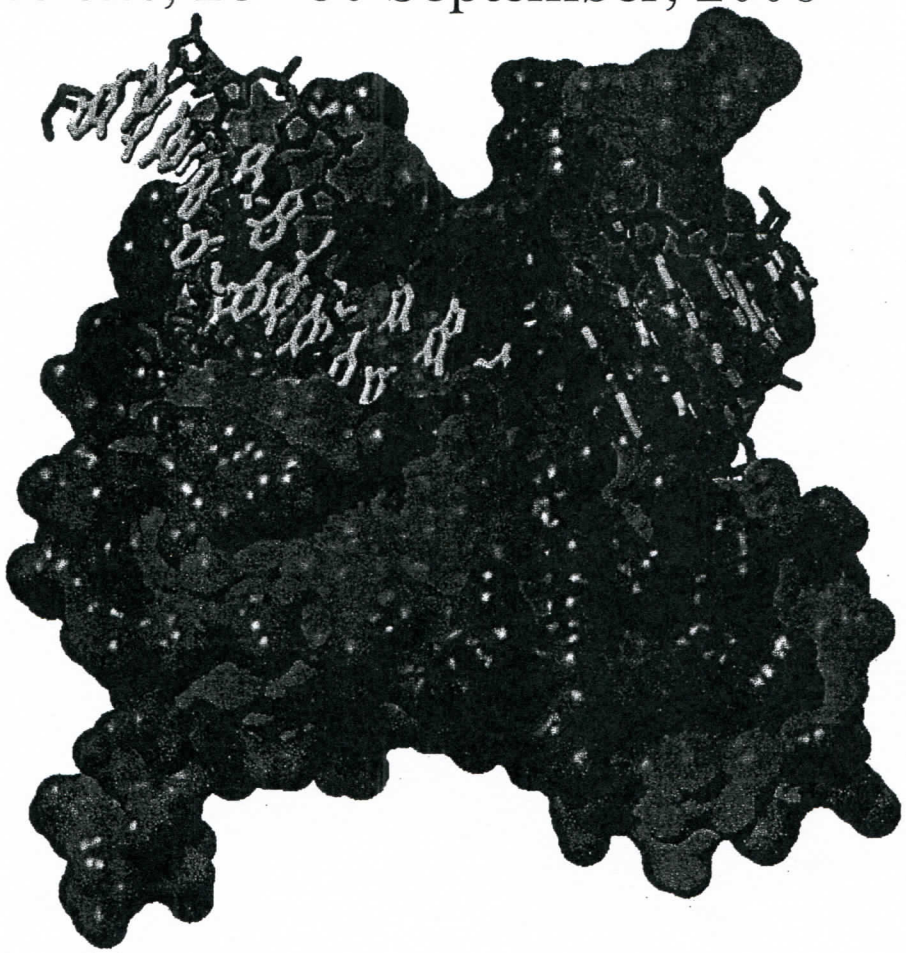

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10.03

THE HIV-1 TAT PROTEIN BROADENS T CELL RESPONSES DIRECTED TO THE HIV-1 ANTIGENS GAG AND ENV: IMPLICATION FOR THE DESIGN OF NEW VACCINATION STRATEGIES AGAINST AIDS

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Aim. Several studies indicate that both HIV-specific CD4- and CD8-mediated T cell responses play a key role during acute and chronic infection, and that long-term non-progressors have consistently higher and broader levels of HIV-specific T cell responses than progressors. Biologically active Tat protein displays immunomodulatory features which make it an attractive adjuvant for other HIV antigens and for the design of new combined subunit vaccines.

Methods. To address the effect of Tat on epitope specific T cell responses directed to OVA/Gag/Env antigen, C57BL6/J or BALB/c mice were vaccinated with the Ovalbumin, HIV-1 Gag or Env protein alone or in combination with the Tat protein. OVA specific T cell responses was evaluated by cytotoxic assay and IFN-Elispot assay.

Results. Mice immunized with OVA alone lysed target cells pulsed with the immunodominant epitope, as did splenocytes from mice immunized with the OVA/Tat combination, albeit to a lesser degree. However, immunization with the OVA/Tat combination generated clear CTL responses to the subdominant and cryptic epitopes which were not detected after immunization with Ova alone. Mice immunized with Gag alone responded to 7 peptide pools, whereas mice immunized with both Gag and Tat responded to 11 peptide pools. We then assayed 36 individual peptides identified as potential targets by the matrix approach. Mice immunized with Gag alone responded to 5 different peptides, whereas mice immunized with Gag/Tat responded to 10. Similarly, mice immunized with Env alone responded to 7 pools, whereas mice immunized with the Env/Tat combination not only responded to these 7 pools but also to an additional 6. Mice immunized with Env alone responded to 5 different peptides, whereas mice immunized with Env and Tat responded to 17. Similar results were obtained when the V2-deleted Env was used.

Conclusions. We demonstrate that Tat is not only an antigen, but also a novel and potent adjuvant capable of broadening the spectrum of epitopes recognized by T cells. This in vivo adjuvant effect may be due to a combination of the immunomodulatory properties of the Tat protein. These observations strongly suggest that Tat should be exploited as a component in the development of subunit-based vaccines against HIV/AIDS.

10.04

THE DIFFERENTIATION PROCESS OF HUMAN MONOCYTE-DERIVED DENDRITIC CELLS: POSSIBLE ROLE OF MITOCHONDRIA AND REACTIVE OXYGEN SPECIES

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Dendritic cells (DC) are highly specialized antigen-presenting cells (APC) with the unique capacity to establish and control T-cell mediated immune responses. DC derive from bone marrow precursors and reside as immature DC (iDC) in peripheral tissues as sentinel cells for incoming antigens (Ag). Following an encounter with an Ag, DC undergo a maturation process characterized by dramatic changes in their phenotype and functions. In particular, mature DC (mDC) present an up regulation of their APC function and the promotion of their migration from peripheral tissues to the draining lymph nodes where they present processed Ag to naïve T cells. Recent studies have implicated ROS in normal physiological signalling by growth factors and cytokines and suggest a role as essential second messengers for DC responses to several physiological stimuli. It is well established that the basal production of cellular ROS is mainly associated to the mitochondrial electron transport chain activity. Two sites of the respiratory chain, namely Complex I and Complex III, have been suggested to be the major source of ROS. We are currently investigating the role of mitochondrial oxidative phosphorylation system in the differentiation process of human DC generated in vitro from immuno-magnetically selected $CD14^+$ monocytes.

Our preliminary results show that i) the differentiation process of DC is characterized by a two-three fold increase of the endogenous respiration as compared to their monocyte precursors, ii) sub-saturating concentrations of rotenone (a specific inhibitor of the complex I of the respiratory chain) seem to inhibit DC differentiation process since rotenone-treated cells showed a decreased expression of the classic DC marker CD1a and the lack of the respiratory activity increase. iii) cells cultured in the presence of rotenone exhibit a lower ROS generation as well as a decreased content of mitochondria compared to control cells. Given the strategic localization of DC at the interface of innate and adaptive immunity, this study may provide the rational for the identification of new targets in the regulation of DC functions.

Reference

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