Gene expression profile of Immature monocyte-derived dendritic cells in response to HIV-1 Virus-Like Particles stimulation.

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Motivation

Lack of small-size animal models for HIV infection pathogenesis and vaccine immunoprotection as well as the restricted availability of primate models (for high costs and low accessibility) infectable only by pseudoviruses (SHIV), led our group to develop ex vivo methodology for evaluating the immunogenicity of anti-HIV vaccine candidates (in particular our VLP-based vaccine) and to identify immuno-genomic pattern related to the vaccinees responsive status. In this respect the genomic transcriptional profile of MDDCs, obtained from normal healthy donors and HIV positive subjects, stimulated by baculovirusexpressed
HIV-VLPAs have been evaluated.

Methods

DC preparation and treatment.

Monocyte-derived DCs, obtained from enrolled subjects under informed consent, were generated as described previously by Cella M et al. (J Exp Med. 1996). In particular, the Ficoll-Hypaque-isolated and CD14+ enriched monocytes were allowed to adhere to plastic, by plating 106 cells per/ml in RPMI 1640 medium, and then cultured for 6 days in DC culture medium supplemented with 50 ng of recombinant GM-CSF (rGM-CSF, R&D Systems, Minneapolis, Minn.) per ml and 1,000 U of recombinant IL-4 (rIL-4; R&D Systems, Minneapolis, Minn.) per ml. After 6-day culture, MDDCs were pulsed with either 5 g/ml of HIV-VLPs or 1 g/ml of LPS for 4 and 8 hours, for gene microarray analysis, and for 16 hours for maturation and activation phenotype analysis.

RNA preparation and microarray hybridization.

DCs were harvested, washed twice in PBS and lysed in 350 ul RLT buffer with fresh addition of 2-Mercaptoethanol per each well of the 6-well plate. Total RNA was isolated using RNeasy minikits (Qiagen), RNA quality and quantity estimated by Agilent Bioanlayzer and NonoDrop. Six ug of amplified antisense RNA (aRNA), obtained from total RNA (0.53 g) via two rounds of in vitro transcription, were labeled with Cy5 (Amersham) while the same amount of reference sample (pooled normal donor PBMCs) was labeled with Cy3. Test- reference sample pairs were mixed and co-hybridized to 17K cDNA microarrays.

Microarrays and statistical analyses.

Hybridized arrays were scanned at 10-m resolution on a GenePix 4000 scanner (Axon Instruments) at variable PMT voltage. Resulting jpeg and data files were deposited at microarray data base (mAdb) http://nciarray.nci.nih.gov and retrieved after median centered, filtering of intensity (>300) and spot elimination (bad and no signal). Data were further analyzed using Cluster and TreeView software and Partek Pro software (Partek). Subsequent low- stringency filtering (80% gene presence across all experiments and at least one experiment with ratio fold change >3), 3.119 genes were selected for further analysis. Hierarchical cluster analysis was conducted on these genes according to Eisen et al. (PNAS. 1998) differential expressed genes were visualized by Treeview and displayed according to the central method.

Results

The results obtained confirm that baculovirus-produced HIV-VLPs induce a maturation pattern in MDDCs consistent with the one triggered by known activators, such as LPS, TNFalpha or cytokine cocktails; the activation, however, of unique genes and cellular pathways, reflects a distinctive cellular and immunological reprogramming.

An unsupervised Eisen's clustering analysis confirms the specificity of the observation, given that the MDDCs samples derived from the HIV-VLP treatment cluster together. Genes involved in the transcriptional profile cluster according preferentially to the donor more than to the length of stimulation (4 or 8 hr). Furthermore, the observed different donor susceptibility to HIV-VLP- treatment may suggest the possibility to select specific gene patterns useful for the identification of 'responsive' vaccinees. Among the pathways and specific genes in VLPs-activated MDDCs, those directly involved in the biological

functions as antigen presenting cells (APCs) have been analyzed in detail. The full functional maturation and activation of MDDCs by HIV-VLPs has been confirmed, in particular the activation of genes involved in cellular control (proliferation, differentiation, migration and homeostasis) as well as in functional activity (antigen presentation, T cell activation and Th polarization).
Microarray approach, which allows quantitative and simultaneous expression analysis of a large amount of genes and the systematic studies of expression patterns, is extremely useful to identify molecular events and key pathways involved in cellular functions induced by specific stimuli. In this study, informative data on the global pattern of gene expression underlying the activation of MDDCs by HIV-VLPs at the early stages of the immune response have been obtained. This approach could contribute to the identification of specific activation markers to trace the biological effects of modifications/optimizations of the HIV-VLP vaccination strategy.
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