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SUPPLEMENTAL MATERIALS AND METHODS

Microarray analysis

Experiment design. Monocytes were cultured for 5 d with 500 U/ml IL-4 and 800 U/ml GM-CSF, and cytokine treatment was repeated at day 3. Ligands were added at the beginning of differentiation, but AGN193109 (AGN) treatment was repeated at day 3. Cells were obtained from three healthy individuals (three biological replicates), and cells were treated with vehicle (DC), 2.5 μM rosiglitazone (DC RSG), 100 nM AM580 (DC AM). RSG treatment was also combined with 1 μM AGN193109 (DC RSG AGN), and in this case two biological replicates were used (RNA was obtained from two and three individuals).

Sample preparation, labeling, and hybridization. Total RNA was isolated using Trizol Reagent (Invitrogen) and further purified with the RNeasy total RNA isolation kit (Qiagen). Integrity of the RNA samples was assessed qualitatively on an Agilent 2100 Bioanalyzer (Agilent). Biotin-labeled cRNA was generated from 5 μg of total RNA by using the SuperScript Choice kit (Invitrogen) and the High Yield RNA transcription labeling kit (Enzo Diagnostics) according to instructions in the Affymetrix technical manual. Fragmented cRNA was sent to the Microarray Core Facility of EMBL (Heidelberg, Germany) for hybridization and preliminary data analyses. 15 μg fragmented cRNA was hybridized to Affymetrix arrays (HU133 Plus 2.0) according to Affymetrix standard protocols.

Data analyses. The resultant images were captured as a data image file and analyzed with GeneChip Operating Software (GCOS version 1.4) using the Affymetrix statistical (MAS5) algorithm. Raw expression value and detection call (present, absent, marginal) of the individual probe sets was determined with MAS5 algorithm using the default settings. These raw data were imported to GeneSping software, and all of the further data analyses were performed with this program. We selected those genes (probe sets) which are significantly up- or down-regulated upon PPARγ ligand (RSG) treatment using the following protocol. Data were normalized using per chip normalization (global scaling). First, we filtered out those genes (probe sets) which had "absent" call all cases (very low expressors). Then, those genes (probe sets) were selected whose expression was at least twofold up- or down-regulated upon RSG treatment (the median expression levels was compared). Finally, ANOVA test was performed and those probe sets were selected whose p values were ≤ 0.05. Using this protocol, 553 out of 54,675 probe sets were significantly changed in a PPARγ-dependent manner (some of the probe sets cover the same transcript and therefore the number of the genes is less). To find characteristic gene expression patterns of the PPARγ- and RARα-activated samples, we performed K-means cluster analysis using the gene list which contains 553 probe sets. First, per gene normalization was performed: expression was normalized to the control sample (DC). The result of the cluster analysis was presented in Fig. 3 A, where red color indicates higher and blue lower levels of gene expression relative to control DCs, whose expression level was designated yellow. Parameters of the cluster analysis, number of clusters 6; similarity measure, change correlation. The average signals of the individual genes of clusters 3 and 6 are presented in Tables S2 and S3.

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