Supplementary Materials

Supplementary microarray methods

Microarray labeling, pre-processing and data sets.

The core collected for gene expression analysis was placed immediately in RNALater (Life Technologies, Carlsbad, CA), kept at 4°C for 4-24 hrs, then stored at -20°C. RNA extraction, quality control and HG_U133_Plus_2.0 GeneChip (Affymetrix, Santa Clara, CA) processing were described previously (1). Detailed protocols are available at (www.affymetrix.com).

All microarray analyses were done using the "R" software package, version 3.0.1 (64-bit), with various libraries from Bioconductor 2.8. Microarray expression files are posted on the Gene Expression Omnibus website (GSE36059 and GSE48581). Microarrays from 703 indication biopsies and 8 nephrectomies were normalized with Robust Multiarray Averaging (RMA). Since the material from 403 biopsy set was labeled with 3' IVT One-Cycle Target Labeling and Control Kit, and 300 biopsy set was labeled with 3' IVT Express kit, we used a correction factor to help minimize batch effects using the Ratio-G method (2), after normalization.

The t-tests (ci>1 vs. cid1) identified 3403 probe sets and 10743 probe sets at the p value<0.05 in early and late biopsies, respectively (679 and 10495 probe sets with Benjamini-Hochberg corrected p-values<0.05). Furthermore, there were 10538 and 10855 probe sets at the p value<0.05 in the TxBx uncorrected and corrected comparison (combined early and late biopsies), respectively (10263 and 10618 probe sets with Benjamini-Hochberg corrected p-values<0.05) (Supplementary tables).

The immunoglobulin (IGT) (3), mast cell (MCAT) (4), acute kidney injury (AKI) (5), and fibrillar collagen transcript (FICOL) set scores (6;7) were each defined as the geometric mean of expression for the probe sets (normalized to the eight normal kidney specimens) included in each respective transcript set.

IGT, MCAT and AKI transcripts are listed on our homepage http://atagc.med.ualberta.ca/Research/GeneLists/Pages/default.aspx. Injury-repair response transcripts (IRRAT950) were previously published (5).

Cultured cells panel

Effector T cells: $CD4^+$ and $CD8^+$ T cells from three and five healthy donors, respectively, were generated through allostimulations starting with PBMCs cultured at a ratio of 3:1 with mitomycin C (Sigma, St. Louis, MO)-treated chronic myelogenous leukaemic B cells (*RPMI8866*, *ATCC*). Recombinant human IL-2 (eBioscience) was added to the cultures at 50 U/mL and cultured for 5 days per round. After four rounds of stimulation, live cells were collected by Ficoll® density gradient centrifugation, followed by $CD4^+$ and $CD8^+$ cell purification using EasySep® negative selection kits (StemCell Technologies) according to manufacturer instructions. Cell purity varied between 92% and 98% (assessed by flow cytometory). Effector phenotype was demonstrated by intracellular staining: 95±3% of $CD8^+$ T cells stained positive for GZMB after the final stimulation, and 96±2% of $CD4^+$ and 90±3% of $CD8^+$ T cells stained positive for IFNG upon restimulation (8).

B cells and NK cells: B and NK cells were purified from PBMCs using EasySep® negative selection kits (StemCell Technologies). Both cell populations remained unstimulated until the time of RNA extraction. B cells were >97% $CD19^+$ and NK cells varied between 90 and 98% $CD56^+CD3^-$. Human NK cells were selected from donors with similar high ratios of $CD56^{lo}$/$CD56^{bright}$ NK cells, suggestive of a cytolytic phenotype (9). The majority (average 96.1%) of NK cells showed a cytotoxic phenotype ($CD56^{dim}$) as expected in whole blood (10).

Monocytes: Monocytes were isolated using EasySep® Human $CD14^+$ Selection Kit (StemCell) direct from the PBMCs.

Macrophages: Monocytes were resuspended in complete RPMI, allowed to adhere on 100 mm plates (BD Falcon), and left for 24 hours, with or without recombinant human IFNG (500 U/mL, eBioscience).

Dendritic Cells: Monocytes were cultured for seven days in the presence of IL-4 (500 U/mL, eBioscience) and recombinant human granulocyte-macrophage colony stimulating factor (500 U/mL, eBioscience). DCs were either immature ("Immature DCs"), or they were matured with LPS for a further 24 hours before harvesting ("Mature DCs"). The DC phenotype was characterized as $CD14^-$ $CD11c^+CD83^+$HLA-DR^{HI} by flow cytometry (11-13).

Parenchymal and endothelial cells: HUVECs (StemCell Technologies, Vancouver, BC Canada) and human renal proximal tubular epithelial cells (RPTECs) (Lonza Inc., Allendale, NJ, USA) were maintained in tissue culture according to supplier recommendations. Cryopreserved primary RPTECs were purchased from Cambrex (Walkersville, MD, USA) and grown in REGM in 50 mL flasks to 50% confluence. At this point, cells were seeded onto collagen-coated inserts of 6-well plates and grown to 80% confluence in REGM. RPTECs and HUVECs were left untreated or treated with recombinant human IFNG (500 U/mL) for 24 hours. After 48 hours the cells were collected and stored in TRIZOL at -70 °C. Total RNA was extracted and used for analysis by microarrays (14).

Fibroblasts: microarray data for keloid fibroblasts was obtained from GEO, GSE7890 (15).

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