

Supplementary Table 1.
Primers and probes used for the Taqman analysis of human genes.

Target gene	Assay ID	5'.....3'	Source
HPRT1	NM_000194RT5	CTGGCGTCGTGATTAGTGAT	Genscript
	NM_000194RT3	CTCGAGCAAGACGTTTCAGTC	
	NM_000194RTP	FAM- CACCCTTTCCAAATCCTCAGCATAATG -TAMRA	
CCL5	NM_002985RT5	CAGCAGTCGTCTTTGTCACC	Genscript
	NM_002985RT3	GTTGATGTACTCCCGAACCC	
	NM_002985RTP	FAM-CGCCAAGTGTGTGCCAACCC-TAMRA	
CCL17	NM_002987RT5	GGCTTCTCTGCAGCACATC	Genscript
	NM_002987RT3	TCTGGTACCACGTCTTCAGC	
	NM_002987RTP	FAM-CACGCAGCTCGAGGGACCAA-TAMRA	
CCL20	NM_004591RT5	GCTTTGATGTCAGTGCTGCT	Genscript
	NM_004591RT3	GATGTCACAGCCTTCATTGG	
	NM_004591RTP	FAM-TGCTGCTTCTGATTCCGCCG-TAMRA	
CCL22	NM_002990RT5	CCCTGGGTGAAGATGATTCT	Genscript
	NM_002990RT3	AATGCAGAGAGTTGGCACAG	
	NM_002990RTP	FAM-ATGACCGTGGCCTTGGCTCC-TAMRA	
CXCL9	NM_002416RT5	ATTGGAGTGCAAGGAACCC	Genscript
	NM_002416RT3	GGATAGTCCCTTGGTTGGTG	
	NM_002416RTP	FAM-AAGGGTGCCTGTTCTGCATCA-TAMRA	
CXCL10	NM_001565RT5	AAGTGGCATTCAAGGAGTACC	Genscript
	NM_001565RT3	TGATCTCAACACGTGGACAA	
	NM_001565RTP	FAM-CCGTACGCTGTACCTGCATCAGC-TAMRA	
CXCL11	NM_005409RT5	AAGAAGAGCAGCAAAGCTGA	Genscript
	NM_005409RT3	GGGAAGCCTTGAACAAGTGA	
	NM_005409RTP	FAM-CAGCCAAGGCTATAGCCATGCCC-TAMRA	
CXCL12	NM_199168RT5	CCAACGTCAAGCATCTCAA	Genscript
	NM_199168RT3	GGGTCAATGCACACTTGTCT	
	NM_199168RTP	FAM-TGCCCTTCAGATTGTAGCCCGG-TAMRA	
COX-2	Hs00153133_m1	-	Applied biosystems
	Hs00153133_m1	-	
	Hs00153133_m1	-	
CTLA4	Hs00175480_m1	-	Applied biosystems
	Hs00175480_m1	-	
	Hs00175480_m1	-	
FOXP3	Hs00203958_m1	-	Applied biosystems
	Hs00203958_m1	-	
	Hs00203958_m1	-	

Legends to Supplementary Figures

Supplementary Figure 1 $\text{TNF}\alpha$ and PGE_2 synergize in inducing high-level CCL22 production in macrophages. Macrophages were generated from peripheral blood monocytes in six-day cultures in the presence of GM-CSF and stimulated for 48 hrs with $\text{TNF}\alpha$, PGE_2 (see M&M), or their combination. CCL22 production was quantified by ELISA.

Supplementary Figure 2. FACS Analysis of FOXP3 in CD4^+ T cells migrated to Recombinant human chemokines and DC sups. **(A)** 0.5×10^6 Purified CD4^+ T cells were allowed to migrate against recombinant human CXCL10 (1000 ng/ml) and CCL22 (200 ng/ml) for 3 hrs. The migrated cells in the lower chamber were harvested and stained for FOXP3 using FOXP3 staining kit (Ebioscience) and analyzed for FOXP3 cells by flow cytometry. The parenthesis denotes net migrated CD4^+ T cell count for each respective chemokine. Net CD4^+ T cell count = (chemokine-induced T cell count) – (spontaneous/medium T cell count). More FOXP3 T cells migrated to CCL22, in spite of more migration of total CD4^+ T cells to CXCL10. This agrees well with Taqman analysis data in figure 4a. **(B)** Photomicrographs of CD3^+ T cells migrated in responses to the medium, αDC1 , and sDC. Please note that αDC1 s attract higher overall numbers of T cells, compared to sDCs.

Supplementary Figure 3. CTLA4^+ CD4^+ T cells preferentially migrate in response to the supernatants from PGE_2 -matured DC. Analysis of CTLA4 mRNA expression in CD4^+ T cells migrated in response to (A) recombinant CXCL11 and CCL22, (B) supernatants from maturing DCs exposed to $\text{IFN}\alpha$ or PGE_2 and (C), Supernatants from αDC1 or sDC. Analysis revealed that CTLA4^+

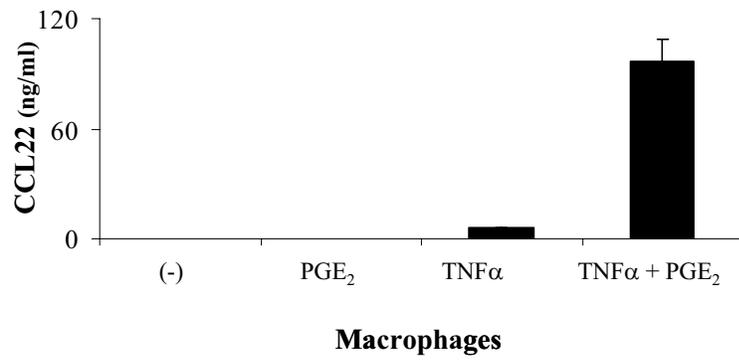
T cells migrated well to CCL22, PGE₂ exposed DC and sDC, this data agrees well with FOXP3 analysis done in the same instances.

Supplementary Figure 4. Tumor-derived prostanoids prime DC for high CCL22 production.

Tumors from patients were cut into pieces using 4mm biopsy punch knife (Miltex Inc., York, PA). 9 pieces were allocated per condition to counteract possible heterogeneity of tumor tissue and were either cultured in presence or absence of Indomethacin (COX inhibitor) for 48 hrs in serum-free CellGenix medium. The resulting supernatants were filtered through 0.22µm filter to generate cell-free tumor-conditioned media. Supernatants from the differentially-treated cultures were added to DCs from day 0 to 6 and secretion of CCL22 protein by DCs obtained in the absence or presence of the differentially-treated tumor supernatants (data from one of three similar experiments) was analyzed. *The inset:* Levels of COX-2 expression (relative to HPRT1) in the untreated tumor and DC exposed to supernatants from the untreated tumor tissue.

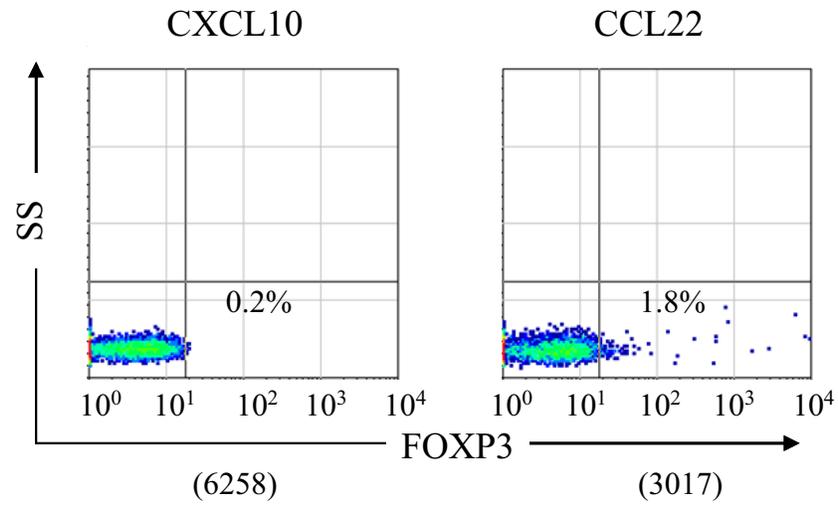
Supplementary Figure 5. Key role of TNFα and PGE₂ in enhancing the CCL22 expression in the TNFα/IL-1β/IL-6/PGE₂-matured “standard” DCs. DC were cultured for 6 days in GM-CSF+IL4, and then treated with IL1β, TNFα, PGE₂, or IL6, individually or in combinations for 48hrs, prior to the analysis of CCL22 mRNA expression by Taqman. Please, note that while PGE₂ alone has a limited CCL22-inducing effect, it strongly enhances the CCL22 expression in the DCs maturing in response to TNFα and IL-1β.

Supplementary Figure 1.

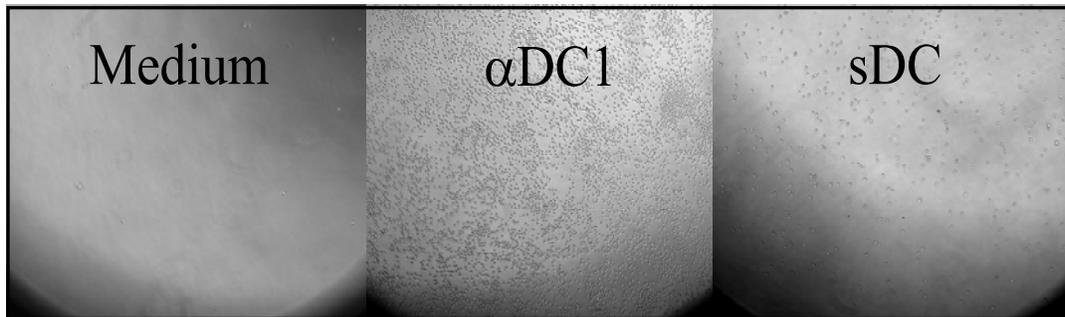


Supplementary Figure 2.

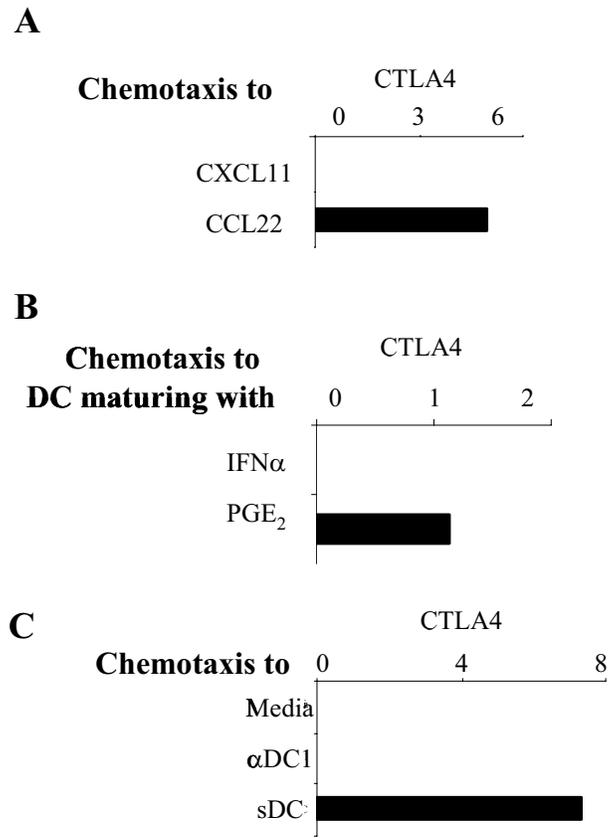
A.



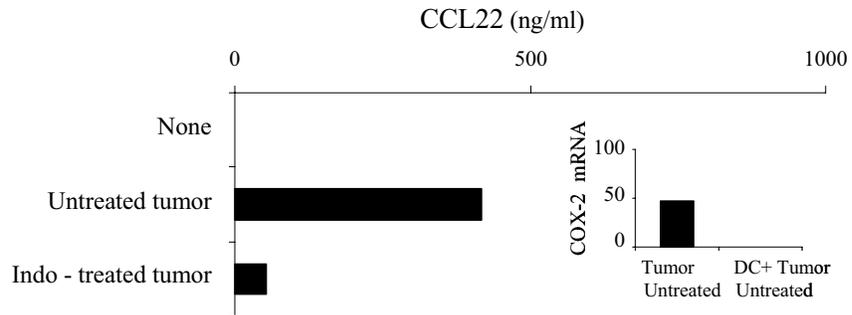
B



Supplementary Figure 3.



Supplementary Figure 4.



Supplementary Figure 5.

