Renal Inflammation in Human Systemic Hypertension Pathogenesis: The Roles of Various T Lymphocyte Populations

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Introduction

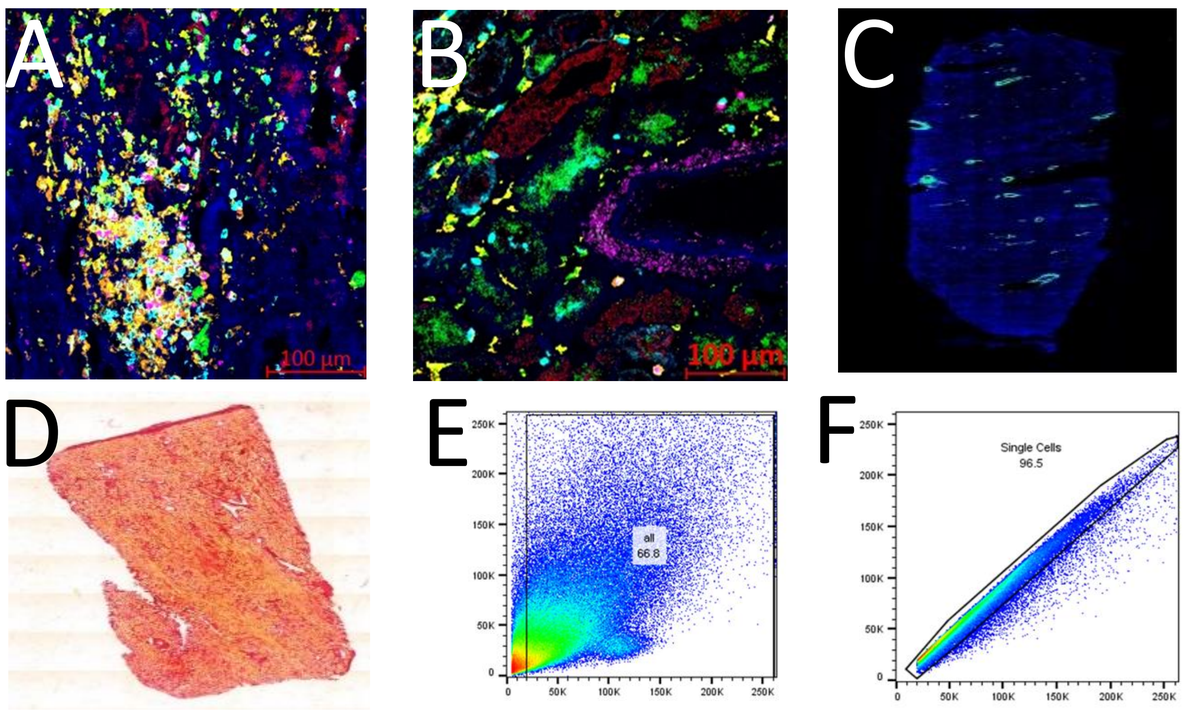
- Hypertension is common affecting over 1 billion people worldwide with recent experimental research linking T lymphocyte mediated renal inflammation to hypertension pathogenesis (Crowley 2007).
- T cell subpopulations have differing roles: CD4+ and CD8+ lymphocytes promote hypertension whereas T regulatory (Treg) lymphocytes protect against hypertension (Zhang 2015).
- Experimental research has found T lymphocytes stimulate renal fibrosis and blood vessel destruction contributing to hypertension development and reduced kidney function (Idris 2014).
- The evidence base detailing the role of T lymphocytes in human systemic hypertension pathogenesis is limited.

Objective

To characterise T lymphocyte populations in human renal tissue and correlate these cells with data regarding hypertension, fibrosis and renal blood vessels.

Research Methods

- Human renal tissue was obtained from patients undergoing nephrectomy for renal cell carcinoma.
- **Multiplexed immunohistochemistry** (mIHC) interrogated specimens histologically for 7 lymphocyte markers – CD3, CD4, CD8, CD45RO, FoxP3, Ki67 and DAPI.
- **Flow cytometry** objectively analysed both cortical and medullary panels for CD3, CD4, CD8, Th17, Treg, Granzyme and Perforin.
- Immunofluorescent **α-smooth muscle actin** (α-sma) staining facilitated blood vessel area quantification.
- **PicroSirius Red** staining enabled renal fibrosis assessment.



A – Hypertensive mIHC, B – Normotensive mIHC, C – Picrosirius red image, D – α-sma image, E – FACS gating all cells F – FACS gating single cells

Results

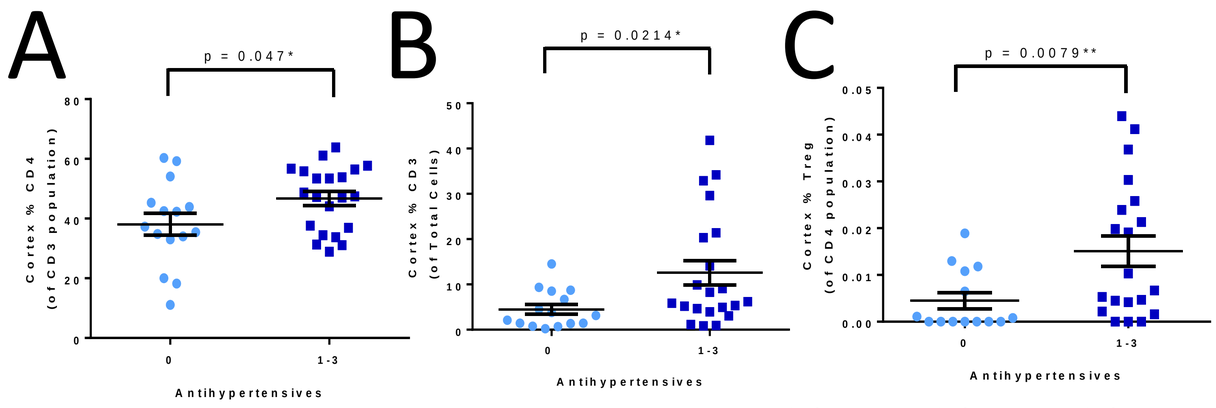
mIHC Results

Cell type	Geometric mean cell number HTN (n=10)	Geometric mean cell number NTN (n=10)	P-value
CD3	34.64	17.94	0.0249*
CD45RO	15.69	7.89	0.0121*
CD4	16.79	8.11	0.0138*
Ki67	4.88	2.00	0.019*
Treg	1.75	1.13	0.14

- Unreliable results due to a **subjective analytical technique**.

FACS Antihypertensive Results

Marker	Region	0 anti-HTN mean % (n=15)	1-3 anti-HTN mean % (n=22)	P-value
CD3	**Cortex**	4.483	12.56	0.0214*
	Medulla	0.05627	0.08170	0.7072
CD4	**Cortex**	38.09	46.69	0.0470*
	Medulla	38.86	40.46	0.7072
CD8	Cortex	45.89	40.43	0.1362
	Medulla	45.43	47.16	0.6301
Th17	Cortex	0.02729	0.06134	0.2564
	Medulla	2.462	4.169	0.2719
Treg	**Cortex**	0.004495	0.01509	0.0079**
	Medulla	0.6976	1.250	0.2413
Granzyme	Cortex	8.947	9.486	0.8088
	Medulla	7.859	8.836	0.5770
Perforin	Cortex	17.32	14.59	0.3602
	Medulla	18.32	17.09	0.6183

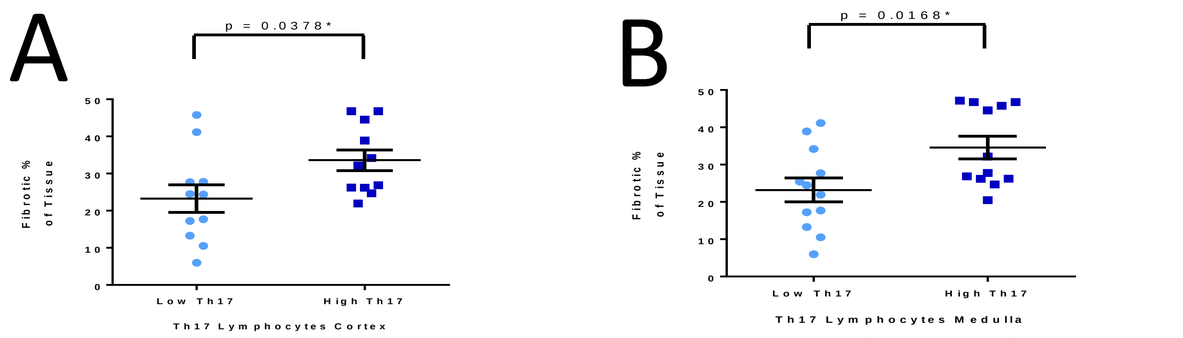


A: Cortical CD8, B: Cortical CD4, C: Cortical Treg

- Patients taking **anti-hypertensives** had significantly increased **cortical CD3, CD4** and **Treg lymphocytes**.

Fibrosis Results

Marker	Region	Low exppression % Fib (n=11)	High expression % Fib (n=11)	P-value
CD3	Cortex	24.78	32.05	0.1549
	Medulla	24.40	33.42	0.0658
CD4	Cortex	28.32	28.52	0.9694
	Medulla	29.46	28.36	0.8301
CD8	Cortex	28.81	28.02	0.8803
	Medulla	27.53	30.29	0.5878
Th17	**Cortex**	23.26	33.57	0.0378*
	Medulla	23.20	34.61	0.0168*
Treg	Cortex	29.96	26,87	0.5549
	Medulla	28.39	29.43	0.8383
Granzyme	Cortex	25.38	31.45	0.2384
	Medulla	31.01	26.81	0.4057
Perforin	Cortex	32.45	24.38	0.1122
	Medulla	30.46	27.36	0.5414



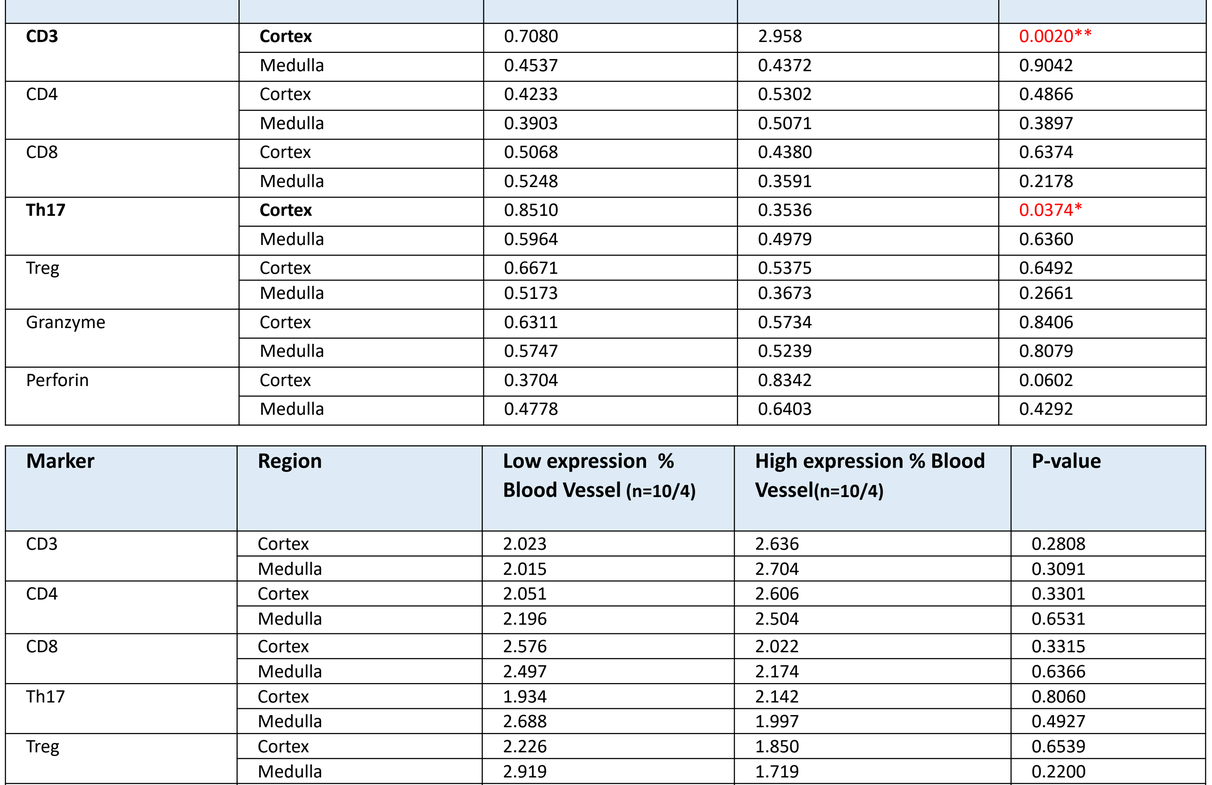
A: Cortical Th17, B: Medullary Th17

- Cortical and medullary specimens with a high **Th17** expression had significantly **increased renal fibrosis**.

Arteriole + Blood Vessel Results

Marker	Region	Low expression % arteriole (n=10/4)	High expression % arteriole (n=10/4)	P-value
CD3	**Cortex**	0.7080	2.958	0.0020**
	Medulla	0.4537	0.4372	0.9042
CD4	Cortex	0.4233	0.5302	0.4866
	Medulla	0.3903	0.5071	0.3897
CD8	Cortex	0.5068	0.4380	0.6374
	Medulla	0.5248	0.3591	0.2178
Th17	**Cortex**	0.8510	0.3536	0.0374*
	Medulla	0.5964	0.4979	0.6360
Treg	Cortex	0.6671	0.5375	0.6492
	Medulla	0.5173	0.3673	0.2661
Granzyme	Cortex	0.6311	0.5734	0.8406
	Medulla	0.5747	0.5239	0.8079
Perforin	Cortex	0.3704	0.8342	0.0602
	Medulla	0.4778	0.6403	0.4292

Marker	Region	Low expression % Blood Vessel (n=10/4)	High expression % Blood Vessel(n=10/4)	P-value
CD3	Cortex	2.023	2.636	0.2808
	Medulla	2.015	2.704	0.3091
CD4	Cortex	2.051	2.606	0.3301
	Medulla	2.196	2.504	0.6531
CD8	Cortex	2.576	2.022	0.3315
	Medulla	2.497	2.174	0.6366
Th17	Cortex	1.934	2.142	0.8060
	Medulla	2.688	1.997	0.4927
Treg	Cortex	2.226	1.850	0.6539
	Medulla	2.919	1.719	0.2200
Granzyme	Cortex	2.114	1.962	0.8573
	Medulla	2.560	2.454	0.9231
Perforin	Cortex	2.119	1.957	0.8489
	Medulla	2.860	1.791	0.2789



A = CD3 Cortex Arteriole, B = Th17 Cortex Arteriole, C = CD3 Cortex Blood Vessel, D = Th17 Cortex Blood Vessel

- High expression of **cortical CD3** was significantly associated with **increased arteriolar area**.
- High expression of **cortical Th17** was significantly associated with **reduced arteriolar area**.
- Objective blood vessel area analyses did not verify these findings.

Summary and Conclusions

- mIHC can successfully interrogate human renal tissue.
- **Cortical CD3, CD4** and **Treg lymphocytes** are implicated in **hypertension pathogenesis**.
- **Th17 lymphocytes** are associated with **renal fibrosis** and **arteriolar changes** promoting hypertension.
- Investigating inflammation's role in human hypertension pathogenesis is challenging due to multiple confounders influencing the renal inflammatory phenotype.

Acknowledgements

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