Background: Hypertension is the leading global risk for mortality, responsible for 12.8% (7.5 million) of total deaths worldwide and is a major health concern because it markedly increases risk of death from stroke, atherosclerosis, and other diseases. Immune cell activation and inflammation are known to be fundamental to the pathogenesis of hypertension and cardiovascular disease development. However, relatively little is known for how immune cells get activated and promote inflammation in the context of hypertension and how factors such as psychological stress may further exacerbate this disease process. Using both clinical and pre-clinical models of hypertension, this project sought to further examine neuro-immune mechanisms in the development of inflammation associated with hypertension and to examine the effects of novel treatment interventions.

Project Objectives and Methodology: Using an animal model of neurogenic hypertension, the modified Annex 1 project objectives were to examine immune cell infiltrates in tissue organs that contribute to the regulation of blood pressure. Secondly, we sought to examine the role of various experimental novel treatment interventions that target the autonomic nervous system in controlling high blood pressure and their effects on immune cell infiltrates in related tissue. Thirdly, we examined immune cell properties in drug-resistant hypertensive patients undergoing experimental treatments that target the autonomic nervous system. To assess these objectives we utilized, developed and integrated flow cytometry based techniques to assess the immune cell infiltrates in tissue of an animal model of neurogenic hypertension and we assessed these effects on blood pressure lowering measures such as renal denervation (RD) and carotid sinus nerve denervation (CSD). Moreover, we utilized transgenic cell specific immune comprised mouse models to examine multiple immune cell types and receptors in the development of hypertension. In parallel to these animal studies, human hypertensive patient blood samples were analyzed before and after treatment interventions using similarly described flow cytometry based methods.

Summary Overview of Main Results: Previous studies have found inflammation, in particular T lymphocytes, to be a contributing factor in the genesis of various forms experimental hypertension, including angiotensin II and neurally mediated chronic hypertension. Currently, clinical interventions such as renal denervation (RD) and carotid sinus nerve denervation (CSD) are aimed at targeting the autonomic nervous system to treat hypertension. However, the effects of these blood pressure lowering strategies on the immune system are unknown. We hypothesized that the sympathoinhibitory effect of CSD contributes to reduced T-cell infiltration in the aorta and brainstem, which could improve vascular compliance including the aortic baroreceptor sensitivity and improved baroreflex transmission centrally. Given the sympathetic innervation of immune system organs such as the spleen, thymus and bone marrow, we suggest that reductions in sympathetic activity may be the trigger for the anti-inflammatory response, thereby breaking this positive (and pathological) feedback loop.

Figure 1

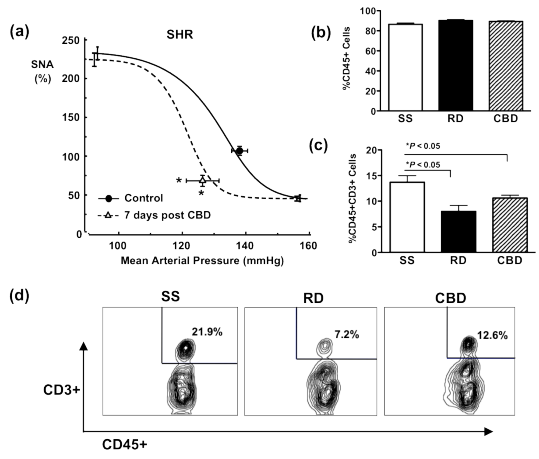


Figure 1 Sympathetic baroreflex and vascular infiltration responses to CSD. (a) The RSA baroreflex function curve is rightward shifted over lower pressure ranges after CSD in SH rats (n=6, *P<0.05 vs baseline) but no changes were observed in Wistar rats. Percent total leukocytes (CD45+ cells) in the aorta of surgical sham (SS), RD and CSD animals (b). There was reduced vascular T cell infiltration following RD and CSD in the SH rats as the percentage of T lymphocytes (CD45+CD3+ cells) in the aorta of RD and CSD animals was less relative to SS (n= 6-7/group; c). Representative flow cytometry contour plot showing T lymphocytes (CD45+CD3+) in aortic samples of each rat group (d). Statistical comparisons were made using one-way ANOVA. *P<0.05.

In our study, RD and CSD were performed in the spontaneously hypertensive rat (SHR) and flow cytometry was used to examine tissue infiltration of T lymphocytes (CD3+) in the aorta and brainstem. Bilateral RD was achieved via a

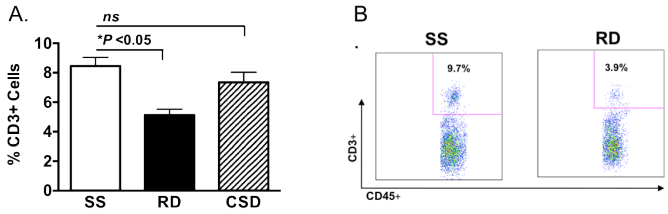
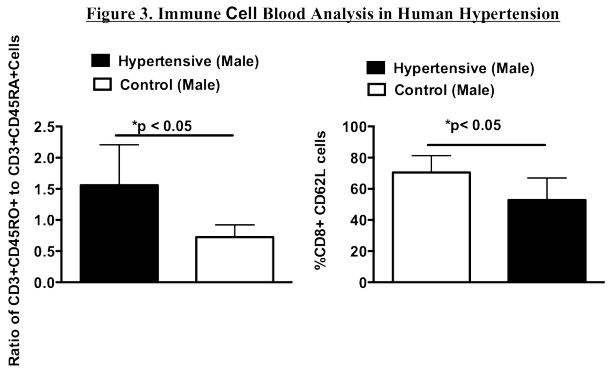


Figure 2. Reduced T cell infiltration in the brainstem following RD in the SH rats expressed as the percentage of total T lymphocytes (CD45+CD3+ cells) (n= 6-12/group; a). Representative flow cytometry scatter plot showing T lymphocytes (CD45+CD3+) in aortic samples of each rat group (b). Statistical comparisons were made using one-way ANOVA. *P<0.05.

retroperitoneal incision to exposure of the renal artery. For CSD, the CS was visualised and branches of the carotid sinus nerve sectioned. Surgical sham-operated SHR (SS) rats underwent the same surgical procedures to expose the kidney and CB but the nerves were left intact. Compared to the SS group, arterial pressure and renal sympathetic nerve activity in the SHR was significantly lowered following both RD and CSD. The percentage of infiltrating CD3+ T lymphocytes in the brainstem (9.6 ± 0.7 vs 6.4 ± 0.8%; $t(9) = 2.79$, $p < 0.05$) as well as the aorta (14.5 ± 1.6 vs 10.0 ± 1.1%; $t(16) = 2.18$, $p < 0.05$) were significantly reduced following RD (Figure 1-2). Following CBD, there was a trend toward reduced percentage of CD3+ cells in the aorta ($t(13) = 1.36$, $p = 0.19$) but unlike RD, there was no change in CD3+ cells in the brainstem (FIGURE 1-2). These data suggest that there is significant systemic CD3+ cell infiltration in the SHR and that some, but not all, procedures targeting the autonomic nervous system may have benefits in lowering tissue inflammation associated with hypertension. We speculate that the sympathoinhibitory effect of CSD contributes to reduced T-cell infiltration in the aorta and brainstem described herein, which could improve vascular compliance including the aortic baroreceptor sensitivity and improved baroreflex transmission centrally.

We next examined the immune cell responses in patients diagnosed with drug resistant hypertension and who were eligible to undergo experimental renal denervation enrolled in the Bristol Heart Institute study. We hypothesized that circulating cells of hypertensive patients exhibit a pro-inflammatory T cell phenotype, as determined by the balance of memory (effector) versus naïve CD3+ cells. Using flow cytometry, subsets of circulating T cells and markers of antigenic memory were analyzed in male hypertensive patients and normotensive subjects. There were no differences in the overall percentage of CD3+ cells in normotensive (63.3 ± 3.7%) (n=5; mean age 34 yrs; mean systolic BP 128.6 mmHg) vs. hypertensive men (62.2 ± 5.5%) (n=6; mean age 53.5 yrs; mean systolic BP 154.7 mmHg) and the percentage of CD4+ and CD8+ T cell subsets was not statistically different between groups. However, T cell subset analysis revealed that the ratio (1.6 ± 0.3 vs 0.7 ± 0.1; *P <0.05*) of memory (CD45RO+CD3+) vs. naïve (CD45RA+CD3+) T cells as well as the percentage of CD8+CD62L(low) cells (70.4 ± 4.5 vs 52.8 ± 6.3; *P <0.05*) were increased in hypertension (FIGURE 3). These results demonstrate an imbalance in effector memory / naïve T cells in hypertensive men and provide further evidence that chronic antigen exposure may be involved in the pathogenesis of the inflammatory response hypertension. Moreover, we plan to follow a subset of these patients at 6 and 12 months following RD and CSD to determine if there is a causal link between lowering blood pressure by targeting the autonomic nervous system in humans and a reduction in pro-inflammatory markers, with a particular emphasis on T lymphocytes. Current preliminary data has also been collected and ongoing mouse animal studies using genetically modified models are ongoing to determine whether the response to specific hypertensive stimuli such as the hormone angiotensin II leads to an antigen specific response in the development of hypertension.



Potential Societal Impact and Conclusions: Our studies show that some but not all interventions targeting the autonomic nervous system provides a potent and persistent anti-hypertensive response in SH rats accompanied by substantial sympathoinhibition in multiple outflows, improved baroreflex and renal function, and reduced T lymphocyte infiltration in the aorta and brainstem. Therefore these studies have direct implications for the potential to provide therapeutic benefit to drug resistant/intolerant human patients with sympathetically mediated diseases, however additional clinical studies are required.

Moreover, our studies explored the antigenic specificity of pathogenic T cells in hypertension, which is critical to know in order to determine the underlying mechanism of the disease process and also for the possible development of therapies based on antigen-induced tolerance, rather than nonspecific immunosuppression. It is therefore possible that drugs that specifically interfere with T cell activation without off target effects could be used to treat refractory hypertension in humans and/or in combination with RD and CSD treatment interventions, however clinical trials and further translational studies are warranted.

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Dissemination of Activities: The dissemination activities during this project period include the publication of abstracts, a manuscript and 2 oral presentations. This information was disseminated through the submission of abstracts to the annual Experimental Biology meeting in April 2013 and 2014 meetings and the IUPS congress meeting in Birmingham, England July 21-26, 2013 (see attached supporting documentation meeting abstract). Moreover, below is the citation for a manuscript that was published in the journal *Nature Communications* during this project period:

McBryde F.D., Abdala, A.P., Hendy, E.B., Pijacka, W., Marvar, P., Moraes, D.J., Sobotka, P.A., and Paton, J.F. The carotid body as a putative therapeutic target for the treatment of neurogenic hypertension. *Nat Commun 2013; 4:239*

Media Coverage for above publication: *iTV News Report September 6, 2013*
http://vimeo.com/73877970#t=4

Oral presentations were given at University of Bristol Seminars in Physiology and Pharmacology series entitled *Autonomic control of blood pressure and inflammation in hypertension* on February 18th, 2013 and on November 7th 2013 in the Cardioniomics Monthly basic and clinical Research meeting.