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The role of Axl in T lymphocyte survival in salt-dependent hypertension

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Abstract

Objective—Survival of immune and non-immune cells relies on Axl, a receptor tyrosine kinase, which is implicated in hypertension. Activated T lymphocytes are involved in regulation of high blood pressure. The goal of the study was to investigate the role of Axl in T lymphocyte functions and its contribution to salt-dependent hypertension.

Approach and Results—For the first time we report increased apoptosis in peripheral blood from $Axl^{-/-}$ mice due to lower numbers of white blood cells mostly lymphocytes. *In vitro* studies showed modest reduction in interferon gamma production in $Axl^{-/-}$ Th1 cells. Axl did not affect basic proliferation capacity or production of interleukin 4 in $Axl^{-/-}$ Th2 cells. However, competitive repopulation of $Axl^{-/-}$ bone marrow or adoptive transfer of $Axl^{-/-}$ $CD4^+$ T cells to $Rag1^{-/-}$ mice showed robust effect of Axl on T lymphocytes expansion *in vivo*. Adoptive transfer of $Axl^{-/-}$ $CD4^+$ T cells was protective in a later phase of deoxycorticosterone-acetate and salt hypertension. Reduced numbers of $CD4^+$ T cells in circulation and in perivascular adventitia decreased vascular remodeling and increased vascular apoptosis in the late phase of hypertension.

Conclusions—These findings suggest that Axl is critical for survival of T lymphocytes especially during vascular remodeling in hypertension.

Keywords

Axl; lymphocyte; hypertension; apoptosis; vascular remodeling

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Conflict of Interest Disclosure

None

Introduction

Tyro3, Axl and Mertk (TAM) family of receptor tyrosine kinases has been implicated in regulation of multiple organ systems[1]. Growth arrest-specific protein 6 (Gas6) and Protein S are potent anticoagulants and ligands for the TAM receptors[2, 3]. However, mice with genetic deletion of all three TAM receptors showed normal hemostasis[1]. It was later reported that Gas6 and TAM receptors are involved in regulation of thrombus stabilization[4]. More severe lymphoproliferative disorder was described in adult TAM triple knockout mice[5]. The observed autoimmune phenotypes was linked to TAM functions in innate immune cells, e.g. macrophages (Mϕs), dendritic cells (DCs), and Natural Killer (NK) cells[6]. Initial data in humans showed that Axl is restricted to innate immune cells and upregulation of Axl is predominant in myeloid leukemias[7]. However, recent reports suggested substantial roles for Axl and Mertk in T and B cell leukemias[8-10]. Thus, the role of TAM family and Axl in particular on lymphocyte functions in homeostasis and under pathological conditions remain unclear.

A recent human study (3,679 untreated hypertensive individuals; The Framingham Heart Study) showed that apoptosis, T cell activation, and T cell differentiation are enriched in a gene-gene interactions network for a mutation in SH2B adaptor protein 3 (SH2B3) that cause increased blood pressure (BP)[11]. Not surprisingly, one of the sub-networks of the whole blood transcriptome included the Gas6/Axl pathway in relation to high BP genetic susceptibility[11]. Previous genetic studies in a Sabra rat model of salt-sensitive hypertension identified Axl as a candidate gene[12]. Experiments in global knockout mice confirmed pathophysiological role for Gas6 and Axl in development of deoxycorticosterone-acetate (DOCA)-salt hypertension[13, 14]. Specifically, our group showed that Axl-dependent signals control vascular smooth muscle cells survival and promote vascular remodeling after 6 weeks of DOCA-salt[13]. We recently reported that Axl expression in bone marrow (BM)-derived cells is responsible for early and late phases of DOCA-salt hypertension[15]. Adaptive immune cells such as T lymphocytes contribute to vascular dysfunction and DOCA-salt hypertension[16]. Recent study showed that both $CD4^+$ and $CD8^+$ T cells producing interferon gamma (IFN-γ) are involved in elevation of blood pressure and kidney damage after repeated hypertensive stimuli[17]. Interestingly, the Th1 response in the retina was also shown to be regulated through Axl/Mertk in mice[18]. The primary focus of this study was on the role of Axl in T lymphocyte functions and its contribution to salt-dependent hypertension.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Increased apoptosis of white blood cells in peripheral blood from $Axl^{-/-}$ mice

Others and we showed that Axl plays a key role in survival of various cell types[19]. We double-stained peripheral leukocytes from Axl littermates with AnnexinV and PI (Fig. 1). The lower-right quadrant of each flow chart shows early apoptotic leukocytes, while the upper-right quadrant necrotic (late apoptotic) cells in Axl mice (Fig. 1A). Quantification of

flow cytometry demonstrated that $Axl^{-/-}$ had significantly higher apoptotic leukocytes compared to $Axl^{+/+}$ mice (Fig. 1B). Of note, the $AnnexinV^{+}PI^{+}$ cell numbers didn't reach statistical significance between Axl genotypes (Fig. 1B). We found that the levels of apoptosis were the same in BM from Axl littermates (not shown). Evaluation of splenocytes from adult Axl littermates showed similar numbers of $CD3^{+}$ T or $NK1.1^{+}$ cells. The frequencies of naive $CD62L^{high}/CD44^{low}$ and $CD62L^{low}/CD44^{high}$ memory T cells were similar between $Axl^{-/-}$ and $Axl^{+/+}$ mice. We also found no differences in B and T lymphocytes in spleens and lymph nodes between Axl genotypes (not shown). However, $Axl^{-/-}$ mice had significantly lower (~40%) total white blood cells and lymphocytes count in peripheral blood compared to their $Axl^{+/+}$ littermates. In addition, Axl deletion resulted in higher $CD3^{+}AnnexinV^{+}$ and $CD3^{-}AnnexinV^{+}$ lymphocytes in blood (Fig. I). Hemoglobin levels were slightly reduced in $Axl^{-/-}$ mice. Taken together, our data suggest that Axl controls survival of T lymphocytes in peripheral blood.

Competitive repopulation of bone marrow cells showed less $Axl^{-/-}$ T cells

Original report on deletion of three TAM receptors resulted in a severe lymphoproliferative disorder in mice[5]. In order to explore the role of Axl on T cells *in vivo* we evaluated homeostatic expansion of $Axl^{+/+}$ ($CD45.1^{+}$) vs. $Axl^{-/-}$ ($CD45.2^{+}$) bone marrow cells in competitive repopulation experiment (Fig. 2). We were able to define Axl genotype origin by dual flow cytometry with anti-CD45.1 and anti-CD45.2 antibodies of white blood cells from peripheral blood from chimeras over 8 weeks time-course (Fig. 2A). Significantly more $CD45.2^{+}$ than $CD45.1^{+}$ cells were found at 6 and 8 weeks in chimeras after bone marrow transplant (BMT) suggesting the role of Axl on immune homeostasis (Fig. 2B). At the end of experiment (8 week) we collected peripheral blood and confirmed higher numbers of $CD45.2^{+}$ cells from $Axl^{-/-}$ mice (Fig. 2C). As shown by original report in triple TAM knockout[5], the immune population of the chimeras was shifted towards increase in $Axl^{-/-}$ B lymphocytes ($CD45.2^{+}CD19^{+}$) both in blood and spleen (Fig. 2D-E). There was dramatic decrease in $Axl^{-/-}$ T cells ($CD45.2^{+}CD3^{+}$) in blood and spleen from chimeric mice after competitive repopulation (Fig. 2F-G). Lymphocyte frequencies in spleen were similar between Axl genotype controls as we observed in naïve vs. memory T cells (Fig. 2E,G). Analyses of innate immune cells suggested the role for Axl in repopulation of blood monocyte/Mϕ ($CD11b^{+}$) but did not affect DCs ($CD11c^{+}$) or NK ($NK1.1^{+}$) cells (Fig. II). There were no differences in innate immune subsets in spleens from Axl genotype controls or repopulated chimeras (Fig. II). These findings confirmed our hematological data and suggest that Axl is required for peripheral T lymphocyte expansion from BM.

Axl regulates $CD4^{+}$ T cell repopulation in vivo

It is possible that the $Axl^{-/-}$ lymphocytes decline could be due to alteration in innate immune cells under competitive repopulation of BM, as was reported in double and triple TAM knockouts before[5, 18]. However, previous studies suggested that dual deletion of Mertk and Axl affected Th1 polarization *in vitro*[18]. In our experiments $Axl^{-/-}$ T cells showed modest but significant decrease in IFN-γ under Th1 polarizing conditions (Fig. 3A). The basic capacity to proliferate in response to T cell receptor stimulation was unaffected in cultured $CD4^{+}$ T cell from $Axl^{-/-}$ vs. $Axl^{-/-}$ mice, with similar cell number yields (Fig. 3B). Although, there was a slight delay in the initial two cell divisions that was compensated in

division 4 in $Axl^{-/-}$ vs. $Axl^{-/-}$ Th1 cells (Fig. 3B; Fig. III). We also found that production of interleukin 4 (IL-4) was similar between Axl genotypes under Th2 polarization (Fig. IV). Thus, Axl showed a modest effect on Th1 polarization of $CD4^+$ T lymphocytes *in vitro*.

To avoid potential effects of Axl depletion in innate immune compartment, we adoptively transferred $Axl^{-/-}$ or $Axl^{+/+}$ $CD4^+$ T cells to recombination activating gene 1 knockout ($Rag1^{-/-}$) mice that lack adaptive immunity but contain Axl-sufficient innate immunity (Fig. 3C-D). In our experiments frequencies of $CD4^+$ T cell were under detection level in peripheral blood from $Rag1^{-/-}$ mice injected with phosphate-buffered saline (PBS; Fig. 3C-D). Adoptive transfer of $Axl^{+/+}$ T cells resulted in significant increase in percentage of $CD4^+$ cells over 10 weeks of repopulation that peaked at the 7 week (Fig. 3C). $Axl^{-/-}$ $CD4^+$ T cell showed slightly slower repopulation after 3 weeks and non-significant increase at 10 weeks after adoptive transfer to $Rag1^{-/-}$ mice (Fig. 3C). Decreased repopulation of $Axl^{-/-}$ $CD4^+$ T cells in blood was confirmed in spleens from adoptively transferred $Rag1^{-/-}$ mice (Fig. 3D). Taken together, our findings suggest that Axl is important for $CD4^+$ T lymphocyte expansion and/or survival *in vivo* in the presence of Axl in innate immune compartment.

Axl is important for $CD4^+$ T cell survival and the late phase of DOCA-salt hypertension

Production of IFN-γ by $CD4^+$ and $CD8^+$ T cells was recently shown to contribute to BP increase and kidney damage after repeated hypertensive stimuli[17]. Similar to our report in Axl chimeras[15], we found reduction in arterial expression of IFN-γ (and Th1-dependent pathways) in $Axl^{-/-}$ mice after 6 weeks of DOCA-salt (not shown). We performed adoptive transfers of $CD4^+$ T cells from $Axl^{+/+}$ or $Axl^{-/-}$ to $Rag1^{-/-}$ mice and compared BP changes after DOCA-salt to that in $Rag1^{-/-}$ injected with PBS (PBS→$Rag1^{-/-}$) or $Axl^{+/+}$ mice (Fig. 4). As expected in this model[16], $Axl^{+/+}$ mice showed significant increase in systolic BP, while PBS→$Rag1^{-/-}$ mice were protected from hypertension after 5-6 weeks of DOCA-salt (Fig. 4A). Adoptive transfer of $Axl^{-/-}$ $CD4^+$ T cells ($Axl^{-/-}$→$Rag1^{-/-}$) was also protective for BP increases as compared to $Axl^{+/+}$→$Rag1^{-/-}$ or $Axl^{+/+}$ mice after 5-6 weeks of DOCA-salt (Fig. 4A). $CD4^+$ T cells were detected in blood in $Axl^{-/-}$→$Rag1^{-/-}$ but were significantly reduced compared to $Axl^{+/+}$→$Rag1^{-/-}$ or $Axl^{+/+}$ mice after 5 weeks of DOCA-salt (Fig. 4B). Levels of BP reflected reduced medial thickening of mesenteric arteries in PBS→$Rag1^{-/-}$ and $Axl^{-/-}$→$Rag1^{-/-}$ vs. $Axl^{+/+}$→$Rag1^{-/-}$ or $Axl^{+/+}$ mice after 6 weeks of DOCA-salt. We also noted significantly reduced adventitial compartment in arteries from $Rag1^{-/-}$ controls and $Axl^{-/-}$→$Rag1^{-/-}$ mice. Previous report suggested that majority of T lymphocytes reside within perivascular adventitia (PVA) in hypertension[16]. As we observed by flow cytometry in peripheral blood, immunohistochemistry mirrored presence of $CD4^+$ in PVA from $Axl^{+/+}$→$Rag1^{-/-}$ or $Axl^{+/+}$ mice after 6 weeks of DOCA-salt (Figs. 4B, 5A). In contrast, PBS→$Rag1^{-/-}$ and $Axl^{-/-}$→$Rag1^{-/-}$ mice showed significantly less $CD4^+$ immunoreactivity in PVA (Fig. 5A). Detected $CD4^+$ staining in PBS→$Rag1^{-/-}$ mice could be due to cross-reactivity of anti-CD4 antibody to endothelial cells in PVA. One of the major pro-survival signals that is controlled by Axl in vasculature is phosphorylated protein kinase B (pAkt) after DOCA-salt[13]. In PVA we observed significant decrease in $pAkt^+$ in PBS→$Rag1^{-/-}$ compared to $Axl^{+/+}$, while $Axl^{-/-}$→$Rag1^{-/-}$ mice had less pAkt relative to $Axl^{+/+}$→$Rag1^{-/-}$ or $Axl^{+/+}$ mice after 6 weeks of DOCA-salt (Fig. 5B). Percentage of apoptotic cells in PVA was elevated in $Axl^{-/-}$→$Rag1^{-/-}$ mice only (Fig. 5C). Thus, Axl is

required for presence of $CD4^+$ T cells not only in circulation but also important for vascular inflammatory response, survival, and remodeling in hypertension.

Discussion

For the first time we report that Axl is required for lymphocyte survival in peripheral blood in a mouse. Single deletion of Axl resulted in modest effect on $CD4^+$ T cells production of IFN-γ. A defective expansion of T lymphocytes was evident in competitive repopulation of $Axl^{-/-}$ BM, which was also resulted in an increase in B cells with decline in circulating myeloid cells. Late repopulation of $CD4^+$ T cells was dependent on Axl after adoptive transfer to $Rag1^{-/-}$ mice. Finally, presence of $CD4^+$ T lymphocytes in blood and PVA could explain Axl-dependent effects on vascular remodeling in the late phase of DOCA-salt hypertension.

The TAM family of receptor tyrosine kinases (particularly Mertk and Axl) are shown in tempering the immune response in murine Mϕs, DCs and NK cells[6, 20]. Genetic defect in the TAM receptors is shown to lead to various autoimmune disorders, including arthritis, experimental autoimmune orchitis, and lupus in mice following a pathological insult[5, 21, 22]. Studies using single or double gene-knockout mice demonstrated that Mertk or Axl suppresses innate immune responses via inhibiting expression of pro-inflammatory cytokines through transcriptional upregulation of the suppressor of cytokine signaling 1 (SOCS1) and SOCS3 proteins[23]. In contrast, we have shown that Axl could inhibit SOCS1 and promote signal transducer and activator of transcription 1 (STAT1) signaling leading to immune modulation and activation of smooth muscle cells in vein grafts or after vascular injury[24, 25]. We also reported that Axl is important for vascular and kidney dysfunction by regulating immune cells in DOCA-salt hypertension[13, 15]. Collectively, our findings indicate distinct roles for the TAM family (e.g., Axl vs. Mertk) on immune modulation, and Mertk likely regulates immunosuppression observed in single or double TAM knockouts. Mertk is the primary TAM receptor that is involved in the engulfment and efficient clearance of apoptotic cells[26], and in an effective resolution of acute inflammation[27]. Our new data suggest that Axl is required for homeostasis of white blood cells (lymphocytes) in peripheral blood.

Initial findings implied that Axl expression is restricted to normal myeloid cells with preference in myeloid (~60%) vs. lymphoid (2%) leukemias[7]. Triple TAM knockout mice exhibited enlarged spleens and lymph nodes with a predominance of $CD4^+$ over $CD8^+$ T cells or B cells after 1-2 months of age[5]. It was concluded that over-activated Mϕs and DCs are responsible for lymphoproliferation and autoimmunity in TAM knockout mice. However, deletion of Mertk exhibited larger spleens compared to lack of Axl or Tyro3 among single or double knockouts[5]. In fact, clinical studies showed that constitutively active Axl expressed in B cells and promotes survival and proliferation in a chronic B lymphocytic leukemia[8]. In addition, Mertk is reported to be ectopically express on B and T cells and might be involved in the progression of a variety of human cancers, including T cell acute lymphoblastic leukemia[9]. Most recent results showed that Mertk and Protein S are expressed in human T cells and upon activation facilitate an autocrine proliferation[10]. Furthermore, lack of Mertk and Axl in mice affected retinal $CD4^+$ T cell polarization towards Th1, but the underlying

mechanism is not clear[18]. In here we described a novel haematological phenotype in a single $Axl^{-/-}$ suggesting a significant role for Axl for T lymphocyte survival that might affect production of IFN-γ. Our *in vivo* repopulation studies in chimeras or $Rag1^{-/-}$ mice strongly support the role of Axl in T lymphocyte expansion. The likely mechanism is related to decrease in Axl-dependent activation of Akt, which is one of the key pro-survival signals in Th1 cells in sepsis[28]. Recent studies argue that a higher proliferation and metabolic activity of T cell[29], and its differentiation to Th1 cells is mediated by the phosphoinositide 3-kinase (PI3K)/Akt signaling[30]. Therefore, Axl-dependent signals could be critical for T cell fitness and might be involved in late phases of vascular pathologies. Slight changes in $Axl^{-/-}$ cell divisions under Th1 polarization *in vitro* could explain more robust effects of Axl deletion *in vivo*. A premature mitosis in antigen-specific Th1 cells was shown to be regulated by the balance between c-fos and nuclear protein kinase Wee1[31]. Similarly, recent pharmacological experiments in several cancer cell lines suggested a synergism between Axl inhibition and blocking cell cycle kinases that control mitotic cell entry[32]. Thus, Axl might regulate long-term activation of T lymphocytes by altering cell division and survival signals.

Increased attention to adaptive immunity revealed a key role for T lymphocytes in regulation of vascular dysfunction and experimental hypertension after Angiotensin II (AngII) or DOCA-salt[16]. As in a typical immune response, hypertensive stimuli increase isoketals in DCs that promote immune activation of T cells and progression of hypertension[33]. Further activated DCs enhance T cell proliferation and promote their polarization towards Th1 contributing to high BP in patients with preeclampsia[34]. There are growing experimental evidence on the primary role for $CD8^+$ T cells in the development of hypertension[35]. However, a recent report[17] showed that both $CD4^+$ and $CD8^+$ T cells-producing IFN-γ increased BP and promoted kidney damage in a repeated hypertensive stimuli model. Likewise, patients with resistant arterial hypertension reported to have increased circulating levels of Th1 cells[36]. High BP is regulated by multiple genomic loci, which was recently shown in untreated hypertensive individuals[11]. The authors identified a causal mutation (rs31184504 C/T) within SH2B3 gene. Gene expression signatures from a whole blood-derived RNA in these individuals lead to generation of co-expression networks and the one of the key drivers was HS2B3. Pathway analyses suggested that apoptosis, T cell activation, and T cell differentiation were the most significant in predisposition to hypertension[11]. The SH2B3 is also known as lymphocyte adaptor protein (LNK) regulates hematopoiesis and lymphocyte differentiation, and implicated in myeloproliferative and inflammatory disorders[37]. Over-production of IFN-γ was restricted to $CD8^+$ and $CD4^+$ T cells in $Lnk^{-/-}$ mice in experimental hypertension[38]. The SH2B3-constructed co-expression network of human peripheral blood also included the Gas6/Axl pathway in relation to high BP genetic susceptibility[11]. We previously showed that deletion of Axl in hematopoietic cells dramatically reduced Mϕs and DCs, and increased accumulation of B cells in the kidney and prevented initiation (1 week) of DOCA-salt hypertension[15]. The protective effects are due to down-regulation of kidney expression of IFN-γ and Th1-dependent pathways in Axl chimeras[15]. However, expression of Axl in BM-derived cells was also contributed to BP and vascular remodeling in the late phase (6 week) of DOCA-salt hypertension[15]. In the current study we showed that Axl is critical for the late survival of $CD4^+$ T cells in peripheral blood and locally via significant increase in pAkt expression and decrease in percentage of

apoptotic cells in the PVA. Observed increases in medial apoptosis in Rag1$^{-/-}$ adoptively transferred with Axl$^{-/-}$ CD4^{+} T cells suggest an important regulatory effect between lymphocytes and smooth muscle cells in late hypertension. On the other hand, an initial vascular response to injury is mostly driven by the immune activation of Axl in smooth muscle cells with modest effect from innate or adaptive immune cells[24, 25]. Collectively, our findings uncover distinct roles for Axl between vascular and immune cells in cardiovascular diseases (vascular injury vs. hypertension).

Measurements of the hemodynamic parameters using tail-cuff plethysmograhy have some limitations associated with stress. However, many laboratories including ours have validated tail-cuff BP measurements by radiotelemetry method. Our recent study in inbred mouse strains showed significant correlation between our tail-cuff training protocol and radiotelemetry measurements of systolic BP[39]. We are confident in our results collected by tail-cuff method because of BP levels reflected degree of arterial remodeling obtained by histology across mice after DOCA-salt. It is possible that Axl-dependent signals in innate immune cells and B lymphocytes are also involved in pathogenesis of DOCA-salt hypertension. A recent report on genetic or pharmacological depletion of B cells attenuated Ang II-induced hypertension and vessel remodeling[40]. Investigation of the TAM family on B lymphocyte function in hypertension warrant additional studies.

In summary, we conclude that Axl is critical for survival of CD4^{+} T cells not only in circulation but also in vascular inflammatory response and vascular remodeling in hypertension. Our study offers a new therapeutic avenue that might target pro-hypertensive T lymphocytes in peripheral blood and ameliorate T cells at the vascular site.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AngII	Angiotensin II
BM	Bone marrow
BMT	Bone marrow transplant
BP	Blood pressure
DC	Dendritic cell
Gas6	Growth arrest-specific protein 6

NK cell	Natural Killer cell
TAM	Tyro3, Axl and Mertk family of receptor tyrosine kinases
Mϕ	Macrophage
SH2B3	SH2B adaptor protein 3
DOCA	Deoxycorticosterone-acetate
IFN-γ	Interferon gamma
IL-4	Interleukin 4
LNK	Lymphocyte adaptor protein
Th1	Type 1 T helper cell
Th2	Type 2 T helper cell
pAkt	Phosphorylated protein kinase B
PBS	Phosphate-buffered saline
PI3K	Phosphoinositide 3-kinase
PVA	Perivascular adventitia
Rag1	Recombination activating gene 1
SOCS1	Suppressor of cytokine signaling 1
SOCS3	Suppressor of cytokine signaling 3
STAT1	Signal transducer and activator of transcription 1

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Highlights

- Axl, a receptor tyrosine kinase, is required for peripheral T lymphocyte survival
- Axl-mediated $CD4^+$ T cell survival is critical for elevated blood pressure and vascular remodeling in the late phase of hypertension

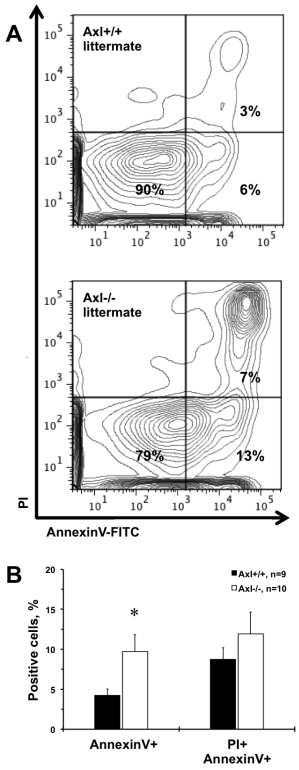

Figure 1. Critical role of Axl in leukocytes survival

Representative flow data for: $Axl^{+/+}$ littermate; $Axl^{-/-}$ littermate (**A**). The lower-right quadrant represents early apoptotic leukocytes ($AnnexinV^{+}$), while the upper-right quadrant – necrotic cells ($PI^{+}AnnexinV^{+}$) from Axl mice peripheral blood. **B.** Quantitation of apoptotic, necrotic and live cells between Axl genotypes. Black bars represent $Axl^{+/+}$ mice. Open bars represent $Axl^{-/-}$ mice. Values are mean±SEM. *, $p<0.05$ vs. $Axl^{+/+}$. n, Number of mice.

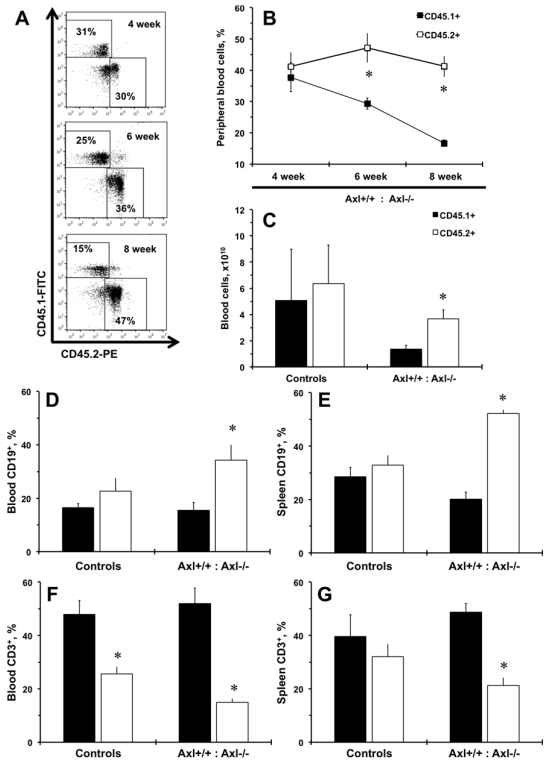

Figure 2. Competitive repopulation of Axl bone marrow cells after bone marrow transplant

A. Representative flow charts of double stained CD45.1 ($Axl^{+/+}$) and CD45.2 ($Axl^{-/-}$) peripheral leukocytes from Axl chimeric mice. **B.** A time-course of competitive repopulation ($CD45.1^+$ and $CD45.2^+$; 50% and 50%) after 8 weeks of bone marrow transplant (BMT). **C.** Total numbers of $CD45.1^+$ and $CD45.2^+$ cells in Axl chimeras at 8 weeks after BMT. **D.** Percentages of $CD19^+$ cells in blood. **E.** Percentages of $CD19^+$ cells in spleen. **F.** Percentages of $CD3^+$ cells in blood. **G.** Percentages of $CD3^+$ cells in spleen. Black bars represent $Axl^{+/+}$ ($CD45.1^+$) cells. Open bars – $Axl^{-/-}$ ($CD45.2^+$) cells. Values are mean ±SEM. *, $p<0.05$ vs. $Axl^{+/+}$. Controls, n=4. Axl chimeras, n=10.

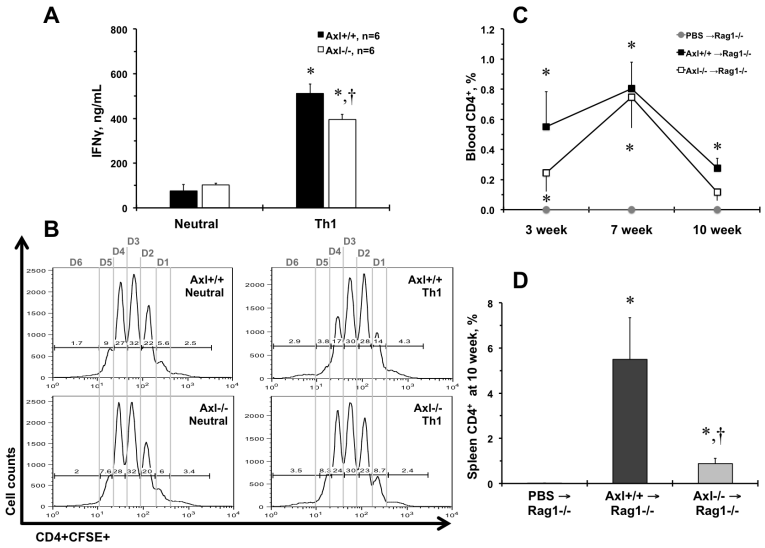

Figure 3. Axl regulates T cell functions

A. Secretion of interferon gamma (IFN-γ) under Th1 conditions. Black bar represent $Axl^{+/+}$ cells. Open bar represent $Axl^{-/-}$ cells. *, $p<0.05$ vs. Neurtal conditions. †, $p<0.05$ vs. $Axl^{+/+}$ Th1 cells. n, Number of replicates. **B.** Representative flow charts of CFSE proliferation of Axl $CD4^+$ T cell in 6 cell divisions (Division 1 – Division 6). n=5. **C.** A time-course of $CD4^+$ T cell repopulation after adoptive transfers to $Rag1^{-/-}$ mice in blood. Grey circle and grey line show $Rag1^{-/-}$ mice injects with PBS (n=4). Black squares and black line – $Axl^{+/+}$ $CD4^+ \rightarrow Rag1^{-/-}$ mice (n=6). Open squares and black line – $Axl^{-/-}$ $CD4^+ \rightarrow Rag1^{-/-}$ mice (n=6). **D.** Percentages of $CD4^+$ T cells in spleen after 10 weeks after adoptive transfer to $Rag1^{-/-}$ mice. Open bar shows PBS $\rightarrow Rag1^{-/-}$ mice. Dark grey bar – $Axl^{+/+}$ $CD4^+ \rightarrow Rag1^{-/-}$ mice. Light grey bar – $Axl^{+/+}$ $CD4^+ \rightarrow Rag1^{-/-}$ mice. Values are mean±SEM. *, $p<0.05$ vs. PBS $\rightarrow Rag1^{-/-}$ mice. †, $p<0.05$ vs. $Axl^{+/+}$ $CD4^+ \rightarrow Rag1^{-/-}$ mice.

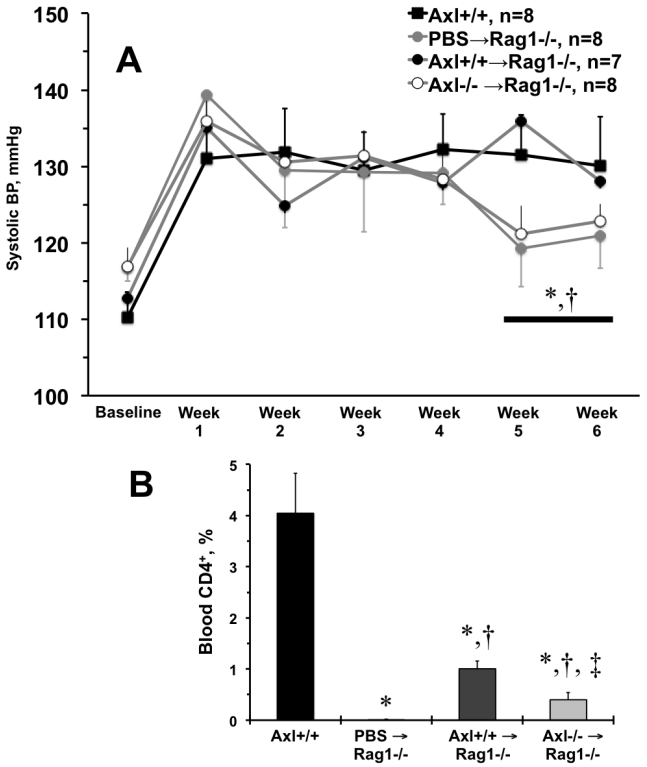

Figure 4. Axl controls circulating CD4$^+$ T cells during late phase of salt-dependent hypertension

A. Changes in systolic blood pressure (BP) after deoxycorticosterone acetate (DOCA) and salt in mice after adoptive transfer of CD4$^+$ T cells to Rag1$^{-/-}$ mice time-course of. Black squares and black line show Axl$^{+/+}$ mice. Grey circles and grey line – PBS → Rag1$^{-/-}$ mice. Black squares and grey line – Axl$^{+/+}$ CD4$^+$ → Rag1$^{-/-}$ mice. Open circles and grey line – Axl$^{-/-}$ CD4$^+$ → Rag1$^{-/-}$ mice. **B.** Percentage of CD4$^+$ cells in peripheral blood from experimental mice. Black bar shows Axl$^{+/+}$. Open bar – PBS → Rag1$^{-/-}$ mice. Dark grey bar – Axl$^{+/+}$ CD4$^+$ → Rag1$^{-/-}$ mice. Light grey bar – Axl$^{-/-}$ CD4$^+$ → Rag1$^{-/-}$ mice. Values are mean±SEM. *, $p<0.05$ vs. Axl$^{+/+}$. †, $p<0.05$ vs. PBS → Rag1$^{-/-}$ mice. ‡, $p<0.05$ vs. Axl$^{+/+}$ CD4$^+$ → Rag1$^{-/-}$ mice. n, Number of mice in each group.

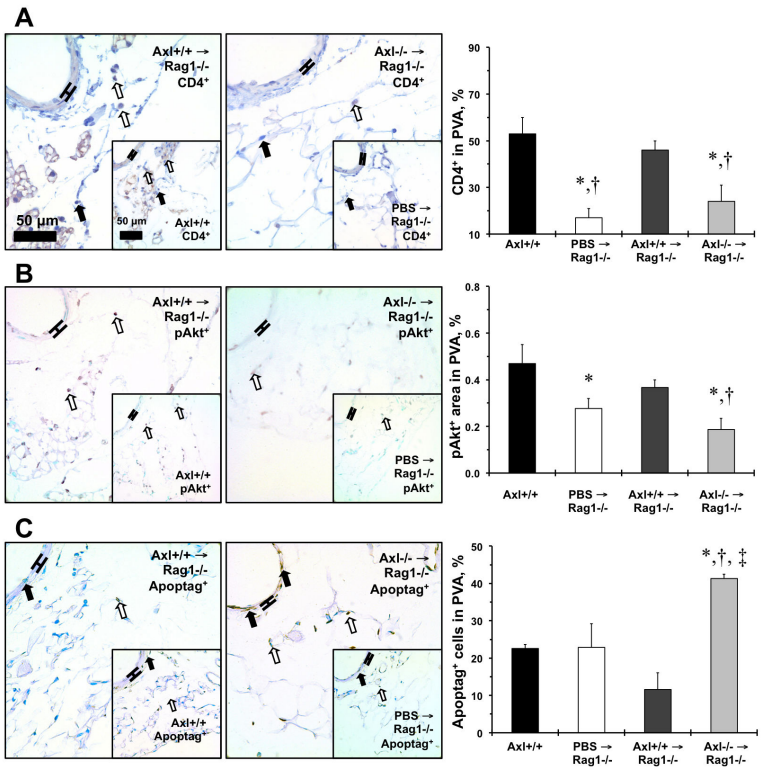

Figure 5. Immunohistochemical evaluation of the arteries from mice after CD4$^+$ T cell repopulation and DOCA- salt hypertension

A. CD4$^+$ cells and quantification in the perivascular adventitia (PVA) of mesenteric artery. **B.** p-Akt+ cells and quantification in the PVA of mesenteric artery. **C.** Apoptag$^+$ cells and quantification in the PVA of mesenteric artery. Black bars show Axl$^{+/+}$. Open bars – PBS → Rag1$^{-/-}$ mice. Dark grey bars – Axl$^{+/+}$ CD4$^+$ → Rag1$^{-/-}$ mice. Light grey bars – Axl$^{-/-}$ CD4$^+$ → Rag1$^{-/-}$ mice. Values are mean±SEM. *, $p<0.05$ vs. Axl$^{+/+}$. †, $p<0.05$ vs. PBS → Rag1$^{-/-}$ mice. ‡, $p<0.05$ vs. Axl$^{+/+}$ CD4$^+$ → Rag1$^{-/-}$ mice. n=3 per each group.