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IMMUNODEFICIENT R2G2 MOUSE STRAIN YIELDS SPLEENS WITH UNUSUAL CYTOARCHITECTURE AND SYMPATHETIC INNERVATION

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IMMUNODEFICIENT R2G2 MOUSE STRAIN YIELDS SPLEENS WITH UNUSUAL CYTOARCHITECTURE AND SYMPATHETIC INNERVATION

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Abstract

The nervous system and immune system contact one another through two-way communication in order to establish and preserve homeostasis. The sympathetic neurotransmitter norepinephrine has an impact on how the immune system responds by affecting regional blood flow and activation of adrenergic receptors on leukocytes. Former studies showed that immune cells are capable of releasing nerve growth factor allowing for the establishment and continuation of sympathetic nerves in targeted tissues. From this gathered information, it was hypothesized that sympathetic nerves would prove to be less frequent in spleens from the immunodeficient R2G2 mouse strain (Envigo) when compared to 129P3/J (129) and C57BL/6 (C57) strains. R2G2 mice are an immunodeficient strain that lacks functional T, B, and natural killer cells. Ten to eleven week aged-matched male mice were measured by body weight, spleen weight, and temperature. Spleens were cut and fixed for histological investigation. Sympathetic nerves were labeled by immunostaining tyrosine hydroxylase (TH). Hematoxylin & eosin (H&E) was used to stain spleen sections in order to evaluate cytoarchitecture. Von Willebrand factor (VWF) was used to immunostain for megakaryocytes. R2G2 mice showed slightly higher temperatures and body weights but yielded a significantly smaller spleen weight (R2G2, 38.20 ± 1.48; 129, 65.08 ± 11.71; C57, 81.33 ± 8.38; P< 0.0001, ANOVA). TH stain revealed sympathetic innervation in all strains but location and morphology differed in R2G2 mice compared to controls. Control spleens had nerves which entered white pulp regions of the spleen and were closely related to leukocytes. Fiber profiles in the controls were filamentous with small acute bends. R2G2 differed by having (TH+) nerve fibers more associated with arteries and less localized in the surrounding parenchyma. The fibers were abnormally swollen and held a more granular shape instead of a filamentous shape. The H&E stain showed clear red and white pulp zones in the control spleens with 129 showing more distinct germinal centers than C57. R2G2 H&E sections showed cytoarchitecture with indistinct pulp areas. VWF staining revealed R2G2 mice had an abundant amount of megakaryocytes versus control mice megakaryocyte counts (R2G2, 11.28 ± 3.87 per 20X field; 129, 1.73 ± 0.70; C57, 1.42 ± 0.13; P< 0.0001, ANOVA) and extramedullary hematopoiesis was highly prominent. This evidence supports that leukocytes secrete neurotrophic factors or are vital to establishing normal growth of TH+ nerves toward the white pulp. Leukocytes may not be required for sympathetic innervation of blood vessels in the spleen, however, lack of leukocytes shows TH+ nerve fibers with abnormal morphology in severely immune threatened mice.

Introduction

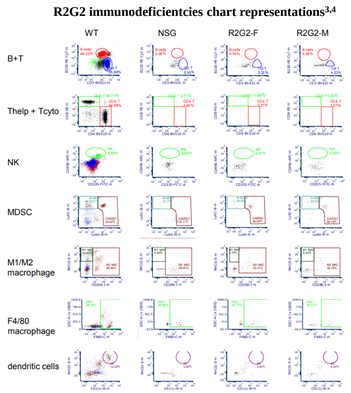
The nervous system and immune system interact through two-way communication in order to establish and preserve homeostasis. The sympathetic neurotransmitter norepinephrine has an impact on how the immune system responds by affecting regional blood flow and activating adrenergic receptors on leukocytes. Its is well-known that nerve growth factor (NGF) is required for development and maintenance of sympathetic neurons throughout life. NGF is a target-derived neurotrophic factor, and recent evidence has shown that it is released by leukocytes, which are targets for norepinephrine in the spleen. Previous studies from our laboratory have shown that NGF upregulation occurs during the acute phase of sepsis in a model model and that increased NGF expression is associated with sympathetic nerve sprouting.[1] In contrast, loss of leukocytes in patients who died from end stage sepsis was associated with loss of sympathetic nerves.[2] These findings suggest that changes in supply of NGF from leukocytes can alter sympathetic innervation of the spleen.

Hypothesis:
It was hypothesized that sympathetic nerves would prove to be less frequent in spleens from the immunodeficient R2G2 mouse strain (Envigo) when compared to 129P3/J (129) and C57BL/6 (C57) control strains.

Materials and Methods

Animals:
Ten to eleven week old Rag2/IL2RG (R2G2) mice and control 129P3/J (129) and C57BL/6 (C57) mice from Jackson Labs were studied. After collecting weights and rectal temperatures, mice were euthanized with isoflurane, and the spleen was removed and weighed.
The R2G2 mouse model is a double knockout mouse with an ultra immunodeficient phenotype. These mice are deficient in T cells and B cells, lack NK cells, and have decreased macrophages, dendritic cells, and neutrophils. R2G2 mice were generated on a mixed background of 129 and C57.[3]

R2G2 immunodeficientcies chart representations[3,4]



Results

R2G2 Mice have Undefined Spleen White Pulp

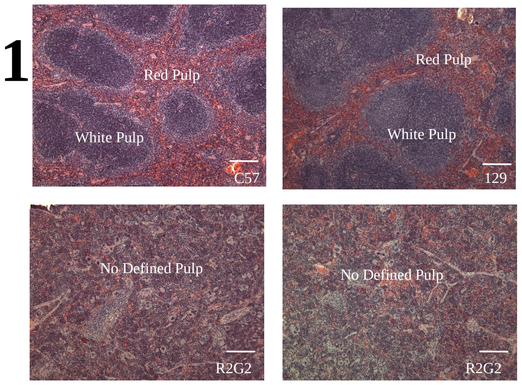


Fig. 1 H&E stain with scale bars have a length of 175 microns. Magnification taken at 10x. Labeled pulp regions.

High Spleen Megakaryocyte Counts in R2G2 Mice

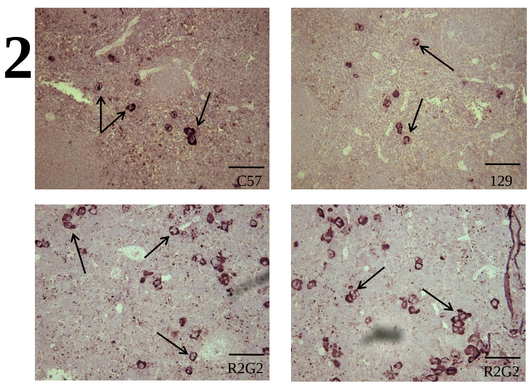


Fig. 2 VWF immunostain with scale bars have a length of 100 microns. Magnification taken at 20x. Arrows point to scattered megakaryocytes.

Irregular Spleen Sympathetic Innervation in R2G2 Mice

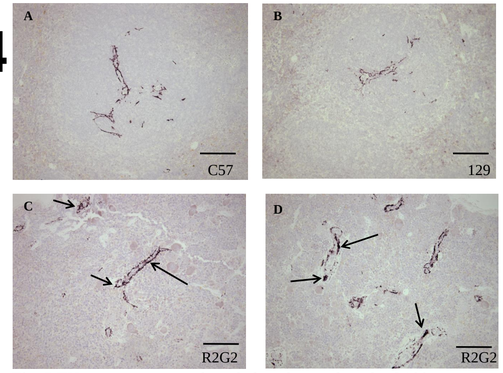


Fig. 5 TH immunostain with scale bars have a length of 100 microns. Magnification taken at 20x. Arrows indicate large abnormalities.

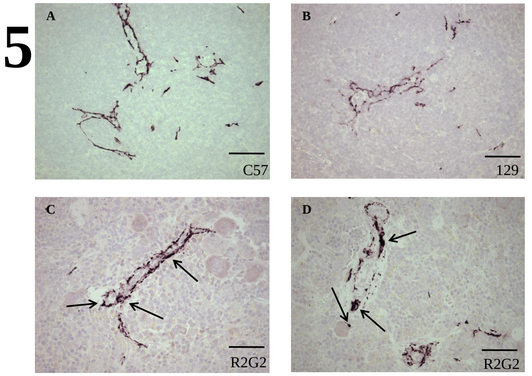


Fig. 6 TH immunostain with scale bars have a length of 50 micron. Magnification taken at 40x. Arrows indicate large abnormalities.

Materials and Methods

Histological Staining:
Spleens were fixed in formalin overnight and embedded in paraffin. Embedded tissues were cut at 5 mm thickness using a microtome, and sections were collected on charged slides. Standard procedures were conducted in order to perform hematoxylin and eosin (H&E) staining in the spleen in order to observe tissue morphology. Images of the H&E stained spleen sections were collected at 10x magnification using an Olympus BX4 microscope equipped with a digital camera and Qcapture software.

Statistical Analysis:
Statistic comparisons were made using one-way analysis of variance (ANOVA). Tukey's post-hoc test was used to further analyze data. A probability level of 0.05 or smaller was used for testing of statistical significance. Prism software was used for statistical analysis and graphing.

Immunohistochemistry:
Spleen sections were immunolabeled for the sympathetic nerve marker tyrosine hydroxylase (TH) using rabbit anti-TH (1:1000, PelFreez) and for the megakaryocytes marker Von Willebrand factor (VWF) using rabbit anti-VWF (1:400, Dako). Immunostaining was done using a ABC Elite kit and VIP chromogen, both from Vector Labs. Stained sections were viewed with an Olympus BX4 microscope and images collected using a digital camera and Qcapture software. Images of VWF staining were collected at 20X used for counting megakaryocytes. Stereo Investigator software was used to accurately count megakaryocytes per slide to prevent repeating counts.

Results

Characteristics of Control and R2G2 Mice Strains

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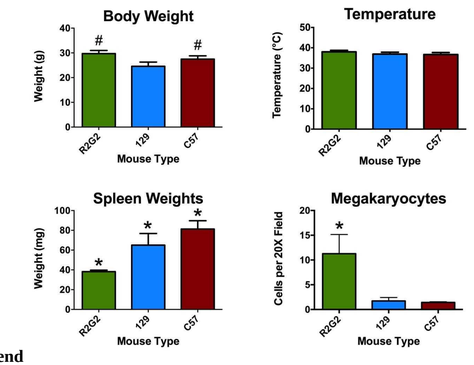

Fig. 3 Legend
Bar graphs comparing body weight, temperature, spleen weight, and abundance of megakaryocytes in R2G2 and control mouse strains. Values are means ± SDs (n=5 per group). For each parameter, data were evaluated by one-way ANOVA with P<0.05 considered a significant difference. Specific differences between groups were identified using Tukey's multiple comparison test. *Different from other strains. #Different from 129 strain.

Summary and Conclusions

- R2G2 mice maintain normal body weight and temperature but have significantly smaller spleens. Lower spleen weight is probably due to leukocyte deficiency.
- The H&E stain showed clear red and white pulp zones in the control spleens with 129 showing more distinct germinal centers than C57. H&E stained sections from R2G2 mice showed cytoarchitecture with indistinct pulp areas. Extramedullary hematopoiesis and large cells, presumed to be megakaryocytes, were highly prominent in R2G2 spleens.
- VWF staining of spleen sections confirmed the presence of megakaryocytes and their greater abundance in R2G2 mice versus control mice (R2G2, 11.28 ± 3.87 per 20X field; 129, 1.73 ± 0.70; C57, 1.42 ± 0.13; P< 0.0001, ANOVA).
- TH stain revealed sympathetic innervation in all strains but location and morphology differed in R2G2 mice compared to controls. Control spleens had nerves which entered white pulp regions of the spleen and were closely related to leukocytes. Fiber profiles in the controls were filamentous with small acute bends. R2G2 differed by having (TH+) nerve fibers more associated with arteries and less localized in the surrounding parenchyma. The fibers were abnormally swollen and held a more granular shape instead of a filamentous shape.
- This evidence supports that leukocytes secrete neurotrophic factors or are vital to establishing normal growth of TH+ nerves toward the white pulp. Leukocytes may not be required for sympathetic innervation of blood vessels in the spleen, however, lack of leukocytes shows TH+ nerve fibers with abnormal morphology in severely immune threatened mice.

References

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