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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 interact through two-way communication in order to establish and preserve homeostasis. The sympathetic nervous system may play an important role in the body's immune response by affecting regional blood flow and activating the adrenergic receptors on leukocytes. It is well-known that nerve growth factor (NGF) is required for development and maintenance of sympathetic nerves throughout life. NGF is a target-derived neurotrophic factor, and recent evidence has shown that it is found in leukocytes, which are targets for neurotrophins in the spleen. Previous studies from our laboratory have shown that NGF expression occurs during the acute phase of sepsis in a model model and that increased NGF expression is associated with sympathetic nerve sprouting. In contrast, loss of leukocytes in patients who died from end stage sepsis has an impact on how the immune system develops and characterizes of the ultrastructure of the spleen in R2G2 mice versus control mice.

Introduction

The nervous system and immune system interact through two-way communication in order to establish and preserve homeostasis. The sympathetic nervous system may play an important role in the body's immune response by affecting regional blood flow and activating the adrenergic receptors on leukocytes. It is well-known that nerve growth factor (NGF) is required for development and maintenance of sympathetic nerves throughout life. NGF is a target-derived neurotrophic factor, and recent evidence has shown that it is found in leukocytes, which are targets for neurotrophins in the spleen. Previous studies from our laboratory have shown that NGF expression occurs during the acute phase of sepsis in a model model and that increased NGF expression is associated with sympathetic nerve sprouting. In contrast, loss of leukocytes in patients who died from end stage sepsis has an impact on how the immune system develops and characterizes of the ultrastructure of the spleen in R2G2 mice versus control mice.

Materials and Methods

Animals:

Ten to twelve week old Rag2L-IgBlg (R2G2) mice and control 129P3/J (129) and C57BL/6 (C57) control strains.

Histological Staining:

Spleens were fixed in formalin overnight and embedded in paraffin. Embedded tissues were cut at 5 μm 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 on an Olympus BX microscope equipped with a digital camera and Qcapture software. Images of H&E staining were collected at 20x used for counting megakaryocytes. Stereo Investigator software was used to accurately count megakaryocytes per slide to prevent repeating counts.

Statistical Analysis:

Statistical 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 the for the megakaryocytes marker Von Willebrand factor (VWF) using rabbit anti-VWF (1:140, Dako). Immunostaining was done using an ABC Elite kit and VIP chromogen both from Vector Labs. Stained sections were viewed with an Olympus BX 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.

Summary and Conclusions

• R2G2 mice maintain normal body weight and temperature but have significantly smaller spleen. Lower spleen weight is probably due to leukocyte deficiency.
• The H&E stained showed 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 ± 1.87 per 20X) (129, 1.73 ± 0.90; 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 more 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, immunolabeled with 4 contrast, showed a more granular shape instead of a filamentous shape.
• This evidence suggests 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.

Materials and Methods

Results

R2G2 Mice have Undefined Spleen White Pulp

High Spleen Megakaryocyte Counts in R2G2 Mice

Irrregular Spleen Sympathetic Innervation in R2G2 Mice

Fig. 1 H&E stain with scale bars have a length of 157 microns. Magnification taken at 10x. Labelled pulp regions.

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

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, 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.

References

4. Brit, N. M. Molecular and Viral Immunology Development and characterization of the ultra immuno-deficient B6-L20%Islet Single Knockout (SKO) mice model.

https://energyresearchmodels.com/resmodels/gmo?opt=22655&func=2

http://nicholasbritt.com/wp-content/themes/MouseTemplate/resources/figures/1578.jpg?t=1522241230655

Fig. 4 TUN immunostain with scale bars have a length of 157 microns. Magnification taken at 10x. Arrows indicate large abnormalities.

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 microns. Magnification taken at 40x. Arrows indicate large abnormalities.