# Comparative analysis of complete blood count, serum chemistry and immunodeficient phenotype between SRG and CD rats

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## 1 INTRODUCTION

The number of cancer drugs in development has doubled over the last decade, increasing the need for well characterized immunodeficient animal models that allow human xenograft engraftment, an important component of the drug development process. The immunodeficient SRG Rat®* is well suited for such studies and has demonstrated utility across multiple research applications from tumor metastasis to drug validation [1-3]. While T, B, and NK cells immunodeficiency in the SRG rat is known, our understanding of its other immune components, such as monocytes as well as the complement cascade and serum chemistry, remains limited.

To further characterize and understand the SRG rat, we examined several parameters focusing on complete blood count, serum chemistry, complement cascade activity, and monocyte levels in naïve animals compared to the CD® (Sprague Dawley) rats. Potential immunophenotypic differences between male and female SRG rats were also explored in this study.

## 2 METHODS

**Complement activity**

Serum was collected from 3 age-matched naïve SRG rats and 3 age-matched naïve CD rats of each sex. Complement activity was measured using the Rat complement pathway assay kit from Hycult® Biotech and performed according to manufacturer's instruction. Statistical analysis was done using unpaired t-test, with a statistical cut-off of $p < 0.05$.

**Complete blood count and serum chemistry**

Naïve animals were not fasted prior to whole blood and serum collection. Whole blood and serum were collected from age-matched (5 weeks) SRG and CD rats (5 males and 5 females of each strain). Samples were processed according to standard procedures established at the test facility. Values of acquired parameters are listed in Tables 1 and 2.

**Immunophenotyping**

Whole blood was collected from 20 4-6 weeks old naïve SRG rats of each sex (n=40) with 2 naïve CD (Sprague Dawley) rats of each sex used as control group (n=4). Whole blood samples were processed according to standard procedures established at the test facility. Samples were analyzed by flow cytometry for CD4 and CD8 positive T cells, B cells, NK cells and monocytes. Prior to immunophenotyping, ACK-lysed blood samples were counted to check viability and yield. Cells were stained, fixed and resuspended in a final volume of 300 µL of BD Stain buffer for acquisition at 100 µL/min on an Attune NxT flow cytometry system (ThermoFisher). Gating strategy was done as previously described[4]. Markers used for flow cytometry are listed in Table 3. Statistical analysis was done using unpaired t-test, with a statistical cut-off of $p < 0.05$.

Animal procedures were reviewed and approved by CRL IACUC and performed in an AAALAC accredited facility.

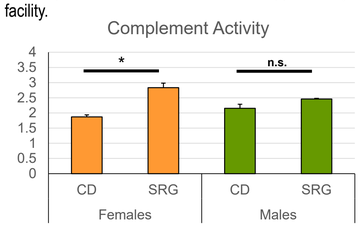


**Figure 1.** Analysis of complement activity in SRG rats and control CD rats. SRG rats demonstrate active complement pathway in both male and female SRG rats. Female SRG rats demonstrated a 1.5-fold increase in activity compared to female CD rats.
*** : p < 0.05; n.s. : not significant.**

## 3 RESULTS

| Sample | CD | | SRG | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| Absolute Reticulocyte (K/uL) | 590.1 | 473-658 | 504.9 | 406-603 |
| Reticulocyte Hemoglobin Content (pg) | 23.32 | 21.4-28 | 22.62 | 21.8-24 |
| RBC (M/uL) | 6.236 | 5.39-6.86 | 7.31 | 6.69-7.84 |
| HGB (g/dL) | 12.23 | 11.3-13.3 | 13.47 | 12.3-14.7 |
| Nucleated RBC (/100 WBC) | 0.1 | 0-1 | 0 | 0-0 |
| WBC (K/uL) | 6.35 | 2.3-9.7 | 1.5 | 0.6-2.2 |
| Lymphocyte (/uL) | 5102.8 | 1739-8488 | 763.7 | 340-1105 |
| Neutrophil (/uL) | 928 | 271-2912 | 497.6 | 169-862 |
| Monocyte (/uL) | 248.3 | 92-428 | 227.9 | 0-412 |
| Eosinophil (/uL) | 52.9 | 0-262 | 8.1 | 0-20 |
| MCV (fL) | 73.9 | 68-81 | 65.5 | 62-68 |
| Basophil (/uL) | 18.1 | 0-67 | 2.9 | 0-19 |
| MCH (pg) | 19.66 | 18.5-21 | 18.44 | 17.9-19.3 |
| MCHC (g/dL) | 26.58 | 25.8-27.3 | 28.13 | 27.4-28.7 |
| Platelet Count (K/uL) | 727 | 438-1138 | 616.1 | 449-771 |

**Table 1.** Summary of complete blood count analysis in naïve CD and SRG rats. Animals were not fasted prior to blood collection. Mean values and range are shown in table. **N=10 of each strain.**

| Sample | CD | | SRG | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| ALP (U/L) | 533.1 | 407-687 | 450.1 | 305-680 |
| AST (U/L) | 190.6 | 103-548 | 196.9 | 67-596 |
| ALT (U/L) | 72.4 | 36-105 | 54.1 | 39-115 |
| Creatine kinase (U/L) | 807.4 | 198-3000 | 1005.8 | 212-3917 |
| GGT (U/L) | 0.5 | 0-1 | 0.1 | 0-1 |
| Amylase (U/L) | 667.4 | 541-866 | 725 | 541-999 |
| Lipase (U/L) | 9.9 | 6-18 | 19.4 | 9-28 |
| Albumin (g/dL) | 3.31 | 2.9-3.6 | 3.44 | 3.3-3.7 |
| Total Bilirubin (mg/dL) | 0.1 | 0.1-0.1 | 0.1 | 0.1-0.1 |
| Total Protein (g/dL) | 5.4 | 4.8-5.9 | 5.86 | 5.6-6.4 |
| Bilirubin - Conjugated (mg/dL) | 0 | 0 | 0.01 | 0-0.1 |
| BUN (mg/dL) | 16.7 | 15-19 | 20 | 12-27 |
| Creatinine (mg/dL) | 0.2 | 0.1-0.3 | 0.26 | 0.2-0.3 |
| Cholesterol (mg/dL) | 135.5 | 109-228 | 184.5 | 114-215 |
| Glucose (mg/dL) | 241.5 | 164-311 | 213.1 | 171-287 |
| Calcium (mg/dL) | 12.89 | 12.5-13.3 | 12.79 | 11.8-13.2 |
| Phosphorus (mg/dL) | 13.46 | 12.2-14.6 | 12.48 | 12.1-13.1 |
| Bicarbonate TCO2 (mmol/L) | 30.9 | 25-34 | 30.7 | 27-35 |
| Chloride (mmol/L) | 99 | 96-101 | 96.8 | 95-99 |
| Potassium (mmol/L) | 8.09 | 7-9.4 | 7.29 | 6.2-8.4 |
| Sodium (mmol/L) | 142.8 | 141-146 | 142.4 | 139-145 |
| LDH (U/L) | 514.7 | 157-1898 | 696.6 | 164-2063 |
| Bilirubin - Unconjugated (mg/dL) | 0.1 | 0.1-0.1 | 0.09 | 0-0.1 |
| Triglycerides (mg/dL) | 97.5 | 69-155 | 89.3 | 59-145 |
| Uric Acid (mg/dL) | 3.8 | 3.2-4.7 | 2.99 | 1.8-3.9 |

**Table 2.** Summary of serum chemistry analysis in naïve CD and SRG rats. Animals were not fasted prior to serum collection. Mean values and range are shown in table. **N=10 of each strain.**

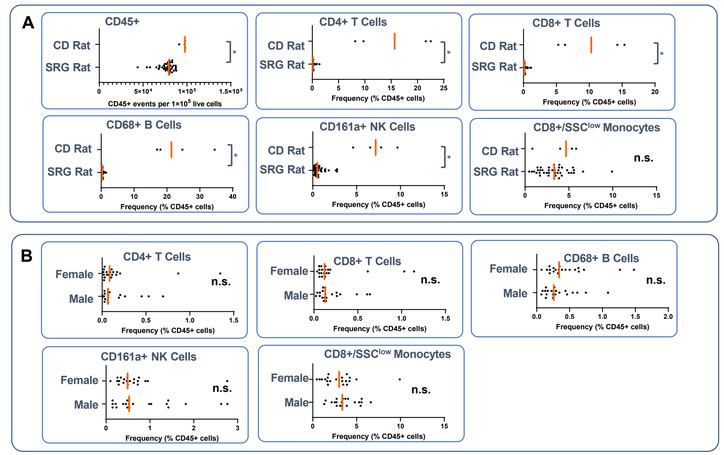


**Figure 2.** Flow cytometry analysis of immune cell subpopulations. **A.** Reduced levels of circulating T, B, and NK cells in were observed in SRG rats compared to CD® rats but demonstrate no statistically significant difference in monocyte levels (previously published)[4]. CD45+ subpopulation are represented as number of CD45 positive events per $10^5$ live cells. All other cell populations are represented as percentage of CD45+ cells. Each dot represents a single animal. Orange line represents mean value. **B.** No statistically significant differences in T, B, NK cells and monocytes were observed between male and female SRG rats.
*** : p < 0.05; n.s. : not significant.**

| Marker | CD rat | SRG rat |
|---|---|---|
| CD45+ (per $10^5$ live cells) | 96050 ± 3307 | 76740 ± 9121 |
| CD4+ (% CD45+) | 15.5 ± 7.6 % | 0.16 ± 0.3 % |
| CD8+ (% CD45+) | 10.3 ± 5.2 % | 0.22 ± 0.3 % |
| B-cells (% CD45+) | 23.6 ± 8.1 % | 0.4 ± 0.3 % |
| NK cells (% CD45+) | 7.2 ± 2.1 % | 0.76 ± 0.7 % |
| Monocytes (% CD45+) | 4.0 ± 2.2 % | 3.4 ± 1.8 % |

**Table 3.** Summary of immune cell subpopulations in CD and SRG rats. CD45+ cells are represented as number of CD45+ events per $10^5$ live cells. Other subpopulations are indicated as percentage of CD45+ cells Mean ± s.d. are shown.

## 4 CONCLUSION

- This is the first known study examining complete blood count and serum chemistry in SRG rats.
- Male and female SRG rats possess an active complement pathway and demonstrate similar immunodeficiencies.
- The SRG Rat® has reduced levels of circulating T, B and NK cells but demonstrated no statistically significant difference in circulating levels of monocytes compared to CD rats.
- Data presented here is a critical first step towards better understanding the SRG rat and aid in its application in future discovery and safety studies.

**References**

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3. Yamamoto, Ami et al. *Proceedings of the National Academy of Sciences of the United States of America* vol. 120,10 (2023): e2214888120. doi:10.1073/pnas.2214888120.
4. Aw Yong, Koh Meng et al. Cancer Res 1 April 2023; 83 (7_Supplement): 51. https://doi.org/10.1158/1538-7445.AM2023-51.

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