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# Total Leucocytes and Lymphocytes Correlates with T cell deficiency and not on B or NK cell deficiency in mice

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## Abstract

The objective of this study was to compare changes in leucocyte and lymphocyte analytes in various models of immunodeficient mice lacking T or B or NK cells or both T and B cells. In this study, we used the following immunodeficient mice (nu; T inactive B+ NK+), (IgH-6$^{-/-}$; T+B inactive NK+) (beige; T+B+NK inactive) and SCID and RAG-1$^{-/-}$ (T inactive B inactive NK$^{+}$). Among the T cell deficient (Ii$^{-/-}$, CD8+, CD4 Inactive) and (TAP-1$^{-/-}$; CD 4 inactive and CD8$^{+}$) were used. FACS analyses of peripheral-blood mononuclear cells were performed to determine the percentage of CD3$^{+}$ T cell, B220 $^{+}$ B cell and NK cell along with analysis of hematological parameters. There were marked differences in the relative proportions of leucocytes and lymphocytes blood cell population among the immunodeficient strains. These results indicate that WBC and lymphocytes population in whole blood depends on T cells percentage. B cells and NK cells deficiency has minor role in the leucocytes and lymphocytes population in immunodeficient status in mouse models. The hematological differences described here are based on the level of CD3, B220 and NK1.1 cells. This study will provide baseline information for researchers who use various immunodeficient mice for immunological, genetic and cancer studies.

**Key words: immunodeficient mouse, Hematology, FACS**

## Introduction

The immune system is the central defender against outside pathogen and neoplasm. However, the genome and the immune system between human and mouse is similar, making mouse the most widely used model to explore the key processes of immune system and in revealing the molecular mechanisms of immunological diseases (Venter *et al*., 2001). Immunodeficient rodents are essential models for investigators for various studies. Today, biomedical researchers use genetically modified mice and rats to study the immune system, rejection of tissue transplants, infections, cancer, and tumor growth. In humans, hematological parameters have heritability's >50% (Evans *et al*., 1999, Lin *et al*., 2007) and mutations in key genes have important phenotypic consequences. Various hematological parameters are tightly regulated traits with high clinical relevance. Values outside normal ranges are diagnostic for disorders, including cancer, immune disease, and cardiovascular disease. Also peripheral blood provides an overall assessment of leukocyte homeostasis in circulation. Previous studies in mouse have shown that the relative percentage of peripheral blood leukocyte populations varies among mouse strains of young age and also vary with age and sex (Kile *et al*., 2003; Chen *et al*., 2007). Peripheral leukocyte subsets can be dramatically altered by spontaneous and induced mutations (Kirchgessner *et al*., 1995; Jichun *et al*., 2002) also reported that quantitative trait loci is regulating relative lymphocyte proportions in mouse peripheral blood but the authors did not reported the effect of immunodeficiency on leucocytes and lymphocytes changes of the mouse. In this study, we attempted to evaluate the changes in the leucocyte and lymphocyte values in accordance with the type of immunodeficiency.

## Materials and methods

Eight weeks old male mice (n=10) of different immunodeficiency strains were obtained from The Jackson Laboratory USA and maintained in centralized animal facility of National Institute of Immunology (NII). The animals were housed in individually ventilated cages and had an *ad libitum*

access to acidified autoclaved water (pH 2.8-3.1). The animal room was maintained at 23°C on a 12 h light-12 h dark cycle and all procedures were carried in accordance with the CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals) guidelines in a CPCSEA registered animal facility. The strains of mice employed for the current study and their key of immunodeficiency are detailed below:

***T-cell deficiency mice - Nude mice (nu)*** **:** The mutation at Foxn1 (winged-helix/forkhead transcription factor) gene blocks thymus-derived T cells. These mice are athymic, however, they have highly activated NK cells.

***TAP-1 $^{-/-}$ mice (B6.129S2-$^{Tap1tm1Arp}$/J)*** **:** The antigen peptide transporter 1 (TAP1), together with TAP2, constitutes a major histocompatibility complex class 1 antigens (MHC1) transporter maintaining the major pathway of MHC class I surface expression. TAP1 is essential for thymic differentiation of T cells and its deficiency result in a diminished supply of peripheral $CD8^+$ cells.

***Ii$^{-/-}$ mice (B6.129S6-$^{Iitm1Liz}$/J) :*** The invariant chain (Ii) plays a critical role in this process by influencing the expression and peptide loading of the MHC class II molecules. Therefore, coordinate expression of these molecules is believed to play an important role in antigen presentation. $Ii^{-/-}$ mice clearly have a deficiency in the CD4+ T cell compartment as a result of defective positive selection in the thymus.

***B-cell deficient mice - IgH6$^{-/-}$ mice (B6.129S2-$^{Igh-6tm1Cgn}$/J)*** **:** Mice homozygous for the $Ighm^{tm1Cgn}$ targeted mutation are viable and fertile. Homozygous mutant mice lack mature B cells. There is no expression of membrane-bound IgM. It may be useful as a model for B cell immunodeficiency.

***NK cell deficient mice- Beige mice (C57BL/6J-$^{Lystbg-J}$/J*** **:** Mice homozygous for the beige-J spontaneous mutations (Lystbg-J) are identical to the original beige mutation ($Lyst^{bg}$). The phenotype closely resembles Chediak-Higashi disease in man and similar conditions in mink and cattle. They have abnormal NK cell physiology and lower NK cell activity.

***T and B cell deficient mice - SCID mice (NOD.CB17-$^{Prkdc<scid>}$/J)*** **:** SCID mice have defects in T and B cell development due to a mutation in the gene for DNA-dependent protein kinase (DNA PK) on chromosome16.

***Rag-1$^{-/-}$ mice (B6.129S7-$^{Rag1tm1Mom}$/J) :*** Rag1 is essential for the V (D) J gene rearrangements that generate functional antigen receptors in T and B cells; homozygous $Rag1^{tm1Mom}$ mutants have no mature, functional T and B cells.

## Flow cytometric analysis

Flow cytometric (FACS) analysis was done to examine the proportion of various immune cells in the peripheral blood of different strains of mice. Blood samples (200 ul) were drawn from the retro-orbital plexus under Ketamine and Xylazine anesthesia. One hundred microliter of blood was used for FACS analysis using citrate phosphate dextrose (CPD) buffer and rest 100 ul of blood was used for hematology analysis. The following fluorochrome tagged antibodies were used for analysis: fluorescein isothiocyanate (FITC) conjugated anti-mouse CD3, phycoerythrin (PE) conjugated anti-mouse NK 1.1, perdinin chlorophyll Cy 7.7 (PerCp Cy 7.7) conjugated anti-mouse B220, allophycocyanin (APC) conjugated anti-mouse CD4 and APC-Cy7 conjugated anti-mouse CD8 (all from BD Biosciences). All the antibodies were used at a dilution of 1:100. For staining, 50ul of this anti-coagulated whole blood was mixed with 50ul of phosphate buffered saline (PBS) containing the respective antibody and incubated in dark for 30 min. at room temperature. RBC lysis was done using RBC lyse fix solution (BD Biosciences). Appropriate single colour controls were included for compensation purpose. The samples were run in FACS Verse and analyzed using FACS Diva software. The proportion of CD4+ and CD8+ cells were evaluated in CD3+ gated cells. The proportion of B220 cells were evaluated in the lymphocyte gated population.

## Hematology

One hundred microliter of blood were dispensed in the tube containing EDTA as anticoagulant and samples were analyzed (within 10 min of collection) using automated veterinary haematology analyzer MS 4e automated cell counter (Melet Schloesing Laboratories, France). Intra-assay CV (%) of each parameter was tested to evaluate the results of the hematology analyzer.

## Statistical analyses

Statistical analysis was performed using Graph Pad Prism (Version 5 Graph Pad Software. Results are presented as mean ± S.D. One way ANOVA followed by Bonferroni's multiple comparison tests was applied for comparing immunodeficient strain with 95 per cent CI of difference. Statistical significance were considered if $p < 0.05$.

# Results

The CD3, B220 and NK.1.1 cells from the representative immunodeficient strain is stated in figure 1 and 2. The levels of WBC, lymphocytes, monocytes and granulocytes in percentages and absolute values were illustrated in table-1 and figure -3.

The WBC count is reduced in mice lacking T cells as in nude, SCID and Rag-1 (1.5-3 m/mm$^3$). Whereas B and NK cells deficient mice have higher value of WBC count (6-8 m/mm$^3$). The lymphocyte percentage is drastically reduced in SCID, RAG-1$^{-/-}$ and nude mice (24 –35 per cent and 0.4-1.2 m/mm$^3$). However, mice deficient in B cells and NK cells have lymphocyte population ranging (60-80% and 3.2 -9 m/mm$^3$).

Even in the absence of either $CD4^+$ or $CD8^+$ population in T cells the percentage of lymphocytes has no effect. From this study, we could observe that the percentage of lymphocytes independently depend on T cells . The percentage of monocytes was increased (< 10% and 0.4 -0.6 $m.mm^3$) in mice deficient in RAG-$1^{-/-}$ as well as in B cell deficient Igh6 mice as compared to NK cells or T cell- deficient mice. There was no correlation among percentages of either monocyte or absolute monocyte count in immunodeficient mice. The granulocytes population in blood was largely affected (increased 55-60%) in mice lacking RAG-$1^{-/-}$, SCID and nude mice as compared to other immunodeficient mice. There was no correlation within the results (percentages and absolute) of monocytes and granulocytes count in immunodeficiency

## Discussion

Lymphocytes forms a part of immune system and includes T cells (for cell-mediated immunity) B cells (for humoral immunity) and natural killer cells (NK cells) (which function in cell-mediated, cytotoxic innate immunity). Lack of any of these immune cells may lead to immunodeficiency. In humans, various immunodeficiency (primary and secondary) has been developed due to malfunction of the immune cells. Further, (Hennewig *et al*., 2007) reported that lymphopenia is a classical feature of person suffering from T cell or SCID deficiency in infants and adults. The same phenomenon appears in the mice having either SCID or T cell deficiency. The deficiency of either $CD4^+$ or $CD8^+$ in T cells does not have any effect on WBC or lymphocytes population. Similarly, in the current study, B cells or NK cells also does not have any impact on the percentage of lymphocytes. The reason may be due to the fact that T cells contribute for the majority of lymphocytes population in peripheral blood. There was no correlation within the results (percentages and absolute) of monocyte sand granulocytes; hence we could not interpret the changes in immunodeficient mice. The Jackson laboratory (Peter's *et al*., 2002) have analyzed hematological parameters, including per cent population of lymphocytes, neutrophils, and monocytes across 43 inbred strains and concluded that marked variations exists in total white blood cell counts among the inbred strains. In addition, few other mouse strains characteristics have been displayed in the website [The Jackson Laboratory, Bar Harbor, Maine (URL: http://www.informatics.jax.org/)]. One of the limitations of this study is that the hematological parameters are studied in few immunodeficiencies, and only male mice were used. The reason for use of male mice alone is that few studies indicated that sex steroid hormones in females have important regulatory functions in circulating leukocytes (Jilma *et al*.,1994; Chernyshov *et al*., 2002).

To conclude, our observation would help the investigators to understand the levels of WBC and deferential leukocyte count in immunodeficient mice models and know how various deficiencies affects the hematology indices.


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**Figure 1:** Representative percentages of CD3, B220 and NK cells from Nude, Igh$^{-/-}$ , Beige, SCID, RAG-1$^{-/-}$ mice

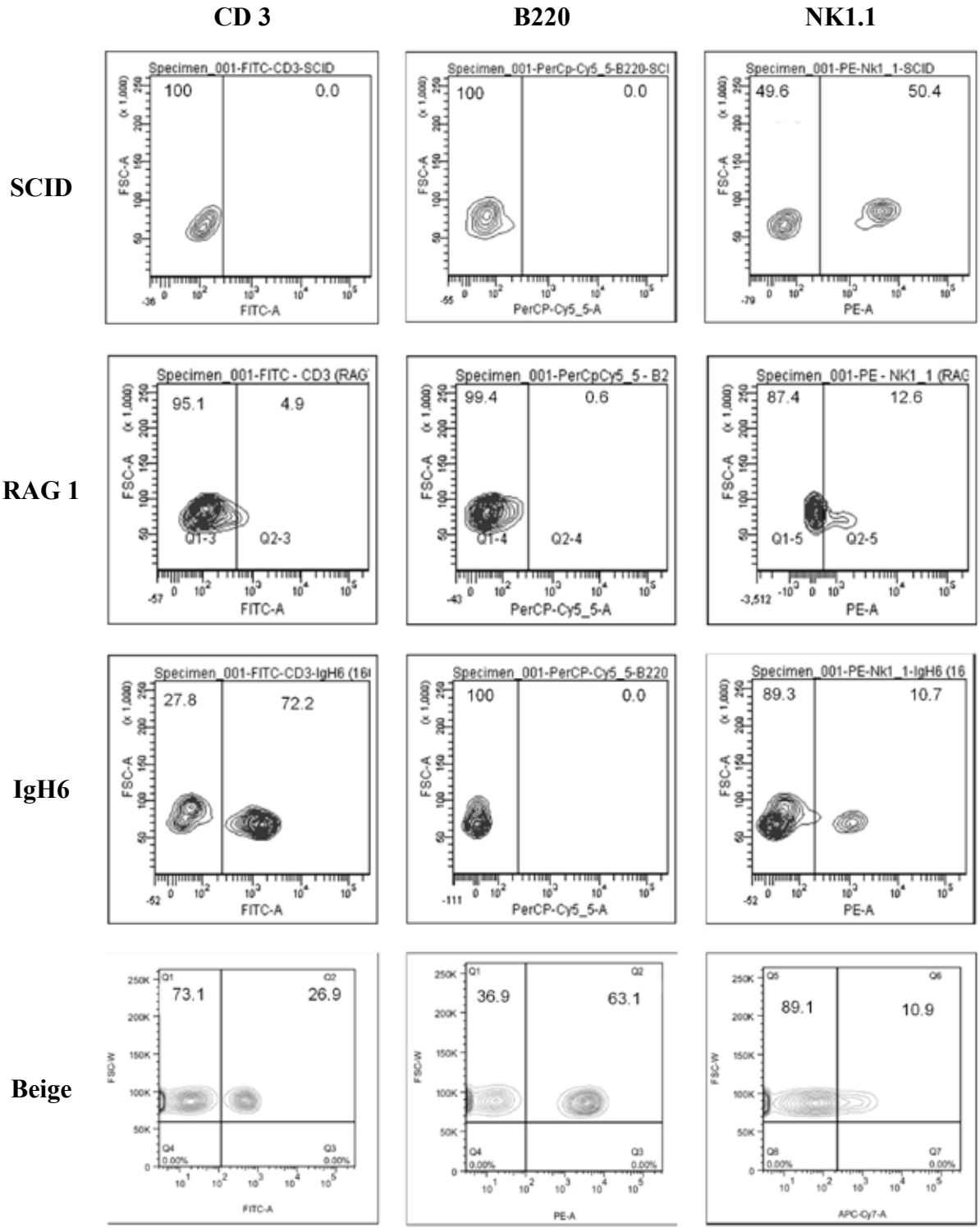


**Figure 2:** Representative percentage of CD4 and CD8 from TAP-1$^{-/-}$ and Ii mice

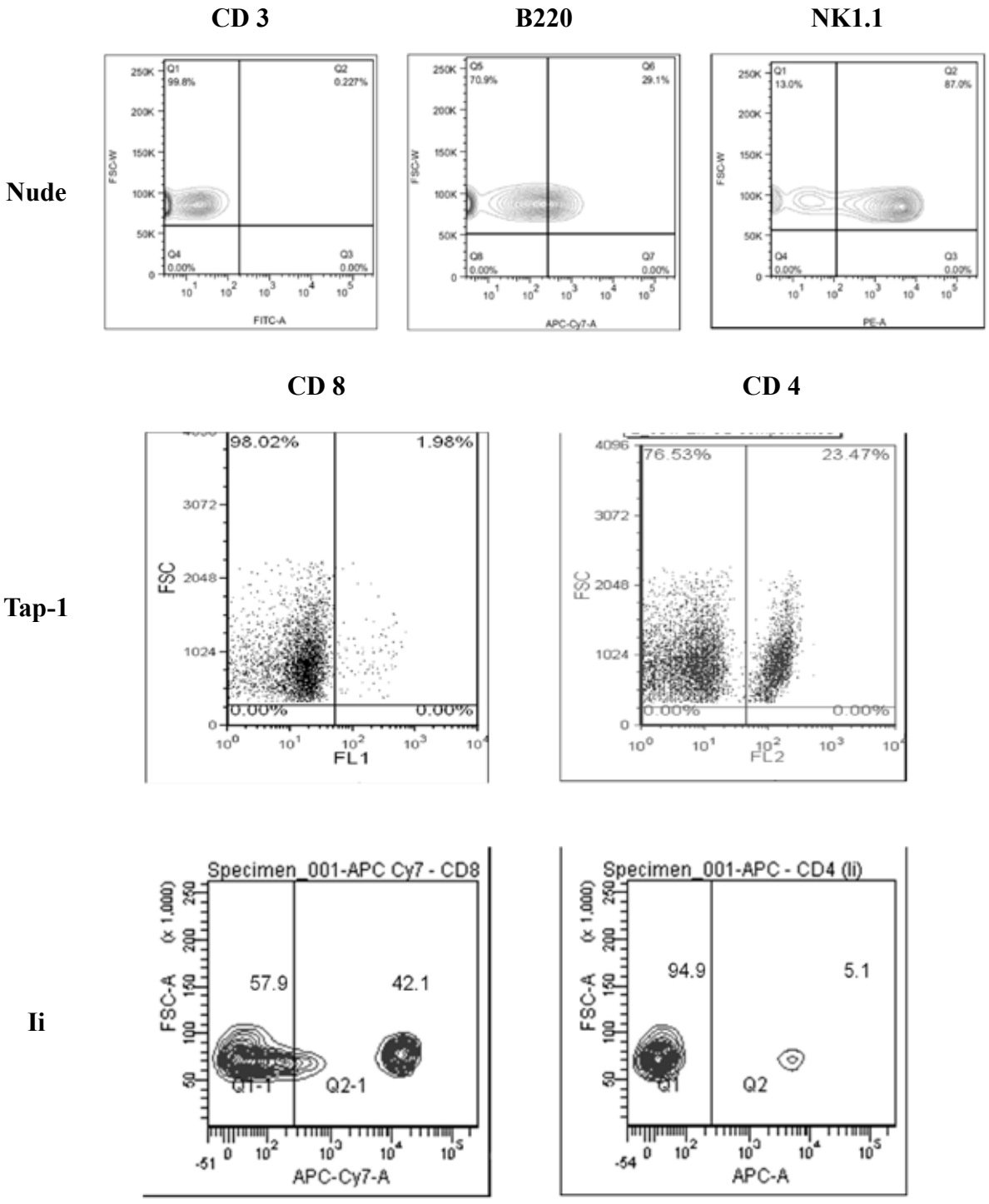
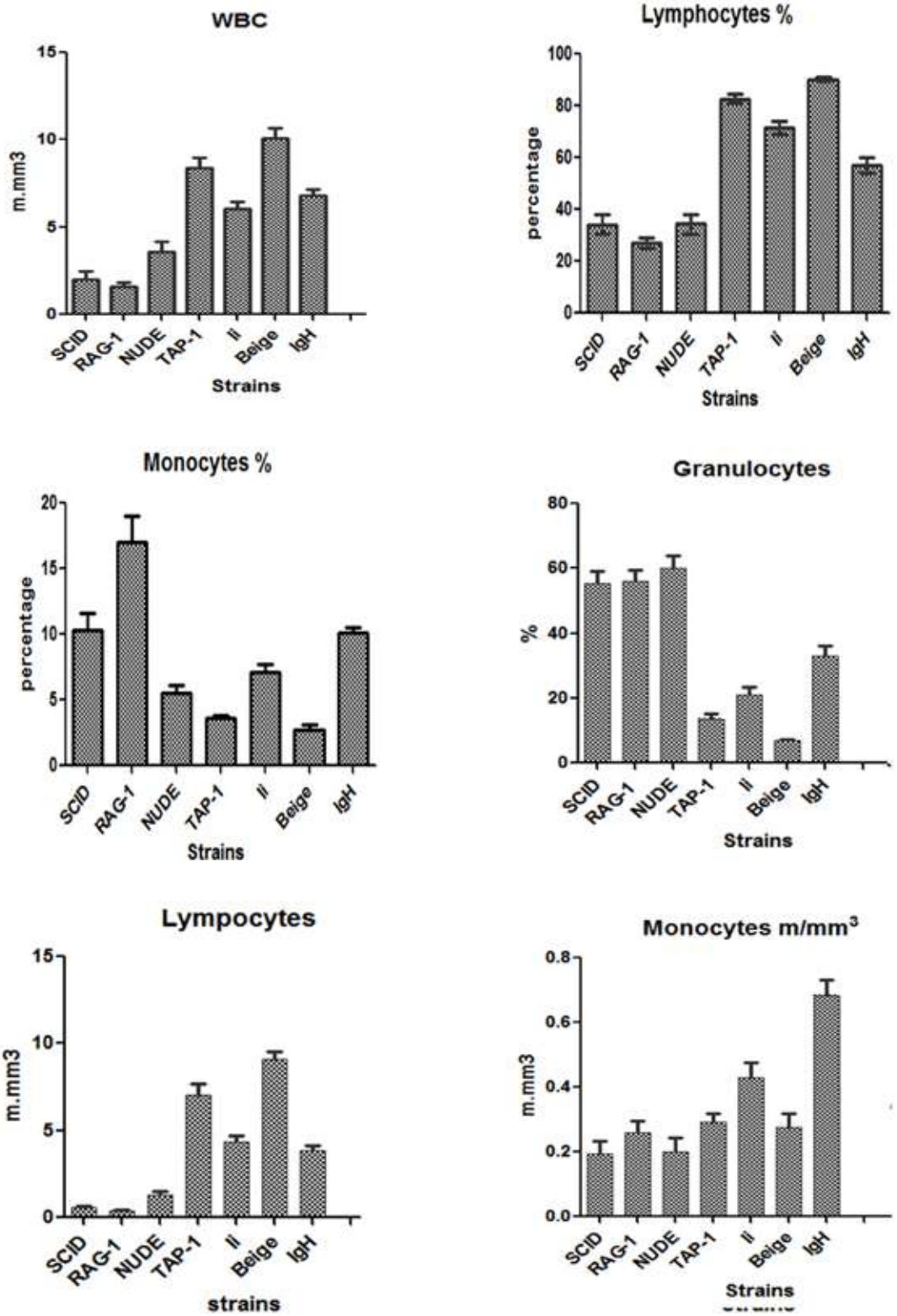


**Figure-3:** Graphical representation of percentages and absolute number of WBC, lymphocytes, monocytes and granulocytes



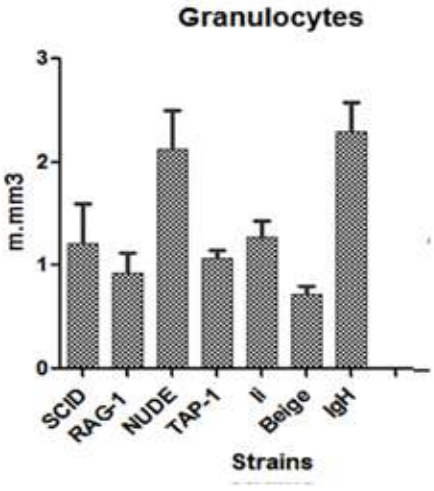



**Table-1:** The hematology analytes of WBC, lympocytes, monocytes and granulocytes of immunodeficiency strains. Values were expressed as Mean ±SD (n=10)

| Parameters | Strains of mice | | | | | | |
|---|---|---|---|---|---|---|---|
| | Nude – T cell deficient | TAP-1 $^{-/-}$ CD8 deficient | Ii $^{-/-}$ CD4 deficient | IgH6 $^{-/-}$ B cell deficient | Beige NK cell deficient | SCID T and B cell deficient | Rag-1 T&B cell deficient |
| WBC (m/mm $^{3}$) | 3.59±1.44 | 8.39±2.1 | 6.04±1.28 | 6.83±1.12 | 10.11±1.86 | 2±1.29 | 1.588±0.76 |
| LYM (%) | 34.42 ± 9.28 | 82.83±5.85 | 71.74±8.99 | 56.96±8.73 | 90.26±2.03 | 34.31±9.93 | 26.91±6.47 |
| MON (%) | 5.5±1.4 | 3.6±0.6 | 7.15±1.84 | 10.09±1.08 | 2.75±1.03 | 10.31±3.33 | 16.976.55 |
| GRA (%) | 60.08±9.51 | 13.57±5.52 | 21.11±7.32 | 32.95±8.93 | 6.99±1.25 | 55.37±9.95 | 56.12±11.8 |
| LYM(m/mm $^{3}$) | 1.26±0.54 | 7.03±2.15 | 4.33±1.22 | 3.85±0.71 | 9.07±1.55 | 0.597±0.19 | 0.397±0.14 |
| MON(m/mm $^{3}$) | 0.2±0.1 | 0.29±0.08 | 0.43±0.14 | 0.68±0.14 | 0.27±0.13 | 0.193±0.11 | 0.259±0.11 |
| GRA(m/mm $^{3}$) | 2.12±0.91 | 1.06±0.26 | 1.273±0.48 | 2.29±0.79 | 0.72±0.22 | 1.21±1.03 | 0.932±0.59 |