Data in Brief 14 (2017) 653–658

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

Data on the effects of eIF6 downmodulation on the proportions of innate and adaptive immune system cell subpopulations and on thymocyte maturation

Nicola Manfrini [a,*,1], Sara Ricciardi [a,1], Annarita Miluzio [a], Maya Fedeli [b], Alessandra Scagliola [a,c], Simone Gallo [a,c], Thure Adler [d,2], Dirk H. Busch [e], Valerie Gailus-Durner [d], Helmut Fuchs [d], Martin Hrabě de Angelis [d,f,g], Stefano Biffo [a,c,**]

[a] National Institute of Molecular Genetics "Romeo ed Enrica Invernizzi" - INGM, via F. Sforza 35, 20122 Milan, Italy
[b] Experimental Immunology Unit, Division of Immunology, Transplantation and Infectious Diseases, DIBIT, H. San Raffaele Scientific Institute, via Olgettina 58, 20132 Milan, Italy
[c] Department of Biosciences, University of Milan, Via Celoria 26, 20133 Milan, Italy
[d] German Mouse Clinic, Institute of Experimental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany
[e] Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Trogerstrasse 30, 81675 Munich, Germany
[f] Center of Life and Food Sciences Weihenstephan, Technische Universität München, 85354 Freising, Germany
[g] German Center for Diabetes Research, 85764 Neuherberg, Germany

ARTICLE INFO

Article history:
Received 24 July 2017
Received in revised form
2 August 2017
Accepted 24 August 2017
Available online 30 August 2017

ABSTRACT

The data described in this article are related to "High levels of eukaryotic Initiation Factor 6 (eIF6) are required for immune system homeostasis and for steering the glycolytic flux of TCR-stimulated $CD4^+$ T cells in both mice and humans" (Manfrini et al., in press) [1]. eIF6 is a translation initiation factor required for ribosomal biogenesis (Sanvito et al., 1999) [2] and for proper translational initiation

DOI of original article: http://dx.doi.org/10.1016/j.dci.2017.07.022
* Corresponding author. Fax: +39 0200660216.
** Corresponding author at: National Institute of Molecular Genetics "Romeo ed Enrica Invernizzi" - INGM, via F. Sforza 35, 20122 Milan, Italy. Fax: +39 0200660216.
E-mail addresses: manfrini@ingm.org (N. Manfrini), biffo@ingm.org (S. Biffo).
[1] The two authors contributed equally to the work.
[2] Present address: Comprehensive Pneumology Center, Institute of Lung Biology and Disease, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany.

http://dx.doi.org/10.1016/j.dib.2017.08.023

Keywords:
eIF6
Immune system
Innate immunity
Adaptive immunity
Thymocyte

(Gallo and Manfrini, 2015; Miluzio et al., 2016) [3,4] whose protein abundance requires tight regulation. Here we analyze by flow cytometry the effects of eIF6 depletion on proportions of specific innate and adaptive immune system subpopulations and on thymocyte maturation in mice.

Specifications Table

Subject area	*Biology*
More specific subject area	*Molecular Biology, Immunology*
Type of data	*Figure*
How data was acquired	FACS analysis of peripheral blood leukocytes (PBLs) isolated from the blood and of thymocytes isolated from thymi of wt and eIF6 heterozygous (het) mice. Samples were acquired on a FACSCantoII flow cytometer (from BD Biosciences) and data were analyzed using the FlowJo software (Tree star).
Data format	*Analyzed*
Experimental factors	*Not applicable*
Experimental features	*After PBL and thymocyte extraction, samples were stained with appropriate antibodies and analyzed by FACS.*
Data source location	*Not applicable*
Data accessibility	*Data is with this article*

Value of the data

- Albeit preserving precise levels of eIF6 is of physiological importance [1–6], our data indicate that eIF6 depletion does not alter the proportions of specific innate and adaptive immune system cell subpopulations nor the capability of the thymus to correctly produce mature thymocytes.
- These data could be a useful starting point for further characterization of the role of eIF6 and of translation in general in immune system regulation.
- The data can be used for comparison to other studies on translation factors affecting immune system homeostasis.

1. Data

The data presented in this article show the effects of eIF6 depletion on the proportions of innate and adaptive immune cell subtypes and on thymocyte maturation. All data support the research article "High levels of eukaryotic Initiation Factor 6 (eIF6) are required for immune system homeostasis and for steering the glycolytic flux of TCR-stimulated $CD4^+$ T cells in both mice and humans" [1].

Percentages of blood granulocytes and monocytes in wt and eIF6 heterozygous (het) mice are shown in Fig. 1A. Data on the percentages of blood B cells and $CD3^+$ T cells in both wt and eIF6 het mice are presented in Fig. 1B.

Fig. 2 focuses on thymocyte development. Thymocyte precursors are present in the thymus as double negative (DN) $CD4^-CD8^-$ cells. When both CD4 and CD8 co-receptor molecules are expressed, precursor cells become $CD4^+CD8^+$ double positive (DP) thymocytes. DP thymocytes then develop

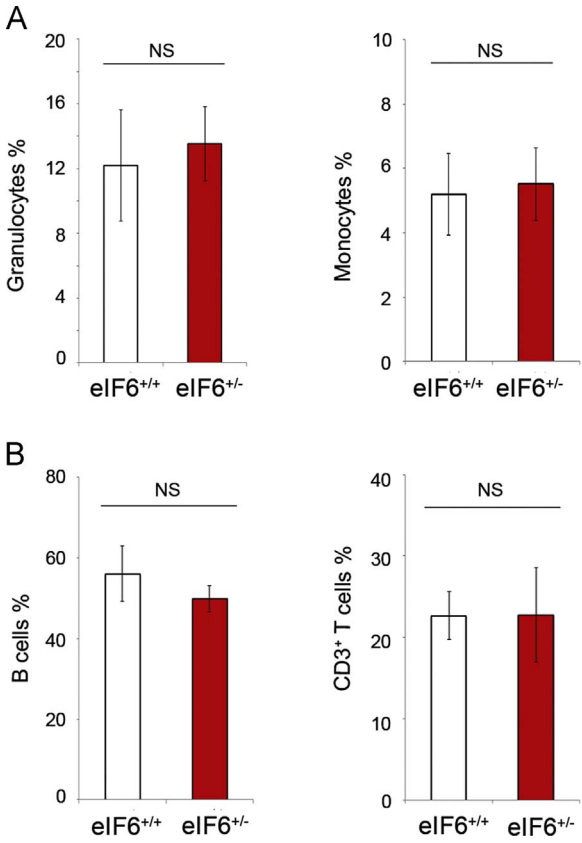

Fig. 1. eIF6 het mice show no overall differences in the proportions of innate and adaptive immune cell subpopulations compared to control animals. Peripheral blood leukocytes were purified from blood samples of both male wt ($eIF6^{+/+}$) ($n=10$) and eIF6 het mice ($eIF6^{+/-}$) ($n=8$) (14 weeks old). Proportions of innate and adaptive immunity subpopulations were assessed. After intact cells identification by FSC/SSC profile, dead cells were gated out according to propidium iodide/PE signal. (A) Among the living cell population, for innate immunity subsets the percentages of granulocytes and monocytes were monitored by FACS analysis and are relative to the total number of living leukocytes. (B) For adaptive immunity subpopulations, the percentages of $CD3^+$ T and $CD19^+$ B cells were assessed. Error bars represent Standard Deviation. Statistical *p*-values were calculated using the two-tailed *t*-test (NS: *p*-value >0.05; granulocytes % *p*-value: 0.37; monocytes % *p*-value: 0.58 ; B cell % *p*-value: 0.87; T cell % *p*-value: 0.96).

into either $CD4^+$ or $CD8^+$ single positive (SP) T cells [7]. Data regarding the proportions of specific DN thymic cell subpopulations in both wt and eIF6 het animals are shown in Fig. 2A, while percentages of overall thymic DN, DP and SP thymocytes are shown in Fig. 2B.

2. Materials and methods

2.1. Mouse colony

eIF6+/- mice [5] were backcrossed to the C57BL6/N strain for 22 generations, in order to establish a pure genetic background. Mice health was monitored on monthly bases according to the instructions given by the Federation of European Laboratory Animal Science Associations (FELASA). MNV infection was diagnosed by PCR. After genotyping, mice were randomly analyzed and experiments were carried-out on age-matched male mice. All primary cells were derived from thymi and blood of mice littermates.

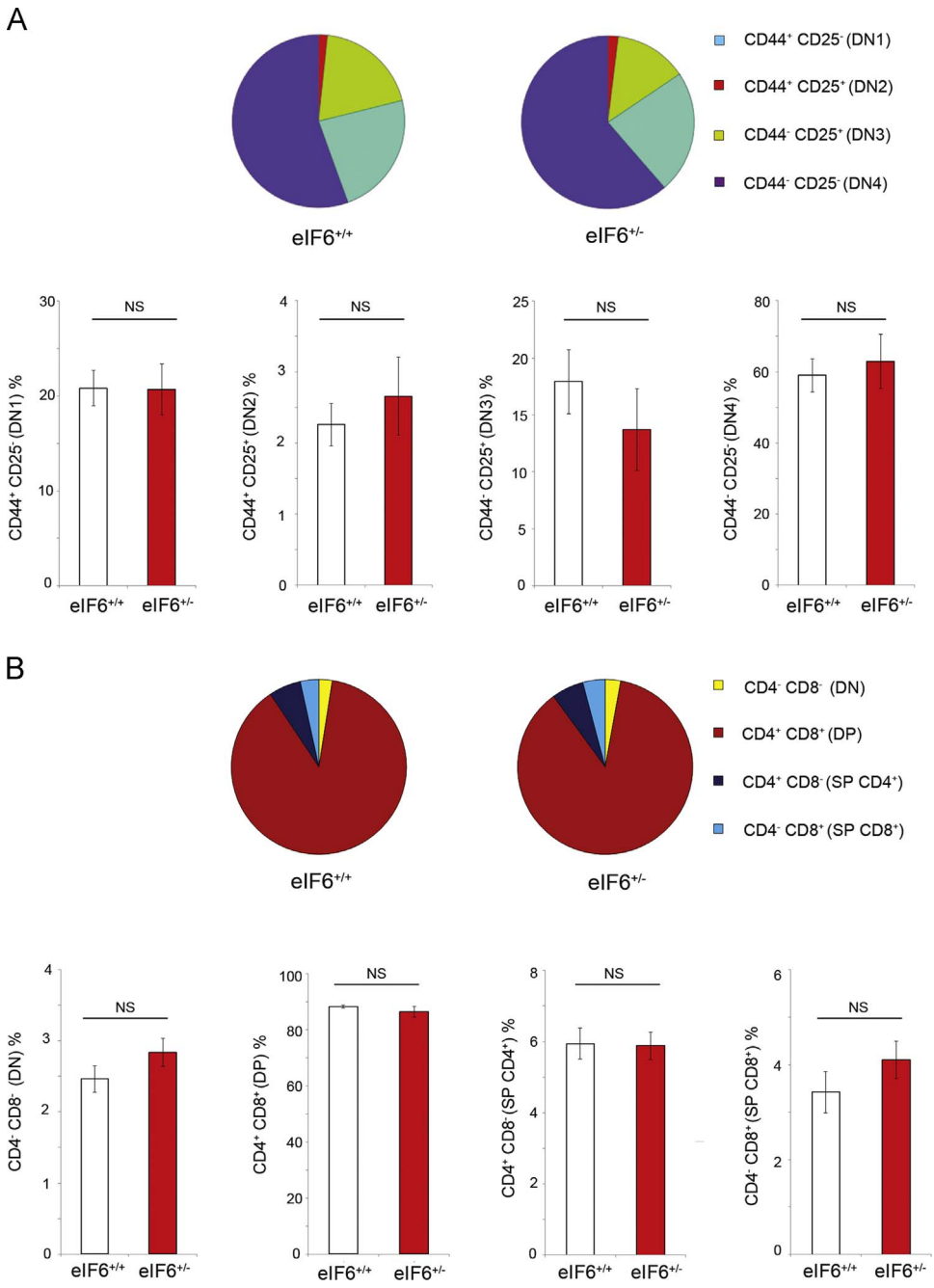

Fig. 2. eIF6 het mice show no overall differences in the proportions of DN, DP or SP cell subpopulations in the thymus. (A) Proportions of DN subpopulations and (B) proportions of overall DN, DP and SP populations were detected by FACS analysis on thymocytes isolated from thymi of eIF6 het ($n=3$) and wt ($n=3$) mice. Among the DN population DN1, DN2, DN3 and DN4 subpopulations were detected with anti-CD44 and anti-CD25 antibodies, while DN, DP and SP populations were detected with anti-CD4 and anti-CD8 antibodies. Percentages of DN subpopulations were calculated on the total DN population while percentages of DN, DP and SP populations were calculated on the total number of living thymocytes. Representative pie charts show population percentages. Each pie chart slice is also depicted as a bar graph. Error bars represent Standard Deviation. Statistical p-values were calculated using the two-tailed t-test (NS: p-value >0.05; $CD44^+$ $CD25^-$ % p-value: 0.8; $CD44^+$ $CD25^+$ % p-value: 0.09; $CD44^-$ $CD25^+$ % p-value: 0.064; $CD44^-$ $CD25^-$ % p-value:0.33; $CD4^-$ $CD8^-$ % p-value: 0.074; $CD4^+$ $CD8^+$ % p-value: 0.20; $CD4^+$ $CD8^-$ % p-value: 0.86; $CD4^-$ $CD8^+$ % p-value: 0.20) .

Experiments involving mice and their relative experimental procedures were approved by the local Institutional Animal Care and Use Committees of the San Raffaele Research Institute (IACUC n. 688) and performed in accordance to Italian national regulations and experimental protocols. At the German Mouse Clinic (GMC), mice were maintained according to housing conditions and German laws. Tests performed at the GMC were approved by the responsible authorities.

2.2. Isolation of blood samples from mice and FACS analysis

Mice peripheral blood samples (14 weeks old males; 10 wt, 8 eIF6 het) were collected by retro-bulbar sinus puncturing with non-heparinized glass capillaries (1.0 mm in diameter; Neolab; Munich, Germany) after isofluraneanesthetization. After collection in heparinized tubes (Li-heparin, KABE, Art. No. 078028; Nümbrecht, Germany), each blood sample was immediately and vigorously inverted and then left at RT for approximately two hours. Cells were then separated from plasma through centrifugation (10 min, 5000*g*; 8 °C). The plasma supernatant was removed while the cell pellet was used for FACS analyses. Peripheral blood leukocytes (PBLs) were isolated from cell pellets of 500 μl whole blood samples. Pellets were then dissolved in 600 μl NH_4Cl-based and TRIS-buffered red cell lysis solution. 150 μl were transferred into 96-well micro titer plates. After washing the FACS staining buffer, PBLs were incubated for 20 min with the Fc blocker (clone 2.4G2, PharMingen, San Diego, USA) and then stained with fluorescence-conjugated monoclonal antibodies (PharMingen). Propidium iodide was added for the identification of dead cells [8,9]. Samples were acquired and measured with a three laser 10-color flow cytometer (LSRII, Becton Dickinson, USA; Gallios, Beckman Coulter, USA). 30,000 living $CD45^+$ cells were analyzed for each sample. Whole intact cells were identified by their FSC/SSC profile while dead cells were excluded from the analysis by gating out the propidium iodide positive population. Living cells were then gated on their SSC/CD45 signal [10].

The different leukocyte populations were identified using the following stainings:

1) T cells ($CD3^+$), $CD4^+$ T cells, $CD8^+$ T cells (see also [1]).
2) NK cells (NKp46 and/or $NK1.1^+$ $CD5^-$), B cells (CD19 and/or $B220^+$), granulocytes ($CD11b^+Gr1^+$) and monocytes (non-NK cells, non-granulocytes).

2.3. Purification of thymocytes from mice and FACS analysis

After mechanical thymus dissection total thymocytes were isolated from mice (4 weeks old males; 3 wt and 3 eIF6 het). Cells were stained with anti-CD4, anti-CD8, anti-CD44 and anti-CD25 (from Biolegend), in the presence of Rat anti-Mouse CD16/CD32 Fc Blocker 2.4G2 mAb. Dead cells and doublets were excluded through DAPI staining and physical gating respectively. Samples were acquired on a FACSCantoII flow cytometer (from BD Biosciences) and data were analyzed using the FlowJo software (Tree star).

2.4. Statistical analysis

All the results were analyzed with the two-tailed *t*-test. A *p*-value < 0.05 was considered significant. *p*-values > 0.05 were considered not significant (NS).

Acknowledgements

Studies were funded by the European Research Council (ERC) under grant “Translate” n. 338999 to S.B. and by the German Federal Ministry of Education and Research to the GMC (Infrafrontier grant 01KX1012).

Transparency document. Supplementary material

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2017.08.023.

References

[1] N. Manfrini, S. Ricciardi, A. Miluzio, M. Fedeli, A. Scagliola,, S. Gallo, D. Brina, T. Adler, D.H. Busch, V. Gailus-Durner, H. Fuchs, M. Hrabě de Angelis, S. Biffo, High levels of eukaryotic Initiation Factor 6 (eIF6) are required for immune system homeostasis and for steering the glycolytic flux of TCR-stimulated $CD4^+$ T cells in both mice and humans, Dev. Comp. Immunol. (2017) (In press).
[2] F. Sanvito, S. Piatti, A. Villa, M. Bossi, G. Lucchini, P.C. Marchisio, S. Biffo, The beta4 integrin interactor p27(BBP/eIF6) is an essential nuclear matrix protein involved in 60S ribosomal subunit assembly, J. Cell Biol. 144 (1999) (1999) 823–837.
[3] S. Gallo, N. Manfrini, Working hard at the nexus between cell signaling and the ribosomal machinery: an insight into the roles of RACK1 in translational regulation, Translation (Austin) 3 (2015) e1120382.
[4] A. Miluzio, S. Ricciardi, N. Manfrini, R. Alfieri, S. Oliveto, D. Brina, S. Biffo, Translational control by mTOR-independent routes: how eIF6 organizes metabolism, Biochem Soc. Trans. 44 (2016) 1667–1673.
[5] V. Gandin, A. Miluzio, A.M. Barbieri, A. Beugnet, H. Kiyokawa, P.C. Marchisio, S. Biffo, Eukaryotic initiation factor 6 is rate-limiting in translation, growth and transformation, Nature 455 (2008) 684–688.
[6] D. Brina, A. Miluzio, S. Ricciardi, K. Clarke, P.K. Davidsen, G. Viero, T. Tebaldi, N. Offenhauser, J. Rozman, B. Rathkolb, S. Neschen, M. Klingenspor, E. Wolf, V. Gailus-Durner, H. Fuchs, M. Hrabe de Angelis, A. Quattrone, F. Falciani, S. Biffo, eIF6 coordinates insulin sensitivity and lipid metabolism by coupling translation to transcription, Nat. Commun. 6 (2015) 8261.
[7] R.V. Luckheeram, R. Zhou, A.D. Verma, B. Xia, CD4(+)T cells: differentiation and functions, Clin. Dev. Immunol. (2012) 925135.
[8] L.L. Zamai, E.E. Falcieri, G.G. Marhefka, M.M. Vitale, Supravital exposure to propidium iodide identifies apoptotic cells in the absence of nucleosomal DNA fragmentation, Cytom. Part A 23 (1996) 303–311.
[9] D. Diaz, A. Prieto, H. Barcenilla, J. Monserrat, P. Prieto, M.A. Sánchez, E. Reyes, M.P. Hernandez-Fuentes, A. de la Hera, A. Orfao, M. Alvarez-Mon, Loss of lineage antigens is a common feature of apoptotic lymphocytes, J. Leukoc. Biol. 76 (2004) 609–615.
[10] J.L. Weaver, D.D. Broud, K. McKinnon, D.R. Germolec, Serial phenotypic analysis of mouse peripheral blood leukocytes, Toxicol. Mech. Methods 12 (2001) 95–118.