ISSN 0006-2979, Biochemistry (Moscow), 2024, Vol. 89, No. 5, pp. 853-861 © Pleiades Publishing, Ltd., 2024.

MINI-REVIEW

Reverse Genetics Applied to Immunobiology of Tumor Necrosis Factor, a Multifunctional Cytokine

Sergey A. Nedospasov[1,2,a]*, Andrei A. Kruglov[3], Alexei V. Tumanov[4], Marina S. Drutskaya[1,2], Irina V. Astrakhantseva[1], and Dmitry V. Kuprash[2]

[1]*Division of Immunobiology and Biomedicine, Sirius University of Science and Technology, 354340 Federal Territory Sirius, Russia*

[2]*Center for Precision Genome Editing and Genetic Technologies for Biomedicine, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russia*

[3]*Laboratory of Systems Rheumatology, German Rheumatism Research Center (DRFZ), a Leibniz Institute, 10117 Berlin, Germany*

[4]*Department of Microbiology, Immunology and Molecular Genetics, University of Texas Health Science Center at San Antonio, 79229 San Antonio, TX, USA*

[a]*e-mail: sergei.nedospasov@gmail.com*

Received November 21, 2023
Revised December 28, 2023
Accepted February 19, 2024

Abstract—Tumor necrosis factor (TNF) is one of many cytokines – protein molecules responsible for communication between the cells of immune system. TNF was discovered and given its grand name because of its striking antitumor effects in experimental systems, but its main physiological functions in the context of whole organism turned out to be completely unrelated to protection against tumors. This short review discusses "man-made" mouse models generated by early genome-editing technologies, which enabled us to establish true functions of TNF in health and certain diseases as well as to unravel potential strategies for improving therapy of TNF-dependent diseases.

DOI: 10.1134/S0006297924050067

Keywords: tumor necrosis factor, TNF, conditional mice models, gene knockout, reporter mice, gene overexpression

INTRODUCTION

Almost 50 years ago, Lloyd J. Old and his colleagues reported an interesting experiment performed to study the antitumor effect of bacterial endotoxin and other substances, which later would be known as activators of innate immune receptors [1]. As it was shown, combination of the infection with BCG (Tb vaccine mycobacterial strain) and subsequent injection of lipopolysaccharide (LPS) from *Escherichia coli* leads to the appearance in blood serum of an unknown "factor" with marked antitumor activity, which was referred to as Tumor Necrosis Factor (TNF) [2]. This discovery was perceived as further development of the ideas of William B. Coley, who pointed out the possibility of using live bacteria or bacterial lysates in the therapy for some tumors as early as at the beginning of the 20th century [3]. Further studies showed that TNF had a protein nature and could be produced by the cells of immune system in response to various stimuli, e.g., by myeloid cells in response to LPS. Molecular cloning and heterologous expression of the human and mouse *Tnf* genes made it possible to relate antitumor activity to a single protein that could be defined as a "cytokine", i.e., the rapidly increasing superfamily of protein mediators of cell communications. Administration of the recombinant TNF produced in *E. coli* to mice fully reproduced antitumor activity of the native TNF [4], and its high doses resulted in manifestation of toxicity associated with an acute inflam-

Abbreviations: ESCs, embryonic stem cells; LT, lymphotoxin; TNF, tumor necrosis factor; UTR, untranslated region.

* To whom correspondence should be addressed.

matory response in mice (toxicity was not observed in the L. Old's experiments [2], probably because it was impossible to achieve the same high concentrations as in the experiments with recombinant TNF).

TNF has a molecular weight of 17 kDa; however, in order to perform its physiological (including antitumor) functions, it has to form a homotrimer, which is a high-affinity ligand of two different receptors: TNFR1 (p55) and TNFR2 (p75) [5]. These receptors differ both in the type of transmitted intracellular signal and in the patterns of tissue-specific expression.

Following the molecular description of TNF and its receptors in the 1980s, other members of the large families of TNF-like cytokines (most of which are membrane-bound molecules) and TNFR-like receptors were soon discovered; they are able to transmit several types of intracellular signals, thereby determining biological activity of the ligands of this family [6]. There were hundreds of papers describing TNF activity in different situations *in vitro* and *in vivo*; however, physiological function of TNF in the context of a whole organism was established in 1990s mainly through two strategies of reverse genetics: creating mice with the *Tnf* gene knockout and generating transgenic mice with its overexpression. Moreover, several interesting mouse lines were isolated by the methods of direct genetics. Below we will discuss numerous mouse models that have been independently created and characterized in various laboratories, as well as the so-called "reporter" mice, which represent additional tools for studying immunobiology and physiology of this interesting cytokine.

MICE WITH COMPLETE, PARTIAL OR REGULATED GENETIC DEFICIENCY OF TNF

The knockout techniques that appeared before the era of genomic nucleases and became widespread in the 1980s were based on targeting the genes in mouse embryonic stem cell lines (ESCs) (for other animals, such lines were created much later). Due to the low efficiency of this procedure in the mouse ESCs it became necessary to introduce antibiotic resistance markers to select knockout clones (usually neomycin-resistance); as a result, the expression cassette containing the resistance gene (the so-called "neo-cassette") remained forever in the structure of the targeted locus and could influence the activity of the nearby genes. Noteworthy, it took 10 years from the cloning of the mouse *Tnf* gene to the creation of the first *Tnf* knockout mice. At least four laboratories obtained such mice almost simultaneously and independently (Table 1), but the palm of victory obviously went to the laboratory of G. Kollias in Greece. When creating the *Tnf* knockout mice, one had to take into consideration two significant peculiarities of this gene. Firstly, the *Tnf* gene is located very close to the related lymphotoxin genes *Lt*α and *Lt*β [7]; therefore, combined *Tnf/Lt* knockouts cannot be obtained by crossbreeding of mice with single knockouts. Secondly, the *Tnf*/*Lt* locus is within the major histocompatibility complex (MHC) [7]; hence, it is very difficult to obtain congenic mouse lines with modifications of the *Tnf* gene and the required alleles of the MHC genes, so that the knockout mice should be designed with particular reference to the genetic basis of ESCs and mouse lines used for subsequent backcrossing.

Pasparakis et al. [8] described new TNF functions associated with its role in the structural and functional organization of lymphoid tissues, about which the discoverers of TNF had no idea. It should be noted that the related cytokine, LTα, was considered for 10 years to be a functional analog of TNF, produced by lymphoid rather than by myeloid cells. This view on lymphotoxin was supported by the fact that the recombinant protein demonstrated an antitumor activity similar to that of TNF in the model of transplantable sarcoma in mice, as well as in cell cultures sensitive to cytotoxic effects of TNF [20]. These results indicated potential redundancy in TNF and LTα functions, inspiring creation of a double knockout of both genes. Table 1 presents some mouse lines with such double knockouts. Note that earlier the lymphotoxin gene knockout produced a sensational phenotype [21]: in mice peripheral lymphoid organs were completely absent, with the exception of spleen. Further comparison of the phenotypes in the knockout mice made it possible to differentiate between the functions of TNF and lymphotoxin, e.g., in the case of organogenesis of Peyer's patches [22], which may be explained by signal transduction from the membrane lymphotoxin complex $LT\alpha_1/LT\beta_2$ [23] through LTβR [24], while a minor overlap in functions is due to the fact that the soluble lymphotoxin ($LT\alpha_3$) is able to trigger the signaling cascade *in vivo* through TNFR1 and TNFR2 [17].

The technique of conditional (tissue-specific or inducible) genetic recombination developed in the early 1990s in mammalian cell culture [25] and then in the cells of transgenic [26, 27] and knockout [28-30] mice was based on LoxP/Cre recombination system of bacteriophage P1, has truly revolutionized biological studies. It became possible, firstly, to circumvent the problem of embryonic lethality typical for a great number of genes, even those with unknown non-redundant functions in embryogenesis, and, secondly, to relate particular functions of the genes and their products to particular types of producer cells. This strategy proved to be especially fruitful for studying the genes whose products manifested pleiotropic functions, which is typical for cytokines and other regulatory molecules.

Development of a panel of conditional mouse lines for the *Tnf* gene began almost 20 years ago [14],

Table 1. Mouse models with TNF deficiency

Genotype of the *Tnf/Lt* locus	Description	References
Mice with the complete TNF gene knockout generated by reverse genetics		
$Tnf^{-/-}$	complete knockout created by targeting in ESCs of the EK.CCE line of mice of the 129/Sv lineage, with one backcrossing at C57BL/6	[8]
$Tnf^{-/-}$	complete knockout created by targeting in ESCs of the W9.5 line from mice of the 129/Sv line, with one backcrossing at C57BL/6	[9]
$Tnf^{-/-}$	complete knockout created by targeting in ESCs from the hybrid of the first generation of C57BL/6 and CBA mice, with selection of the *Tnf* gene knockout in the C57BL/6 haplotype and 5 rounds of backcrossing at C57BL/6	[10, 11]
$Tnf^{-/-}$	complete knockout created by targeting in ESCs from the BL/6-III line on the genetic basis of C57BL/6	[12]
$Tnf^{-/-}$	derived from the mouse line created for conditional targeting of TNF on the genetic basis of 129/Sv, with multiple backcrossing at C57BL/6; the targeted locus does not contain neo-cassettes	[13, 14]
Mouse models with combined TNF/LT knockout		
$Tnf^{-/-}Lta^{-/-}$ (TNF/LTα double KO)	complete knockout of the *Lta* and *Tnf* genes created by targeting in ESCs of the BL/6-III line on the genetic basis of C57BL/6	[12]
$Tnf^{-/-}Lta^{-/-}$ (TNF/LTα double KO)	complete knockout of the *Lta* and *Tnf* genes created by targeting in ESCs from the mouse 129/Sv line, with one backcrossing at C57BL/6	[15]
$Tnf^{-/-}Lta^{-/-}$ (TNF/LTα double KO)	complete knockout of the *Lta* and *Tnf* genes created by targeting in ESCs from the mouse 129/Sv line, with one backcrossing at C57BL/6	[16]
$Tnf^{-/-}Ltb^{-/-}$ (TNF/LTβdouble KO)	complete knockout of the *Ltb* and *Tnf* genes created by targeting in ESCs from the mouse 129/Sv line, with multiple backcrossing at C57BL/6	[17]
$Tnf^{-/-}Lta^{-/-}Ltb^{-/-}$ (TNF/LTα/LTβ triple KO)	complete knockout of the *Lta*, *Tn*, and *Ltb* genes created by targeting in ESCs from the mouse129/Sv line, with multiple backcrossing at C57BL/6	[18]
Hypomorphic allele of the *Tnf* gene selected with the help of direct genetics		
$Tnf^{PanR1/PanR1}$, $Tnf^{PanR1/+}$	in the random mutagenesis experiment using N-ethyl-N-nitrosourea, a dominant-negative mutant with the P138T amino acid substitution in the mature TNF protein was found; the mutation prevents TNF binding to the receptor TNFR1	[19]
Basic “platform” for generating conditional deletions of the *Tnf* gene in mice		
$Tnf^{flox/flox}$	using genetic knockout in ESCs from the mouse 129/Sv line, with multiple backcrossing at C57BL/6, the *Tnf* gene is framed by LoxP sites (“floxed”); the modification does not affect activity of the gene but allows for its subsequent elimination by Cre recombinase in particular types of cells and/or in inducible fashion	[14]

first for the major populations of immunocytes: myeloid cells and lymphocytes. Using these mouse models, it was shown that the particular homeostatic or pathogenic functions of TNF are associated with the particular type of cells producing this cytokine. For example, myeloid cells (primarily macrophages and neutrophils) are necessary for protection against intracellular infections and formation of granulomas but,

at the same time, proved to be the major sources of systemic TNF in different pathological states, e.g., LPS-induced toxicity [14]. On the other hand, TNF produced by T and B cells performs homeostatic functions, including organization of lymphoid tissue [31]. Later, mice with the constitutive or induced deletion of the *Tnf* gene in dendritic cells, monocytes, lymphocytes [32-34], basophiles, microglia, mast cells [35], epithelial cells [36, 37], smooth muscle cells [38, 39], etc., were created. Interestingly, in all cases there were unique phenotypic traits associated with the TNF production by a particular cellular source.

MOUSE MODELS BASED ON TNF OVEREXPRESSION AND/OR "HUMANIZATION"

The first and the best-known transgenic mouse system utilized the mechanism of posttranscriptional regulation of the *Tnf* gene [40], confirmed the hypothesis of a relationship between the overexpression of TNF and the development of arthritis [41] and became a widespread preclinical model for studying TNF blockers. It was created in the Kollias's laboratory [42] in 1991. This model was an important addition to the clinical results obtained by Maini et al. [43] and Feldmann et al. [44] and made it possible to substantiate anti-TNF therapy as an innovative strategy for treating rheumatoid arthritis. In the original work of Kollias's team [42], the number of transgene insertions in mice was several dozens, which resulted in manifestation of the TNF-dependent polyarthritis in all mice at the age of several months. Therefore, the drawbacks of this model included the early development of only one type of the TNF-dependent diseases, preventing modeling of other autoimmune diseases. Later, the same genetic construct was used to create transgenic mice with a smaller number of transgenic insertions, making it possible to ameliorate the pathogenic phenotype and to extend the range of applications of this preclinical model [45] (Table 2).

Note that in the works by Keffer et al. [42] and Hayward et al. [45] the human *Tnf* gene was overexpressed in mice; at the same time, the mouse *Tnf* gene was not deleted and could be expressed, while both TNF receptors remained murine. In essence, these were partially "humanized" preclinical models allowing treatment of the experimental TNF-mediated pathologies with involvement of the human TNF blockers, most of them (with the exception of etanercept) being species-specific and not working against mouse TNF.

Table 2. Mice with TNF overexpression

Mouse model	Description	References
Tgl278	transgenic line with the human *Tnf* gene under control of a strong promoter, with modification of 3′-untranslated region (UTR), which resulted in high expression of human TNF and caused severe polyarthritis in mice; preclinical model for studying the effects of TNF blockers *in vivo*	[42]
B6.Cg-Tg(TNF)#Xen	the same but with moderate levels of human TNF expression; improved preclinical model	[45]
BPSM1	the line with spontaneous insertion of retrotransposon in the mouse 3′-UTR *Tnf*, impairing the previously unknown mechanism of posttranscriptional regulation; TNF overexpression in this mouse line leads not only to severe polyarthritis but also to heart valve disease	[46]
TNF^{del4}, TNF^{del5}, TNF^{del6} and their combinations	the panel of mouse lines with impairment of three regions in the 3′-UTR of the mouse *Tnf* gene regulating mRNA stability; it has been shown that impairment of this system may result in the extremely high TNF production causing death of some embryos	[47]
$TNF^{\Delta ARE}$	mice with deletion of the AT-rich region in 3′-UTR of the mouse *Tnf* gene by the LoxP/Cre technique; phenotypically similar to the Tgl278 and BPSM1 line but, in addition to arthritis, developed inflammation in the ileum of the small intestine	[48]
Transgenic mice with the entire human *Tnf*/*lta*/*ltβ*-locus	all three genes are under control of the intrinsic promoters/enhancers; there was a moderate overexpression of genes of the human *Tnf*/*Lta*/*Ltβ* locus; cortical atrophy of the thymus	[49]

Table 3. Reporter mice for visualization of TNF expression

Genetic name of mouse model	Description	References
TNF-2A-Kat (B6.FRFPK+)	bicistronic expression of TNF and fluorescent protein Katushka using the viral 2A peptide sequence	[58, 59]
hTNF.LucBAC	recombination with the human TNF BAC clone, which resulted in substitution of the luciferase gene for exon 1 of the *Tnf* gene	[60]
FVB/N-Tg(CAG-EGFP,-Tnf)1Kul/J	mice with the "floxed" *eGFP* cassette, which prevents transcription of the mouse *Tnf* cDNA; TNF is expressed only after Cre-mediated recombination	[61]

The more advanced "humanized" model included mice with the human *Tnf* gene inserted precisely at the site of the mouse *Tnf* gene (genetic "knock-in") and with conservation of all regulatory elements [50]. These mice did not demonstrate any abnormalities in the development of lymphoid tissue microarchitecture; at the same time, the human TNF performed protective function in the case of intracellular infections, which was indicative of normal *in vivo* signaling through the mouse TNFR1 receptor. On the other hand, the human TNF is unable to effectively activate the mouse TNFR2 [51], which was a disadvantage of this humanized model. For quite a number of experimental pathologies (arthritis, acute hepatotoxicity, etc.) such limitation of the model is not a problem, because most of the biological effects of TNF are mediated by TNFR1. Nevertheless, in some cases, TNFR2 may play a significant role; therefore, the mice with "humanization" of both TNF and the extracellular domain of TNFR2 with the possibility of conditional activation of TNFR2 were developed to eliminate this limitation and to specify the role of TNFR2 [52]. Transgenic mice expressing Cre recombinase in FoxP3-positive cells were used to demonstrate that signal transduction via TNFR2 in the T-regulatory cells contributes to protection of the central nervous system in the model of autoimmune pathology [52]. One more interesting line contained the modified *Tnf* gene, the product of which had certain amino acid variations and, as a result, almost no soluble TNF was formed [53]. The experiments with this mouse line have demonstrated for the first time that many TNF functions *in vivo* are mediated by the membrane-bound but not soluble form.

Several mouse models with overexpression of the endogenous mouse TNF due to impaired 3′-UTR regions of the *Tnf* gene, which are responsible for mRNA stability, were described (Table 2). In the BPSM1 mice, there was spontaneous integration of retrotransposon in the 3′-UTR [46] leading to the development of severe polyarthritis, as well as heart valve disease. The panel of mouse lines with combinatorial damage to several regions of 3′-UTR involved in the control of mRNA stability demonstrates not only polyarthritis and heart diseases but, in some cases, also embryonic lethality as a result of extremely high TNF production [47]. All these regions are affected by the Cre/LoxP-mediated deletion in the $TNF^{\Delta ARE}$ line developed in the Kollias's laboratory [48] 25 years ago; therefore, with regard to arthritis this model is equivalent to the BPSM1 line and resembles the Tgl278 line, but with more pronounced symptoms of systemic inflammation.

Taking into consideration complexities in regulation of the genes encoding TNF and lymphotoxins, detailed elucidation of the mechanism of accelerated atrophy of the thymus in the mice carrying the entire human locus *Tnf/Lt* as a transgene insertion (Table 2) might need further investigation [49].

"REPORTER" MICE ARE AN IMPORTANT TOOL FOR STUDYING TNF FUNCTIONS

Genetic constructs encoding different luminescent or fluorescent proteins considerably expanded the possibilities of detection and visualization of gene activity *in vitro* and *in vivo* [54]. Transgenic mice, with expression of the protein under study being accompanied by expression of the reporter protein, have become a widespread tool for such studies [55]. In most cases, transgenesis utilized bicistronic constructs that control constitutive or conditional expression of the studied protein and the fluorescent reporter protein. However, it is also possible to design more complex constructs that allow monitoring cells of a particular lineage of cellular differentiation, with the fluorescence of one protein switching to the florescence of another protein in the course of differentiation [56].

Several variants of the reporter mice for TNF studies are known (Table 3). Luciferase, green fluorescent protein GFP, and far-red fluorescent protein Katushka were used as reporter proteins [57]. Detection of reporter proteins is possible not only by flow cytometry and histological identification of TNF-producing cells, but also by *in vivo* visualization.

CONCLUSIONS

TNF proved to be a surprisingly complex cytokine with numerous functions both in homeostasis of the immune system and in some nonimmune organs as well as in different pathologies. Mouse models have made it possible to link the protective and homeostatic functions of TNF and its proinflammatory effects (which may facilitate the development of certain pathologies), to the particular types of the TNF-producing cells. One conclusion that can be drawn from a large number of studies discussed in this review is that the systemic TNF blockade *in vivo* will inevitably demonstrate side effects due to neutralization of homeostatic and protective functions of TNF. Consequently, elucidating how to neutralize TNF only from a "pathogenic" cellular source [62], may allow us to apply improved therapeutic strategies to a variety of TNF-dependent diseases.

Acknowledgments. The authors are grateful to their colleagues S. I. Grivennikov, L. Tessarollo, A. A. Kuchmiy, A. R. Galimov, S. V. Kozlov, I. R. Mufazalov, and R. Naumann for their contributions to creation and study of some mouse models discussed in this review.

Contributions. S.A.N. the concept; S.A.N., I.V.A., and D.V.K. writing the manuscript; A.A.K., A.V.T., and M.S.D. editing the manuscript and making important additions. All authors participated in creation or study of the mouse lines discussed in the review.

Funding. The work was supported by the Ministry of Science and Higher Education of the Russian Federation, project "Development of Bioresource Collection 'Collection of Laboratory Rodents with SPF-status for Fundamental, Biomedical and Pharmacological Research' of the Institute of Biological Chemistry, Russian Academy of Sciences" (agreement no. 075-15-2021-1067). The study of the phenotype of some genetically modified mouse lines was supported by the Russian Science Foundation (project no. 19-75-30032).

Ethics declarations. All manipulations were carried out in accordance with the guideline of the Federation of European Laboratory Animals Science Associations. The experiments were approved by the Bioethics Committee of the Institute of Molecular Biology, Russian Academy of Sciences. The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Parr, I., Wheeler, E., and Alexander, P. (1973) Similarities of the anti-tumour actions of endotoxin, lipid A and double-stranded RNA, *Br. J. Cancer*, **27**, 370-389, doi: 10.1038/bjc.1973.45.
2. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N., and Williamson, B. (1975) An endotoxin-induced serum factor that causes necrosis of tumors, *Proc. Natl. Acad. Sci. USA*, **72**, 3666-3670, doi: 10.1073/pnas.72.9.3666.
3. Coley, W. B. (1910) The treatment of inoperable sarcoma by bacterial toxins (the mixed toxins of the *Streptococcus erysipelas* and the *Bacillus prodigiosus*), *Proc. R. Soc. Med.*, **3**, 1-48, doi: 10.1177/003591571000301601.
4. Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Palladino, M. A., Kohr, W. J., Aggarwal, B. B., and Goeddel, D. V. (1984) Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin, *Nature*, **312**, 724-729, doi: 10.1038/312724a0.
5. Brenner, D., Blaser, H., and Mak, T. W. (2015) Regulation of tumour necrosis factor signalling: live or let die, *Nat. Rev. Immunol.*, **15**, 362-374, doi: 10.1038/nri3834.
6. Locksley, R. M., Killeen, N., and Lenardo, M. J. (2001) The TNF and TNF receptor superfamilies, *Cell*, **104**, 487-501, doi: 10.1016/S0092-8674(01)00237-9.
7. Nedospasov, S. A., Shakhov, A. N., Turetskaya, R. L., Mett, V. A., and Georgiyev, G. P. (1985) Molecular cloning of the genes encoding human tumor necrosis factors: tandem localization of the alpha and beta genes in a short segment (6 k.b.p.) of the human genome, *Dokl. Akad. Nauk SSSR*, **285**, 1487-1490.
8. Pasparakis, M., Alexopoulou, L., Episkopou, V., and Kollias, G. (1996) Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response, *J. Exp. Med.*, **184**, 1397-1411, doi: 10.1084/jem.184.4.1397.
9. Marino, M. W., Dunn, A., Grail, D., Inglese, M., Noguchi, Y., and Richards, E. (1997) Characterization of tumor necrosis factor-deficient mice, *Proc. Natl. Acad. Sci. USA*, **94**, 8093-8098, doi: 10.1073/pnas.94.15.8093.
10. Taniguchi, T., Takata, M., Ikeda, A., Momotani, E., and Sekikawa, K. (1997) Failure of germinal center formation and impairment of response to endotoxin in tumor necrosis factor alpha-deficient mice, *Lab. Invest.*, **77**, 647-658.
11. Iraqi, F., Sekikawa, K., Rowlands, J., and Teale, A. (2001) Susceptibility of tumour necrosis factor-alpha genetically deficient mice to *Trypanosoma congolense* infection, *Paras. Immunol.*, **23**, 445-451, doi: 10.1046/j.1365-3024.2001.00401.x.
12. Körner, H., Cook, M., and Riminton, D. S. (1997) Distinct roles for lymphotoxin-α and tumor necrosis factor in organogenesis and spatial organization of lymphoid tissue, *Eur. J. Immunol.*, **27**, 2600-2609, doi: 10.1002/eji.1830271020.
13. Kuprash, D. V., Tumanov, A. V., Liepinsh, D. J., Lemckert, F. A., Hoek, R. M., Ledermann, B., Köntgen, F.,

De St Groth, B. F., and Sedgwick, J. D. (2005) Novel tumor necrosis factor-knockout mice that lack Peyer's patches, *Eur. J. Immunol.*, **35**, 1592-1600, doi: 10.1002/eji.200526119.

14. Grivennikov, S. I., Tumanov, A. V., Liepinsh, D. J., Kruglov, A. A., Marakusha, B. I., Shakhov, A. N., Murakami, T., Drutskaya, L. N., Förster, I., Clausen, B. E., Tessarollo, L., Ryffel, B., Kuprash, D. V., and Nedospasov, S. A. (2005) Distinct and nonredundant *in vivo* functions of TNF produced by T cells and macrophages/neutrophils, *Immunity*, **22**, 93-104, doi: 10.1016/j.immuni.2004.11.016.
15. Eugster, H-P., Muller, M., Karrer, U., Car, B. D., Schnyder, B., Eng, V. M., Woerly, G., Hir, M. L., Padova, F. D., Aguet, M., Zinkernagel, R., Bluethmann, H., and Ryffel, B. (1996) Multiple immune abnormalities in tumor necrosis factor and lymphotoxin-α double-deficient mice, *Int. Immunol.*, **8**, 23-36, doi: 10.1093/intimm/8.1.23.
16. Amiot, F., Bellkaid, Y., Lebastard, M., Ave, P., Dautry, F., and Milon, G. (1996) Abnormal organisation of the splenic marginal zone and the correlated leukocytosis in lymphotoxin-alpha and tumor necrosis factor alpha double deficient mice, *Eur. Cytokine Netw.*, **7**, 733-739.
17. Kuprash, D. V., Alimzhanov, M. B., Tumanov, A. V., Anderson, A. O., Pfeffer, K., and Nedospasov, S. A. (1999) TNF and lymphotoxin beta cooperate in the maintenance of secondary lymphoid tissue microarchitecture but not in the development of lymph nodes, *J. Immunol.*, **15**, 6575-6580, doi: 10.4049/jimmunol.163.12.6575.
18. Kuprash, D. V., Alimzhanov, M. B., Tumanov, A. V., Grivennikov, S. I., Shakhov, A. N., Drutskaya, L. N., Marino, M. W., Turetskaya, R. L., Anderson, A. O., Rajewsky, K., Pfeffer, K., and Nedospasov, S. A. (2002) Redundancy in tumor necrosis factor (TNF) and lymphotoxin (LT) signaling *in vivo*: mice with inactivation of the entire TNF/LT locus versus single-knockout mice, *Mol. Cell. Biol.*, **22**, 8626-8634, doi: 10.1128/MCB.22.24.8626-8634.2002.
19. Rutschmann, S., Hoebe, K., Zalevsky, J., Du, X., Mann, N., Dahiyat, B. I., Steed, P., and Beutler, B. (2006) PanR1, a dominant negative missense allele of the gene encoding TNF-α (*Tnf*), does not impair lymphoid development, *J. Immunol.*, **176**, 7525-7532, doi: 10.4049/jimmunol.176.12.7525.
20. Gray, P. W., Aggarwal, B. B., Benton, C. V., Bringman, T. S., Henzel, W. J., Jarrett, J. A., Leung, D. W., Moffat, B., Ng, P., Svedersky, L. P., Palladino, M. A., and Nedwin, G. E. (1984) Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumour necrosis activity, *Nature*, **312**, 721-724, doi: 10.1038/312721a0.
21. De Togni, P., Goellner, J., Ruddle, N. H., Streeter, P. R., Fick, A., Mariathasan, S., Smith, S. C., Carlson, R., and Shornick, L. P. (1994) Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin, *Science*, **264**, 703-707, doi: 10.1126/science.8171322.
22. Gogoleva, V. S., Kuprash, D. V., Grivennikov, S. I., Tumanov, A. V., Kruglov, A. A., and Nedospasov, S. A. (2022) LTα, TNF, and ILC3 in Peyer's patch organogenesis, *Cells*, **11**, 1970, doi: 10.3390/cells11121970.
23. Browning, J., Ngam-ek, A., Lawton, P., DeMarinis, J., Tizard, R., Chow, E. P., Hession, C., O'Brine-Greco, B., Foley, S. F., and Ware, C. F. (1993) Lymphotoxin beta, a novel member of the TNF family that forms a heteromeric complex with lymphotoxin on the cell surface, *Cell*, **72**, 847-856, doi: 10.1016/0092-8674(93)90574-A.
24. Crowe, P. D., VanArsdale, T. L., Walter, B. N., Ware, C. F., Hession, C., Ehrenfels, B., Browning, J. L., Din, W. S., Goodwin, R. G., and Smith, C. A. (1994) A lymphotoxin-β-specific receptor, *Science*, **264**, 707-710, doi: 10.1126/science.8171323.
25. Sauer, B., and Henderson, N. (1988) Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1, *Proc. Natl. Acad. Sci. USA*, **85**, 5166-5170, doi: 10.1073/pnas.85.14.5166.
26. Lakso, M., Sauer, B., Mosinger, B., Lee, E. J., Manning, R. W., Yu, S. H., Mulder, K. L., and Westphal, H. (1992) Targeted oncogene activation by site-specific recombination in transgenic mice, *Proc. Natl. Acad. Sci. USA*, **89**, 6232-6236, doi: 10.1073/pnas.89.14.6232.
27. Orban, P. C., Chui, D., and Marth, J. D. (1992) Tissue- and site-specific DNA recombination in transgenic mice, *Proc. Natl. Acad. Sci. USA*, **89**, 6861-6865, doi: 10.1073/pnas.89.15.6861.
28. Gu, H., Zou, Y. R., and Rajewsky, K. (1993) Independent control of immunoglobulin switch recombination at individual switch regions evidenced through Cre-loxP-mediated gene targeting, *Cell*, **73**, 1155-1164, doi: 10.1016/0092-8674(93)90644-6.
29. Kühn, R., Schwenk, F., Aguet, M., and Rajewsky, K. (1995) Inducible gene targeting in mice, *Science*, **269**, 1427-1429, doi: 10.1126/science.7660125.
30. Gu, H., Marth, J. D., Orban, P. C., Mossmann, H., and Rajewsky, K. (1994) Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting, *Science*, **265**, 103-106, doi: 10.1126/science.8016642.
31. Tumanov, A. V., Grivennikov, S. I., Kruglov, A. A., Shebzukhov, Y. V., Koroleva, E. P., Piao, Y., Cui, C.-Y., Kuprash, D. V., and Nedospasov, S. A. (2010) Cellular source and molecular form of TNF specify its distinct functions in organization of secondary lymphoid organs, *Blood*, **116**, 3456-3464, doi: 10.1182/blood-2009-10-249177.
32. Wolf, Y., Shemer, A., Polonsky, M., Gross, M., Mildner, A., Yona, S., David, E., Kim, K.-W., Goldmann, T., Amit, I., Heikenwalder, M., Nedospasov, S., Prinz, M., Friedman, N., and Jung, S. (2017) Autonomous TNF is critical for *in vivo* monocyte survival in steady state and inflammation, *J. Exp. Med.*, **214**, 905-917, doi: 10.1084/jem.20160499.

33. Kruglov, A., Drutskaya, M., Schlienz, D., Gorshkova, E., Kurz, K., Morawietz, L., and Nedospasov, S. (2020) Contrasting contributions of TNF from distinct cellular sources in arthritis, *Ann. Rheum. Dis.*, **79**, 1453-1459, doi: 10.1136/annrheumdis-2019-216068.
34. Wen, Y., Rudemiller, N. P., Zhang, J., Robinette, T., Lu, X., Ren, J., Privratsky, J. R., Nedospasov, S. A., and Crowley, S. D. (2020) TNF-α in T lymphocytes attenuates renal injury and fibrosis during nephrotoxic nephritis, *Am. J. Physiol. Renal. Physiol.*, **318**, F107-F116, doi: 10.1152/ajprenal.00347.2019.
35. Dudeck, J., Kotrba, J., Immler, R., Hoffmann, A., Voss, M., Alexaki, V. I., Morton, L., Jahn, S. R., Katsoulis-Dimitriou, K., Winzer, S., Kollias, G., Fischer, T., Nedospasov, S. A., Dunay, I. R., Chavakis, T., Müller, A. J., Schraven, B., Sperandio, M., and Dudeck, A. (2021) Directional mast cell degranulation of tumor necrosis factor into blood vessels primes neutrophil extravasation, *Immunity*, **54**, 468-483.e5, doi: 10.1016/j.immuni.2020.12.017.
36. Ninnemann, J., Winsauer, C., Bondareva, M., Kühl, A. A., Lozza, L., Durek, P., Lissner, D., Siegmund, B., Kaufmann, S. H. E., Mashreghi, M.-F., Nedospasov, S. A., and Kruglov, A. A. (2022) TNF hampers intestinal tissue repair in colitis by restricting IL-22 bioavailability, *Mucosal. Immunol.*, **15**, 698-716, doi: 10.1038/s41385-022-00506-x.
37. Lakin, R., Polidovitch, N., Yang, S., Parikh, M., Liu, X., Debi, R., Gao, X., Chen, W., Guzman, C., Yakobov, S., Izaddoustdar, F., Wauchop, M., Lei, Q., Xu, W., Nedospasov, S. A., Christoffels, V. M., and Backx, P. H. (2023) Cardiomyocyte and endothelial cells play distinct roles in the tumour necrosis factor (TNF)-dependent atrial responses and increased atrial fibrillation vulnerability induced by endurance exercise training in mice, *Cardiovasc. Res.*, **119**, 2607-2622, doi: 10.1093/cvr/cvad144.
38. Kroetsch, J. T., Levy, A. S., Zhang, H., Aschar-Sobbi, R., Lidington, D., Offermanns, S., Nedospasov, S. A., Backx, P. H., Heximer, S. P., and Bolz, S.-S. (2017) Constitutive smooth muscle tumour necrosis factor regulates microvascular myogenic responsiveness and systemic blood pressure, *Nat. Commun.*, **8**, 14805, doi: 10.1038/ncomms14805.
39. Dinh, D. D., Lidington, D., Kroetsch, J. T., Ng, C., Zhang, H., Nedospasov, S. A., Heximer, S. P., and Bolz, S.-S. (2020) Experimental subarachnoid hemorrhage drives catecholamine-dependent cardiac and peripheral microvascular dysfunction, *Front. Physiol.*, **11**, 402, doi: 10.3389/fphys.2020.00402.
40. Han, J., Brown, T., and Beutler, B. (1990) Endotoxin-responsive sequences control cachectin/tumor necrosis factor biosynthesis at the translational level, *J. Exp. Med.*, **171**, 465-475, doi: 10.1084/jem.171.2.465.
41. Yocum, D. E., Esparza, L., Dubry, S., Benjamin, J. B., Volz, R., and Scuderi, P. (1989) Characteristics of tumor necrosis factor production in rheumatoid arthritis, *Cell. Immunol.*, **122**, 131-145, doi: 10.1016/0008-8749(89)90154-8.
42. Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D., and Kollias, G. (1991) Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis, *EMBO J.*, **10**, 4025-4031, doi: 10.1002/j.1460-2075.1991.tb04978.x.
43. Maini, R. N., Elliott, M., Brennan, F. M., Williams, R. O., and Feldmann, M. (1994) Targeting TNF alpha for the therapy of rheumatoid arthritis, *Clin. Exp. Rheumatol.*, **12**, S63-S66.
44. Feldmann, M., Elliott, M. J., Maini, R. N., and Woody, J. N. (1997) Anti-tumor necrosis factor-α therapy of rheumatoid arthritis, *Adv. Immunol.*, **64**, 283-350, doi: 10.1016/S0065-2776(08)60891-3.
45. Hayward, M. D., Jones, B. K., Saparov, A., Hain, H. S., Trillat, A.-C., Bunzel, M. M., Corona, A., Li-Wang, B., Strenkowski, B., Giordano, C., Shen, H., Arcamone, E., Weidlick, J., Vilensky, M., Tugusheva, M., Felkner, R. H., Campbell, W., Rao, Y., Grass, D. S., and Buiakova, O. (2007) An extensive phenotypic characterization of the hTNFα transgenic mice, *BMC Physiol.*, **7**, 13, doi: 10.1186/1472-6793-7-13.
46. Lacey, D., Hickey, P., Arhatari, B. D., O'Reilly, L. A., Rohrbeck, L., Kiriazis, H., Du, X.-J., and Bouillet, P. (2015) Spontaneous retrotransposon insertion into *TNF* 3′UTR causes heart valve disease and chronic polyarthritis, *Proc. Natl. Acad. Sci. USA*, **112**, 9698-9703, doi: 10.1073/pnas.1508399112.
47. Clayer, E., Dalseno, D., Kueh, A., Lacey, D., Tsai, M., Arvell, E. H., Wimmer, V. C., and Bouillet, P. (2020) Severe impairment of TNF post-transcriptional regulation leads to embryonic death, *iScience*, **23**, 101726, doi: 10.1016/j.isci.2020.101726.
48. Kontoyiannis, D., Pasparakis, M., Pizarro, T. T., Cominelli, F., and Kollias, G. (1999) Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies, *Immunity*, **10**, 387-398, doi: 10.1016/s1074-7613(00)80038-2.
49. Liepinsh, D. J., Kruglov, A. A., Galimov, A. R., Shakhov, A. N., Shebzukhov, Y. V., Kuchmiy, A. A., Grivennikov, S. I., Tumanov, A. V., Drutskaya, M. S., Feigenbaum, L., Kuprash, D. V., and Nedospasov, S. A. (2009) Accelerated thymic atrophy as a result of elevated homeostatic expression of the genes encoded by the TNF/lymphotoxin cytokine locus, *Eur. J. Immunol.*, **39**, 2906-2915, doi: 10.1002/eji.200839191.
50. Olleros, M. L., Chavez-Galan, L., Segueni, N., Bourigault, M. L., Vesin, D., Kruglov, A. A., Drutskaya, M. S., Bisig, R., Ehlers, S., Aly, S., Walter, K., Kuprash, D. V., Chouchkova, M., Kozlov, S. V., Erard, F., Ryffel, B., Quesniaux, V. F. J., Nedospasov, S. A., and Garcia, I. (2015) Control of mycobacterial infections in mice ex-

pressing human tumor necrosis factor (TNF) but not mouse TNF, *Infect. Immun.*, **83**, 3612-3623, doi: 10.1128/IAI.00743-15.

51. Ameloot, P., Fiers, W., De Bleser, P., Ware, C. F., Vandenabeele, P., and Brouckaert, P. (2001) Identification of tumor necrosis factor (TNF) amino acids crucial for binding to the murine p75 TNF receptor and construction of receptor-selective mutants, *J. Biol. Chem.*, **276**, 37426-37430, doi: 10.1074/jbc.M102020200.
52. Atretkhany, K-S. N., Mufazalov, I. A., Dunst, J., Kuchmiy, A., Gogoleva, V. S., Andruszewski, D., Drutskaya, M. S., Faustman, D. L., Schwabenland, M., Prinz, M., Kruglov, A. A., Waisman, A., and Nedospasov, S. A. (2018) Intrinsic TNFR2 signaling in T regulatory cells provides protection in CNS autoimmunity, *Proc. Natl. Acad. Sci. USA*, **115**, 13051-13056, doi: 10.1073/pnas.1807499115.
53. Ruuls, S. R., Hoek, R. M., and Ngo, V. N. (2001) Membrane-bound TNF supports secondary lymphoid organ structure but is subservient to secreted TNF in driving autoimmune inflammation, *Immunity*, **15**, 533-543, doi: 10.1016/s1074-7613(01)00215-1.
54. Chudakov, D. M., Matz, M. V., Lukyanov, S., Lukyanov, K. A., Zambito, G., and Mezzanotte, L. (2010) Fluorescent proteins and their applications in imaging living cells and tissues, *Physiol. Rev.*, **90**, 1103-1163, doi: 10.1152/physrev.00038.2009.
55. Chawda, C., McMorrow, R., Gaspar, N., Zambito, G., and Mezzanotte, L. (2022) Monitoring immune cell function through optical imaging: a review highlighting transgenic mouse models, *Mol. Imaging Biol.*, **24**, 250-263, doi: 10.1007/s11307-021-01662-5.
56. Rubtsov, Y. P., Niec, R. E., Josefowicz, S., Li, L., Darce, J., Mathis, D., Benoist, C., and Rudensky, A. Y. (2010) Stability of the regulatory T cell lineage *in vivo*, *Science*, **329**, 1667-1671, doi: 10.1126/science.1191996.
57. Shcherbo, D., Merzlyak, E. M., Chepurnykh, T. V., Fradkov, A. F., Ermakova, G. V., Solovieva, E. A., Lukyanov, K. A., Bogdanova, E. A., Zaraisky, A. G., Lukyanov, S., and Chudakov, D. M. (2007) Bright far-red fluorescent protein for whole-body imaging, *Nat. Methods*, **4**, 741-746, doi: 10.1038/nmeth1083.
58. Shebzukhov, Y. V., Kuchmiy, A. A., Kruglov, A. A., Zipp, F., Siffrin, V., and Nedospasov, S. A. (2014) Experimental applications of TNF-reporter mice with far-red fluorescent label, in *The TNF Superfamily* (Bayry, J., ed.) New York, Springer New York, pp. 151-162, doi: 10.1007/978-1-4939-0669-7_13.
59. Kuchmiy, A. A., Kruglov, A. A., Galimov, A. R., Shebzukhov, Yu. V., Chudakov, D. M., Lukyanov, S. A., and Nedospasov, S. A. (2011) A new line oftransgenic reporter mice for the study of expression of the tumor necrosis factor, *Ross. Immunol. Zh.*, **5**, 3-4.
60. Minshawi, F., White, M. R. H., Muller, W., Humphreys, N., Jackson, D., Campbell, B. J., Adamson, A., and Papoutsopoulou, S. (2019) Human TNF-Luc reporter mouse: a new model to quantify inflammatory responses, *Sci. Rep.*, **9**, 193, doi: 10.1038/s41598-018-36969-x.
61. Hall, B. E., Zhang, L., Sun, Z. J., Utreras, E., Prochazkova, M., Cho, A., Terse, A., Arany, P., Dolan, J. C., Schmidt, B. L., and Kulkarni, A. B. (2016) Conditional TNF-α overexpression in the tooth and alveolar bone results in painful pulpitis and osteitis, *J. Dent. Res.*, **95**, 188-195, doi: 10.1177/0022034515612022.
62. Drutskaya, M. S., Efimov, G. A., Astrakhantseva, I. V., Kruglov, A. A., and Nedospasov, S. A. (2018) Making anti-cytokine therapy more selective: studies in mice, *Cytokine*, **101**, 33-38, doi: 10.1016/j.cyto.2016.08.022.