 DOI 10.1002/eji.201040980 Eur. J. Immunol. 2010. 40: 2680–2681

Commentary

European Journal of Immunology

At last—rats lacking B cells

Rikard Holmdahl

Medical Inflammation Research, MBB, Karolinska Institutet, Stockholm, Sweden

The rat model is a widely used animal model in research, its popularity perhaps only surpassed by the mouse model. Rats are the preferred models for the study of transplantation and certain tumor and autoimmune diseases. In particular, the understanding of cardiovascular-related diseases and chronic inflammation has depended widely on the rat, from which models for both multiple sclerosis and rheumatoid arthritis have been derived. Until now, research in the rat has been hampered by a lack of precise gene-targeting technology in this model. This limitation, however, is rapidly changing; a recent example is the availability of B-cell-deficient rat strains obtained by the newly established zinc finger nucleases-targeting technology. As described in this issue of the *European Journal of Immunology*, genetic targeting of the rat, for example, using zinc finger nucleases-targeting technology, is likely to rapidly drive progress in the understanding of not only B-cell biology but also in the general understanding of rat disease models.

Key words: B-cell · Disease models · Gene targeting · Rats · Transplantation

See accompanying article by Ménoret *et al.*

The use of both inbred and newly derived mouse lines with specifically targeted and modified gene functions has been of crucial importance for the understanding of biological functions and has resulted in the popularity of the mouse as a research tool. What is often not acknowledged, however, is that rats are historically – and probably still now – more commonly used than mice as experimental animals [1]. As rats are larger than mice, they allow for more sophisticated surgery; moreover, some pathophysiologic pathways seem to be better studied in rats than in mice. For example, there is a wide usage of rats in transplantation studies [1]. Rats are also commonly used as models for cardiovascular diseases and hypertension and in autoimmune diseases such as type I diabetes, multiple sclerosis and rheumatoid arthritis. Several highly relevant models for therapy of human diseases are only available in the rat, *e.g.* the adjuvant arthritis model, in which arthritis is induced with various innate stimuli such as pristane, alkanes, squalenes or -glucan fulfill the classification criteria of rheumatoid arthritis, but no corresponding model exists in the mouse [2]. Some genes targeted in therapy, such as the potassium channel genes, are more conserved between rat and human than between mouse and human [3]. Today, there is also a large toolbox available for rat research, including over 350 inbred strains, numerous congenic and transgenic strains, as well as the possibility of gene mapping using heterogenous stock and recombinant inbred strain panels [1, 4]. So far, the only missing tool, as compared with the mouse, has been the possibility of specifically targeting genes in the rat to modify their function. Progress, however, has now been made and the first rat-knockouts, generated using the zinc finger nuclease (ZFN) technology, were recently reported [5]. This technology is based on the injection into embryonal cells of mRNA ZFN targeting and cleaving specific sequence on double stranded DNA, followed by an induced repair mechanism that introduces site-specific mutations or deletions [6]. Very recently, the first rat with a knockout p53 gene was also reported using classical homologous recombination using established ES cell lines [7].

In this issue of the *European Journal of Immunology*, Ménoret *et al.* [8] describe the first rat models with deficient B-cell function. To generate these rats, the authors used ZFN technology for DNA/RNA microinjection into oocytes. In one model, IgM, and subsequently the whole IgH locus, were rendered non-functional and in the other, the JH locus was deleted. In both cases, the rats were made deficient in both peripheral B cells and in circulating antibodies of all subclasses. The first IgM heavy chain deletion mouse (μMT) was produced some 20 years ago [9] and has been an important tool for the dissection of B-cell function in mouse models. Interestingly, the current rat models

Correspondence: Prof. Rikard Holmdahl
e-mail: Rikard.Holmdahl@ki.se

are slightly different and more complete compared to the corresponding mouse model. In the μMT mouse strain, the deficiency is incomplete as IgA expressing B cells develops [10], a population that at least in some genetic backgrounds occurs in bone marrow and intestine as well as in peripheral lymphoid organs and which is expanded by the influence of estrogen [11]. In human IgM-locus-deficiencies a complete lack of B-cell function is found [12].

The currently reported rat B-cell-deficient strain is likely to be useful in many areas of research since it will now, for the first time, be possible to conclusively study the role of B cells. One such example is given in the current report by Ménoret *et al.* [8], in which the authors demonstrate that the hyperacute heart transplantation model is antibody dependent.

A word of caution is, however, also needed. The ES cell-technology in the mouse constitutes a very powerful tool, but also introduced artifacts due to the genetic manipulation inherent to the technology, but sometimes also to the lack of correct experimental testing leading to over-interpretation of data [13–17]. Most commonly, mouse genes have been targeted using an ES cell from one strain and tested in another after backcrossing. This has sometimes led to spurious results, not only due to artifacts of the targeted genes but also to polymorphic linked genes. Until now, rat research has had more limited tools, but these have been used well, leading to the positional mapping of several genes of importance in disease development [1].

With the introduction of new technical possibilities for gene-targeting, and with the experiences learned from mistakes in the mouse, the possibility to target rat genes may contribute to a revival of the usage of the rat as a prime research tool. The currently reported B-cell-deficient rat strain has been long awaited and can now be used as a tool for dissecting the role of B cells in immunological rat diseases.

Acknowledgements: The author thanks the EU FP7 project EURATRANS (HEALTH-F4-2010-241504) for support.

Conflict of interest: The authors have declared no conflict of interest.

References

1 **Aitman, T. J., Critser, J. K., Cuppen, E., Dominiczak, A., Fernandez-Suarez, X. M., Flint, J., Gauguier, D. *et al.***, Progress and prospects in rat genetics: a community view. *Nat. Genet.* 2008. **40**: 516–522.

2 **Holmdahl, R., Lorentzen, J. C., Lu, S., Olofsson, P., Wester, L., Holmberg, J. and Pettersson, U.**, Arthritis induced in rats with non-immunogenic adjuvants as models for rheumatoid arthritis. *Immunol. Rev.* 2001. **184**: 184–202.

3 **Beeton, C., Wulff, H., Standifer, N. E., Azam, P., Mullen, K. M., Pennington, M. W., Kolski-Andreaco, A. *et al.***, Kv1.3 channels are a therapeutic target for T cell-mediated autoimmune diseases. *Proc. Natl. Acad. Sci. USA* 2006. **103**: 17414–17419.

4 **Johannesson, M., Lopez-Aumatell, R., Stridh, P., Diez, M., Tuncel, J., Blazquez, G., Martinez-Membrives, E. *et al.***, A resource for the simultaneous high-resolution mapping of multiple quantitative trait loci in rats: the NIH heterogeneous stock. *Genome Res.* 2009. **19**: 150–158.

5 **Geurts, A. M., Cost, G. J., Freyvert, Y., Zeitler, B., Miller, J. C., Choi, V. M., Jenkins, S. S. *et al.***, Knockout rats *via* embryo microinjection of zinc-finger nucleases. *Science* 2009. **325**: 433.

6 **Geurts, A. M., Cost, G. J., Remy, S., Cui, X., Tesson, L., Usal, C., Menoret, S. *et al.***, Generation of gene-specific mutated rats using zinc-finger nucleases. *Methods Mol. Biol.* 2009. **597**: 211–225.

7 **Tong, C., Li, P., Wu, N. L., Yan, Y. and Ying, Q. L.**, Production of p53 gene knockout rats by homologous recombination in embryonic stem cells. *Nature* 2010. **467**: 211–213.

8 **Ménoret, S., Iscache, A., Tesson, L., Rémy, S., Usal, C., Osborn, M. J., Cost, G. J. *et al.***, Characterisation of immunoglobulin heavy chain knockout rats. *Eur. J. Immunol.* 2010. **40**: 2932–2941.

9 **Kitamura, D., Roes, J., Kühn, R. and Rajewsky, K.**, A B cell deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. *Nature* 1991. **350**: 423–426.

10 **Macpherson, A. J., Lamarre, A., McCoy, K., Harriman, G. R., Odermatt, B., Dougan, G., Hengartner, H. and Zinkernagel, R. M.**, IgA production without mu or delta chain expression in developing B cells. *Nat. Immunol.* 2001. **2**: 625–631.

11 **Lagerquist, M. K., Erlandsson, M. C., Islander, U., Svensson, L., Holmdahl, R. and Carlsten, H.**, 17Beta-estradiol expands IgA-producing B cells in mice deficient for the mu chain. *Scand. J. Immunol.* 2008. **67**: 12–17.

12 **Pan, Q., Matamoros, N., Bjorkander, J., Conley, M. E. and Hammarstrom, L.**, Lack of IgA in C(mu)-deficient patients. *Nat. Immunol.* 2002. **3**: 595; author reply 596.

13 **Ridgway, W. M., Healy, B., Smink, L. J., Rainbow, D. and Wicker, L. S.**, New tools for defining the 'genetic background' of inbred mouse strains. *Nat. Immunol.* 2007. **8**: 669–673.

14 **Kanagawa, O., Xu, G., Tevaarwerk, A. and Vaupel, B. A.**, Protection of nonobese diabetic mice from diabetes by gene(s) closely linked to IFN-gamma receptor loci. *J. Immunol.* 2000. **164**: 3919–3923.

15 **Sonderegger, I., Kisielow, J., Meier, R., King, C. and Kopf, M.**, IL-21 and IL-21R are not required for development of Th17 cells and autoimmunity *in vivo*. *Eur. J. Immunol.* 2008. **38**: 1833–1838.

16 **Holmdahl, R.**, IL-21 and autoimmune disease – hypothesis and reality?. *Eur. J. Immunol.* 2008. **38**: 1800–1802.

17 **Blom, T., Franzen, A., Heinegard, D. and Holmdahl, R.**, Comment on "The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease". *Science* 2003. **299**: 1845.

Abbreviation: **ZFN:** zing finger nuclease

Full correspondence: Prof. Rikard Holmdahl, Medical Inflammation Research, Karolinska Institutet, Scheeles väg 2, S-171 77 Stockholm, Sweden
e-mail: Rikard.Holmdahl@ki.se

See accompanying article:
http://dx.doi.org/10.1002/eji.201040939

Received: 24/8/2010
Revised: 24/8/2010
Accepted: 30/8/2010

