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Pillars of Immunology

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Since the classical thymectomy experiments of the early 1960s, the thymus has been known as an essential component of normal immune system function. Observations from these early experiments stimulated many years of research that helped to shape our current understanding of T cell–mediated immunity. For example, reagents generated by newly emerging Ab technology could be used to study thymus-derived lymphocytes, an approach still in use today that has culminated in a fine-detailed map of the stages in intrathymic T cell development. Additionally, seminal studies in the 1970s demonstrated the importance of the thymus, notably its radioresistant stromal components, in biasing the TCR repertoire toward recognition of self-MHC molecules (1). Whereas experiments involving the surgical removal of the thymus helped to define its immunological importance, the availability of naturally occurring mouse mutants would also prove to be useful in revealing the importance of the thymus. Discovered by Grist at the Ruchill Hospital in Glasgow, a new hairless mouse mutant termed "nude" was studied and reported by Flanagan (2) in 1966. Although analysis of the immune system was not performed in this initial study, nude mice were reported to demonstrate a failure to thrive, together with increased mortality, because of a recessive mutation in a gene that was termed *nu*. Importantly for immunologists, later analysis showed that nude mice lacked detectable thymus tissue as adults and had dramatically reduced blood leukocyte counts (3). Further studies involving bone marrow and thymic transplantation showed that nude hematopoietic precursors could develop normally in a wild-type thymus, suggesting that the absence of T cells in nude mice was due to defective thymic stromal compartments, most likely thymic epithelial cells (TECs) (4). However, although the mid-1990s saw several studies demonstrating the importance of thymocyte-expressed genes as intrinsic regulators of T cell development, no molecular regulators of TEC development had been reported. Consequently, there was a real need to focus attention on identifying the genetic regulators of thymus development to understand the influence of TEC microenvironments on T cell precursors. The 1994 study of Boehm and colleagues (5) reprinted here did exactly that. They identified *whn* (now called *Foxn1*) as the gene responsible for the nude phenotype in mice and rats and thereby provided the first molecular tool to dissect TEC biology.

To begin their search for the murine *nu* gene, Boehm and colleagues focused their attention on a small region of chromosome 11, an area previously shown to contain the nude locus. Using a PCR-based exon-trapping method, they then identified and ordered ~50 exons within this genomic region. With current technology, the next step appears deceptively simple, yet it proved to be highly effective: given the phenotype of nude mice, Nehls et al. reasoned that expression of exons within the *nu* gene would be detectable in the thymus and skin, but absent from other tissues. When they performed RT-PCR analysis, only one of the identified exons was expressed in both thymus and skin. The full genomic structure of the murine *nu* gene was then determined, and the sequence of mouse skin cDNA clones predicted a protein of 648 aa containing a domain homologous to the DNA binding domain of the winged-helix transcription factor family. So, the *nu* gene was named "winged-helix nude" (*whn*). Further experiments showed the mouse and rat *whn* genes to be highly homologous, and that the mutations in *whn* present in nude mice and nude rats resulted in the expression of truncated proteins lacking the DNA binding domain. Thus, the gene responsible for defective thymus development in nude rodents had been identified and shown to be the first member of the winged-helix family to play a role in vertebrate development. Soon afterward, by generating aggregation chimeras from early stage wild-type and nude mouse embryos, work from Blackburn et al. (6) revealed a cell-autonomous role for the *nu* gene in TEC development. Additionally, Nehls et al. (7) went on to demonstrate that the initial stages of thymus organogenesis occurred in a *Foxn1*-independent manner, fitting well with analysis of the thymus anlagen in nude mouse embryos (8). Thus, the product of the *nu* gene was suggested to act as a key regulator in the differentiation, but not specification, of TECs.

Since 1994, *Foxn1* has become a cornerstone in understanding and manipulating the development of TECs. An understanding of the importance of *Foxn1* was extended to humans through analysis of the genetic basis of a peculiar autosomal recessive human primary immunodeficiency reported in two sisters in Italy. Characterized by T cell immunodeficiency, alopecia, and the absence of a thymus shadow in x-rays, the patients were found to have a deleterious mutation in the human *FOXN1* gene (9). Named Nude/SCID, this epithelial disorder has recently been successfully treated by thymus transplantation (10). The discovery of *Foxn1* has also enabled the generation of mice for TEC-specific gene deletion (Foxn1-Cre mice) to identify key molecular regulators of thymic microenvironments. Only the recently described

Medical Research Council Centre for Immune Regulation, Institute for Biomedical Research, University of Birmingham, Birmingham B15 2TT, United Kingdom

Address correspondence and reprint requests to Prof. Graham Anderson, Medical Research Council Centre for Immune Regulation, University of Birmingham, Birmingham B15 2TT, U.K. E-mail address: g.anderson@bham.ac.uk

Abbreviations used in this article: TEC, thymic epithelial cell; *whn*, winged-helix nude.

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β5t-Cre knock-in mice (11), which selectively target epithelial cells in thymus but not skin, surpass Foxn1-Cre mice in terms of thymus specificity. The manipulation of *Foxn1* via the generation of hypomorphic alleles (12) or systems that allow for temporal control of *Foxn1* expression (13) has been important in understanding its role in thymus development and function. Studies of the evolutionary history of the *Foxn1* gene have even allowed a glimpse into the distant past of thymus evolution in vertebrates (14).

In summary, the identification of the nude mouse gene by Nehls et al. (5) is an excellent example of how one study can reveal, and ultimately help to unravel, the complexity of a primary lymphoid organ. That *Foxn1* is still currently the tool of choice to mark and manipulate TECs is a testament to the importance of its discovery in understanding thymus development and function.

Disclosures

The authors have no financial conflicts of interest.

References

1. Miller, J. F. 2011. The golden anniversary of the thymus. *Nat. Rev. Immunol.* 11: 489–495.
2. Flanagan, S. P. 1966. "Nude", a new hairless gene with pleiotropic effects in the mouse. *Genet. Res.* 8: 295–309.
3. Pantelouris, E. M. 1968. Absence of thymus in a mouse mutant. *Nature* 217: 370–371.
4. Wortis, H. H., S. Nehlsen, and J. J. Owen. 1971. Abnormal development of the thymus in "nude" mice. *J. Exp. Med.* 134: 681–692.
5. Nehls, M., D. Pfeifer, M. Schorpp, H. Hedrich, and T. Boehm. 1994. New member of the winged-helix protein family disrupted in mouse and rat nude mutations. *Nature* 372: 103–107.
6. Blackburn, C. C., C. L. Augustine, R. Li, R. P. Harvey, M. A. Malin, R. L. Boyd, J. F. Miller, and G. Morahan. 1996. The nu gene acts cell-autonomously and is required for differentiation of thymic epithelial progenitors. *Proc. Natl. Acad. Sci. USA* 93: 5742–5746.
7. Nehls, M., B. Kyewski, M. Messerle, R. Waldschütz, K. Schüddekopf, A. J. Smith, and T. Boehm. 1996. Two genetically separable steps in the differentiation of thymic epithelium. *Science* 272: 886–889.
8. Owen, J. J., R. K. Jordan, and M. C. Raff. 1975. The development of the thymus in the nude mouse. *Eur. J. Immunol.* 5: 653–655.
9. Frank, J., C. Pignata, A. A. Panteleyev, D. M. Prowse, H. Baden, L. Weiner, L. Gaetaniello, W. Ahmad, N. Pozzi, P. B. Cserhalmi-Friedman, et al. 1999. Exposing the human nude phenotype. *Nature* 398: 473–474.
10. Markert, M. L., J. G. Marques, B. Neven, B. H. Devlin, E. A. McCarthy, I. K. Chinn, A. S. Albuquerque, S. L. Silva, C. Pignata, G. de Saint Basile, et al. 2011. First use of thymus transplantation therapy for FOXN1 deficiency (nude/SCID): a report of 2 cases. *Blood* 117: 688–696.
11. Ohigashi, I., S. Zuklys, M. Sakata, C. E. Mayer, S. Zhanybekova, S. Murata, K. Tanaka, G. A. Holländer, and Y. Takahama. 2013. Aire-expressing thymic medullary epithelial cells originate from β5t-expressing progenitor cells. *Proc. Natl. Acad. Sci. USA* 110: 9885–9890.
12. Su, D. M., S. Navarre, W. J. Oh, B. G. Condie, and N. R. Manley. 2003. A domain of Foxn1 required for crosstalk-dependent thymic epithelial cell differentiation. *Nat. Immunol.* 4: 1128–1135.
13. Chen, L., S. Xiao, and N. R. Manley. 2009. Foxn1 is required to maintain the postnatal thymic microenvironment in a dosage-sensitive manner. *Blood* 113: 567–574.
14. Swann, J. B., A. Weyn, D. Nagakubo, C. C. Bleul, A. Toyoda, C. Happe, N. Netuschil, I. Hess, A. Haas-Assenbaum, Y. Taniguchi, et al. 2014. Conversion of the thymus into a bipotent lymphoid organ by replacement of FOXN1 with its paralog, FOXN4. *Cell Reports* 8: 1184–1197.