Protein Cell 2011, 2(12): 941–943
DOI 10.1007/s13238-011-1123-1

Protein & Cell

NEWS AND VIEWS

Lupus at the molecular level

Mayami Sengupta, Laurence Morel ✉

Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL 32610, USA
✉ Correspondence: morel@pathology.ufl.edu

Systemic lupus erythematosus (SLE) is a debilitating multifactorial autoimmune disease (Shlomchik et al., 2001; La Cava, 2009; Perry et al., 2011). It involves multiple organ systems with tissue damage driven by the activation of autoreactive T and B cells (Bruns et al., 2000; Langer et al., 2007). It can exist for years before being diagnosed and is known to be more common in women than in men. Intense research efforts have uncovered a complex lupus pathogenesis, many aspects of which are not very clear (Perry et al., 2011).

A number of mouse models displaying different phenotypes and genotypes of SLE have collectively contributed a great deal toward our understanding of the disease. Linkage analyses on inbred strains of New Zealand Black (NZB) and New Zealand White (NZW) mice that spontaneously develop lupus-like disease led to the identification of lupus susceptibility loci with more than 20 genomic intervals from which several candidate genes have emerged with indications of pathways related to disease pathogenesis (Theofilopoulos, 1993; Morel and Wakeland, 1998; Vyse and Kotzin, 1998; Tsao and Grossman, 2001; Morel, 2010). In 1994, Morel et al. identified the genomic positions of three key recessive loci (*Sle1*, *Sle2*, and *Sle3* found on chromosomes 1, 4, and 7, respectively) in NZB and NZW-related NZM2410 lupus-prone strain that are strongly associated with SLE-susceptibility (Morel et al., 1994). Congenic analysis of these susceptibility loci demonstrated that a component phenotype of SLE is displayed by each congenic strain carrying a single susceptibility locus on a resistant B6 genetic background, whereas triple congenic strains exhibit fatal lupus nephritis. These studies suggest that the genes in separate pathways can interact to increase or repress the initiation and progression of systemic autoimmunity.

The three congenic strains, B6.NZM*Sle1*, B6.NZM*Sle2*, and B6.NZM*Sle3*, were established by moving each susceptibility interval onto the C57BL/6 (B6) background; these strains were then used to characterize the primary phenotypes associated with the *Sle* loci (Morel et al., 1997). In the NZM2410 model, *Sle1* and *Sle3* are both associated with the production of autoreactive and activated T cells. However, the *Sle1* locus is specifically known to have the strongest association with lupus nephritis and plays a crucial role in the initiation of the breakdown of tolerance to chromatin by the production of antichromatin autoantibodies; without which neither the *Sle2*, *Sle3*, nor their combinations would result in any increased autoimmunity (Morel et al., 2000; Morel, 2010). This leads us to consider *Sle1* as a locus of primary importance in SLE pathogenesis and it is therefore important to be able to identify their corresponding genes.

Within the *Sle1* loci, the *Sle1a* sub-locus induces the production of activated autoreactive CD4 + T cells that help B cells to secrete the anti-chromatin IgG (Chen et al., 2005). The T regulatory (Treg) cells, a distinct population of the T cell hierarchy, maintain order in the immune system by enforcing a dominant negative regulation on other immune cells; they manifest their function through a number of mechanisms that include the secretion of immunosuppressive factors such as IL-10 and TGFβ (Sakaguchi et al., 2006). Tregs are generally associated with peripheral tolerance and act to prevent autoimmune disease by maintaining self tolerance. Defects in Tregs have been documented in SLE despite significant differences in their clinical phenotype and pathogenesis. Moreover, a reduction in Treg homeostasis has been directly linked to lupus pathogenesis in a mouse model (Humrich et al., 2010). The Sle1a sub-locus controls Treg number and function by multiple mechanisms, directly acting on the Tregs themselves and indirectly through the response of effector T cells and the regulatory role of dendritic cells (Cuda et al., 2007). It is the strong association that Sle1a.1 has with lupus nephritis in the NZM2410 mouse models that results in the increase of activated and autoreactive CD4 + T cells, as well as a decrease in the peripheral Tregs, which at the molecular level corresponds to an increase of a novel splice isoform of Pbx1 (pre-B-cell leukemia homeobox protein 1), Pbx1-d (Cuda et al., in press).

Previous work in our lab has established that *Pbx1* is a novel lupus susceptibility gene that affects T cell activation and tolerance, but its contribution to T cell lupus phenotypes is not known (Cuda et al., in press). PBXs are members of the

three-amino acid loop extension superclass of Homeobox proteins (Bürglin, 1997). The Pbx proteins form complexes with Hox factors, where Pbx1 acts as a cofactor in the transcriptional regulation during the patterning of embryonic tissue and the axial skeleton (Qin et al., 2004; Gordon et al., 2010). However, with the deletion of the gene it can result in severe skeleton malformation (Selleri et al., 2004; Gordon et al., 2010). Pbx2 or Pbx3 does not present a skeletal phenotype (Rhee et al., 2004; Selleri et al., 2004); hence, Pbx1 has a distinct role in skeletal development (Gordon et al., 2010). PBX1 amino acid sequence and exon structure are entirely conserved between both mice and humans (Sobel et al., 2011).

The novel splice isoform Pbx1-d is expressed more so in CD4 + T cells from either the lupus prone mice and SLE patients, and its over-expression leads to an increase in CD4 + T cell activation and a reduced Treg number and function. Peripheral Tregs are induced by TGFβ and further expanded by retinoic acid (RA). However, this process is defective in T cells from lupus-prone mice (Cuda et al., in press) and SLE patients (Sobel et al., 2011) that express Pbx1-d. This defective response to RA was verified in human Jurkat T cells transfected with PBX1-d (Cuda et al., in press). These results suggest an impaired integration of the TGFβ and RA signals in SLE T cells and implicate the PBX1 gene in this process (Qin et al., 2004; Sobel et al., 2011). Pbx1-d also induced apoptosis in Jurkat T cells (Cuda et al., in press). Shah et al. (2011) found that increased levels of CD4 + T cell apoptosis are positively associated with disease activity while decreased levels of percentage expression of CD4 + T lymphocytes were negatively associated with disease activity. Autoreactive CD4 + T cells specific for nuclear peptide antigens play an important role in tolerance breakdown (Bruns et al., 2000; Langer et al., 2007) during the course of SLE, which highlights the pivotal role of the balance between autoreactive CD4 T cells and Tregs in the dynamic course of human SLE. Strong evidence also shows that Tregs effectively counteract lupus autoreactivity and improve disease progression (Humrich et al., 2010). Therefore, Pbx1 regulates both production of autoreactive CD4 + T cells and size of the Treg compartment in the NZM2410 model. It also alters the responses of CD4 + T cells to RA.

The ultimate goal of these studies was to determine the mechanisms by which *Sle1a.1* alters CD4 + T cell function and to identify the gene responsible for these alterations. In summary, *Sle1a.1* results in impaired T cell homeostasis in response to RA. The expression of the PBX1-d that is strongly associated with SLE in both murine and human T cells impacts the homeostasis of memory T cells (Cuda et al., in press). This represents a novel mechanism of autoreactive T cell regulation that needs to be elucidated (Sobel et al., 2011) and therefore studies must be carried out focusing on the novel Pbx 1 isoform. *Pbx1-d* is over-expressed by the NZW strain, and is predicted to function as a dominant negative by binding Meis and Prep but not Hox and DNA. Further detailed studies are required to determine the molecular mechanisms and regulatory sequences in the PBX1 gene itself that lead to PBX1-d expression in lupus T cells (Cuda et al., in press). Through extensive research, it has been possible to study SLE pathogenesis, and more specifically autoreactive T cells, right down to the molecular level. There is still a long way to go in deciphering the specific roles of the genes associated with T cell activation, which will eventually give us a better insight into SLE pathogenesis.

REFERENCES

Bruns, A., Bläss, S., Hausdorf, G., Burmester, G.R., and Hiepe, F. (2000). Nucleosomes are major T and B cell autoantigens in systemic lupus erythematosus. Arthritis Rheum 43, 2307–2315.

Bürglin, T.R. (1997). Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. Nucleic Acids Res 25, 4173–4180.

Chen, Y., Cuda, C.M., and Morel, L. (2005). Genetic determination of T cell help in loss of tolerance to nuclear antigens. J Immunol 174, 7692–7702.

Cuda, C.M., Li, S., Liang, S., Yin, Y., Potula, H.H., Xu, Z., Sengupta, M., Chen, Y., Butfiloski, E., Barker, H., Chang, L., Dozmorov, I., Sobel, E.S. and Morel, L. Pre-B cell leukemia homeobox 1 is associated with lupus susceptibility in mice and humans. J Immunol. (In press)

Cuda, C.M., Wan, S., Sobel, E.S., Croker, B.P., and Morel, L. (2007). Murine lupus susceptibility locus Sle1a controls regulatory T cell number and function through multiple mechanisms. J Immunol 179, 7439–7447.

Gordon, J.A.R., Hassan, M.Q., Saini, S., Montecino, M., van Wijnen, A.J., Stein, G.S., Stein, J.L., and Lian, J.B. (2010). Pbx1 represses osteoblastogenesis by blocking Hoxa10-mediated recruitment of chromatin remodeling factors. Mol Cell Biol 30, 3531–3541.

Humrich, J.Y., Morbach, H., Undeutsch, R., Enghard, P., Rosenberger, S., Weigert, O., Kloke, L., Heimann, J., Gaber, T., Brandenburg, S., *et al.*(2010). Homeostatic imbalance of regulatory and effector T cells due to IL-2 deprivation amplifies murine lupus. Proc Natl Acad Sci U S A 107, 204–209.

La Cava, A. (2009). Lupus and T cells. Lupus 18, 196–201.

Langer, S., Langnickel, D., Enghard, P., Undeutsch, R., Burmester, G. R., Hiepe, F., Radbruch, A., and Riemekasten, G. (2007). The systemic and SmD183-119-autoantigenspecific cytokine memory of Th cells in SLE patients. Rheumatol 46, 238–245.

Morel, L. (2010) Genetics of SLE: Evidence from mouse models. Nat Rev Rheumatol. DOI: 10.1038/nrrheum.2010.63.

Morel, L., Croker, B.P., Blenman, K.R., Mohan, C., Huang, G., Gilkeson, G., and Wakeland, E.K. (2000). Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. Proc Natl Acad Sci U S A 97, 6670–6675.

Morel, L., Mohan, C., Yu, Y., Croker, B.P., Tian, N., Deng, A., and Wakeland, E.K. (1997). Functional dissection of systemic lupus erythematosus using congenic mouse strains. J Immunol 158, 6019–6028.

Morel, L., Rudofsky, U.H., Longmate, J.A., Schiffenbauer, J., and

Wakeland, E.K. (1994). Polygenic control of susceptibility to murine systemic lupus erythematosus. Immunity 1, 219–229.

Morel, L., and Wakeland, E.K. (1998). Susceptibility to lupus nephritis in the NZB/W model system. Curr Opin Immunol 10, 718–725.

Perry, D., Sang, A., Yin, Y., Zheng, Y.Y., and Morel, L. (2011). Murine models of systemic lupus erythematosus. J Biomed Biotechnol 2011, 271694.

Qin, P., Haberbusch, J.M., Zhang, Z., Soprano, K.J., and Soprano, D. R. (2004). Pre-B cell leukemia transcription factor (PBX) proteins are important mediators for retinoic acid-dependent endodermal and neuronal differentiation of mouse embryonal carcinoma P19 cells. J Biol Chem 279, 16263–16271.

Rhee, J.W., Arata, A., Selleri, L., Jacobs, Y., Arata, S., Onimaru, H., and Cleary, M.L. (2004). Pbx3 deficiency results in central hypoventilation. Am J Pathol 165, 1343–1350.

Sakaguchi, S., Setoguchi, R., Yagi, H., and Nomura, T. (2006). Naturally arising Foxp3-expressing CD25 + CD4 + regulatory T cells in self-tolerance and autoimmune disease. Curr Top Microbiol Immunol 305, 51–66.

Selleri, L., DiMartino, J., van Deursen, J., Brendolan, A., Sanyal, M., Boon, E., Capellini, T., Smith, K.S., Rhee, J., Pöpperl, H., *et al.* (2004). The TALE homeodomain protein Pbx2 is not essential for development and long-term survival. Mol Cell Biol 24, 5324–5331.

Shah, D., Aggarwal, A., Bhatnagar, A., Kiran, R., and Wanchu, A. (2011). Association between T lymphocyte sub-sets apoptosis and peripheral blood mononuclear cells oxidative stress in systemic lupus erythematosus. Free Radic Res 45, 559–567.

Shlomchik, M.J., Craft, J.E., and Mamula, M.J. (2001). From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol 1, 147–153.

Sobel, E.S., Brusko, T.M., Butfiloski, E.J., Hou, W., Li, S., Cuda, C.M., Abid, A.N., Reeves, W.H. and Morel, L. (2011) Defective response of CD4 + T cells to retinoic acid and TGF in systemic lupus erythematosus. Arthritis Res Ther 27, 13, R106.

Theofilopoulos, A.N. (1993). In: Systemic Lupus Erythematosus. Lahita, R. G. ed. New York: Churchill Livingstone. 121–194.

Tsao, B.P., and Grossman, J.M. (2001). Genetics and systemic lupus erythematosus. Curr Rheumatol Rep 3, 183–190.

Vyse, T.J., and Kotzin, B.L. (1998). Genetic susceptibility to systemic lupus erythematosus. Annu Rev Immunol 16, 261–292.

