limit of 10^{-18} M for the free concentration of copper in the cell (*8*). Assuming a cell volume of 10^{-14} liters, such a concentration corresponds to 10^{-33} mol or 10^{-9} atoms, leading to the conclusion that cellular pools of free copper are virtually nonexistent.

There is considerable interest in factors that control the concentrations of transition metal ions other than copper in the cell. Proteins have been discovered that facilitate the transport of manganese, iron, and zinc as well as copper across the yeast plasma membrane (*2*), and a bacterial metallochaperone that delivers nickel to the enzyme urease has been reported (*11*). Reliable estimates of the free concentrations of these other transition metal ions in cells are not yet available. The acquisition of such information would be greatly facilitated by the development of fluorescent intracellular metal ion sensors, such as those already available for calcium (*12*). Even if free pools of transition metal ions in cells do not exist, the possibility that they might occur in diseased or otherwise altered states of the cell could be determined if such sensors were at hand. Dysregulated transition metal ion concentrations in cells have been associated with several human diseases, including pediatric neurological disorders (manganese), hereditary hemochromatosis, Parkinson's disease, Friedreich's ataxia (iron), Menkes' syndrome, and Wilson's disease (copper) (*2*).

Although the new findings reveal that yCCS loads SOD1 with copper, several questions still need to be addressed. From what source does this metallochaperone receive its copper? What is the structure of yCCS and, in particular, what are the ligands that bind the copper atom(s)? What are the molecular hand-off mechanisms for transferring copper to and from the protein? Are there common strategies evolved by nature that might apply to other copper trafficking proteins in yeast, such as Atx1 and Cox17 (*9*), and to chaperones for the other transition metal ions? And, recalling that SOD1 contains zinc as well as copper, is there a metallochaperone that also delivers zinc to the enzyme? Designing experiments to answer these questions will ensure that this discipline of bioinorganic chemistry will remain a hotbed of research activity for a long time to come.

References

1. S. J. Lippard and J. M. Berg, *Principles of Bioinorganic Chemistry* (University Science Books, Mill Valley, CA, 1994).
2. D. Radisky and J. Kaplan, *J. Biol. Chem.* **274**, 4481 (1999).
3. R. A. Pufahl *et al.*, *Science* **278**, 853 (1997).
4. V. C. Culotta *et al.*, *J. Biol. Chem.* **272**, 23469 (1997).
5. R. L. B. Casareno, D. Waggoner, J. D. Gitlin, *ibid.* **273**, 23625 (1998).
6. J. D. Rothstein *et al.*, *J. Neurochem.* **72**, 422 (1999).
7. P. C. Wong, J. D. Rothstein, D. L. Price, *Curr. Opin. Neurobiol.* **8**, 791 (1998).
8. T. D. Rae, P. J. Schmidt, R. A. Pufahl, V. C. Culotta, T. V. O'Halloran, *Science* **284**, 805 (1999).
9. J. S. Valentine and E. B. Gralla, *ibid.* **278**, 817 (1997).
10. J. Hirose, T. Ohhira, H. Hirata, Y. Kidani, *Arch. Biochim. Biophys.* **218**, 179 (1982).
11. G. J. Colpas, T. G. Brayman, L.-J. Ming, R. P. Hausinger, *Biochemistry* **38**, 4078 (1999).
12. R. Y. Tsien and A. Miyawaki, *Science* **280**, 1954 (1998).

PERSPECTIVES: IMMUNOLOGY

A New Look at MHC and Autoimmune Disease

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A strong genetic association exists between certain autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, and insulin-dependent diabetes mellitus (IDDM), and the expression of certain kinds (haplotypes) of major histocompatibility complexes (MHCs) (genes encoding cell-surface molecules that display peptides for immune recognition) (*1*). The current explanation for this association proposes that disease-associated MHC molecules efficiently bind autoantigens involved in the pathophysiology of the disease. This results in a peripheral T cell–mediated immune response to the autoantigen and autoimmune sequelae.

Enhanced online at www.sciencemag.org/cgi/content/full/284/5415/749

Recent results in an animal model of autoimmune diabetes, the nonobese diabetic (NOD) mouse, however, suggest a new hypothesis to explain the role of MHC in autoimmunity. This hypothesis proposes that the genetic association between MHC and autoimmune disease results from "altered" thymic selection in which high-affinity self-reactive (potentially autoreactive) T cells escape selection in the thymus as a result of the poor self peptide–binding properties of the disease-associated MHC class II molecules (*2*). This model offers an explanation for the unusual requirement of homozygous MHC class II expression in human IDDM and IDDM in NOD mice.

MHC molecules bind peptides for presentation to antigen-specific T cell receptors (TCRs) on T lymphocytes. The TCRs recognize MHC-peptide complexes on the surface of antigen-presenting cells (APCs). There are two major roles for MHC class II gene products: (i) selection of the T cell repertoire in the thymus, and (ii) presentation of foreign antigens in the periphery. By processing and presenting self peptides bound to MHC molecules to developing thymocytes, thymic APCs first select the potential peripheral T cell repertoire (positive selection) and then purge this positively selected repertoire of T cells that react too strongly to self peptide–MHC complexes (negative selection). Less than 1% of precursor T cells entering the thymus survive this selection (*3*). Subsequently, by presenting foreign peptides to the peripheral T cells that have survived thymic selection, peripheral APCs—expressing the same MHC molecules as their thymic counterparts—initiate protective immune responses. This "dual role" of MHC class II molecules has contributed to our difficulty in deciphering their role in autoimmune disease, because the response (or lack of response) of the peripheral T cell population to any antigen is determined both by MHC-mediated thymic selection events and by the capacity of the MHC molecules to bind and present foreign antigen to peripheral T cells. It is generally believed that some autoimmune diseases are caused by "pathologic" T cells, which "inappropriately" recognize and respond to self peptide–MHC complexes, possibly after activation by foreign peptides that closely resemble self peptides (molecular mimics). But it has been difficult to determine whether the MHC association with autoimmune disease lies in the thymus or periphery, or both. Another complexity is that autoimmune diseases are multigenic. Thus, the MHC acts in conjunction with many other genes, none of which are necessary or sufficient, to produce autoimmunity.

Studies in the NOD model of autoimmune diabetes have begun to solve the puzzle. NOD mice spontaneously develop an autoimmune syndrome in which autoreactive $CD4^+$ T cells infiltrate multiple organs, including the pancreas. Autoreactive T cells in NOD mice recognize multiple pancreatic and nonpancreatic self peptides and can transfer at least three distinct autoimmune syndromes to naïve recipients (*4–7*). Self-tolerance of NOD mice can be "broken" by immunization with self peptides (*8*). The immunized NOD mice develop

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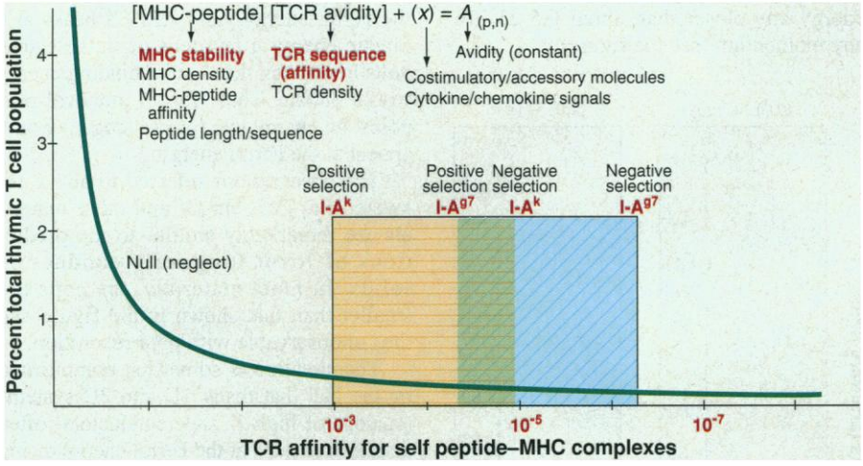
autoreactive CD4+ MHC class II restricted T cells that recognize endogenously processed and presented self peptides, identical to the self peptides used for immunization. However, NOD mice demonstrated normal T cell responses after immunization with foreign antigens. This suggests that there is not a generalized hyperresponsive T cell population in the mice; rather, NOD T cells responded inappropriately to self antigens in the periphery (*8*). The bias of the NOD peripheral T cell repertoire toward autoreactivity after immunization with self peptides, while maintaining normal responses to foreign antigens, suggested that the efficient binder of antigens, I-A^{g7} was found to be a poor peptide binder, and to have structural instability (*13*).

These studies suggest that, in an avidity model of thymic selection—which hypothesizes that the strength (avidity) of a signal transmitted to pre-T cells is the summation of multiple inputs (see the figure) (*14–16*)—unstable I-A^{g7} could produce an effectively decreased density of MHC-peptide complexes on thymic APCs, which would select a population of T cells with increased TCR affinity to attain the avidity threshold required in thymic positive selection (see the figure). These high-affinity population with an increased mean affinity for self peptide–MHC. The resulting increased strength of signal generated by these "high-affinity TCRs" after activation by nonself and subsequent encounter with self antigen, may explain the broad range of T cell autoreactivity in these mice.



An avidity model for thymic selection. The components in color are operative in NOD mice. This model assumes that the avidity necessary to attain positive and negative thymocyte selection thresholds [$A_{(p,n)}$] is constant among mouse strains, reflecting intrinsic thymocyte signaling. Additive contributions to the avidity (x) might differ between NOD and other mouse strains, but are not critical in NOD repertoire selection (see text). Given these constraints, a global decrease in [I-A^{g7} MHC-peptide] stability would necessitate a compensatory increase in the mean population TCR affinity to attain the constant avidity thresholds of positive and negative selection. The result would be a NOD peripheral T cell repertoire biased toward TCR sequences with greater intrinsic affinity for self MHC–peptide. An MHC molecule without global biochemical defects could bind a limited number of self peptides poorly, producing a limited set of higher affinity T cells (*24*).

MHC effect might occur by thymic selection of the T cell repertoire.

Diabetic mice and humans share homozygous expression of MHC class II B chain molecules (*9*). The incidence of diabetes, is lower in heterozygotes (expressing only one copy of the disease associated MHC gene) (*10–12*). Although more than 80% of female NOD mice developed diabetes, only 1 to 3% of NOD F_1 mice developed disease (*12*). How could a twofold reduction in cell surface expression of the class II protein result in a 30-fold (or greater) decrease in disease incidence? Unanue *et al.* approached this question by investigating the biochemical characteristics of the NOD class II molecule, I-A^{g7}. This analysis demonstrated the exact opposite peptide-binding characteristics to those predicted by the good peptide–binding model of MHC. Rather than being an self-reactive T cells would enter the periphery and, in collaboration with multiple other disease-related genes, mediate autoimmunity once an inflammatory event broke self-tolerance. Recent results from several labs support this hypothesis. Kanagawa *et al.* found that I-A^{g7} was associated with quantitatively increased T cell autoreactivity in the peripheral T cell population (*17*). We demonstrated that I-A^{g7} allowed thymic selection of autoreactive T cells, whereas imposition of a single copy of a second, conventional, MHC class II molecule, I-A^{k} (with all other non-MHC NOD genes held constant), eliminated these autoreactive T cells from the periphery, thus explaining the effect of MHC heterozygosity on decreased frequency of IDDM as the result of T cell thymic-selection events (*2*, *18*, *19*). These results suggest that I-A^{g7}acts in the thymus of NOD mice to allow selection of a T cell

This model of defective thymic selection by homozygous NOD I-A^{g7} mice is similar to results obtained in recent knockout and transgenic mouse models. Laufer *et al.* expressed MHC class II exclusively in the thymic cortical epithelium of MHC class II knockout mice, and demonstrated large numbers (up to 5% of total lymphocytes) of self-reactive T cells, representing unopposed positive selection (*20*). Several groups made mice deficient in H2-M, which loads a diverse peptide repertoire into MHC class II molecules. The T cells from these mice underwent positive selection, but demonstrated autoproliferative-like responses to wild-type APCs, again suggesting defective negative selection in the setting of efficient positive selection (*21–23*).

Thus, the association of MHC with autoimmunity may be ascribed to thymic selection of the peripheral TCR repertoire, facilitating the study of other genes required in the pathologic processes of autoimmune inflammation.

References and Notes

1. A. Svejgaard, P. Platz, L. P. Ryder, *Immunol. Rev.* **70**, 193 (1983).
2. W. M. Ridgway, H. Ito, M. Fassò, C. Yu, C. G. Fathman, *J. Exp. Med.* **188**, 2267 (1998)
3. S. C. Jameson, K. A. Hogquist, M. J. Bevan, *Annu. Rev. Immunol.* **13**, 93 (1995).
4. R. Tisch *et al.*, *Nature* **366**, 72 (1993).
5. D. L. Kaufman *et al.*, *ibid.*, p. 69.
6. M. G. Humphreys-Beher, Y. Hu, Y. Nakagawa, P. L. Wang, K. R. Purushotham, *Adv. Exp. Med. Biol.* **350**, 631 (1994).
7. S. Costagliola, M. C. Many, M. Stalmans-Falys, G. Vassart, M. Ludgate, *Endocrinology* **137**, 4637 (1996).
8. W. M. Ridgway, M. Fassò, A. Lanctot, C. Garvey, C. G. Fathman, *J. Exp. Med.* **183**, 1657 (1996).
9. H. Acha-Orbea and H. O. McDevitt, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2435 (1987).
10. S. Makino, Y. Muraoka, Y. Kishimoto, Y. Hayashi, *Jikken Dobutsu* **34**, 425 (1985).
11. M. Prochazka, E. H. Leiter, D. V. Serreze, D. L. Coleman, *Science* **237**, 286 (1987).
12. L. S. Wicker *et al.*, *J. Exp. Med.* **165**, 1639 (1987).
13. E. Carrasco-Marin, J. Shimizu, O. Kanagawa, E. R. Unanue, *J. Immunol.* **156**, 450 (1996).
14. P. G. Ashton-Rickardt *et al.*, *Cell* **76**, 651 (1994).
15. E. Sebzda *et al.*, *Science* **263**, 1615 (1994).
16. D. T. Kim, J. B. Rothbard, D. D. Bloom, C. G. Fathman, *J. Immunol.* **156**, 2737 (1996).
17. O. Kanagawa, S. M. Martin, B. A. Vaupel, E. Carrasco-Marin, E. R. Unanue, *Proc. Natl. Acad. Sc.i U.S.A.* **95**, 1721 (1998).
18. W. M. Ridgway and C. G. Fathman, *Clin. Immunol. Immunopathol.* **86**, 3 (1998).
19. W. M. Ridgway, H. Ito, M. Fassò, C. Yu, C.G. Fathman, *Can. J. Diab. Care Suppl.* **3**, 6 (1998).
20. T. M. Laufer, J. DeKoning, J. S. Markowitz, D. Lo, L. H. Glimcher, *Nature* **383**, 81 (1996).
21. W. P. Fung-Leung *et al.*, *Science* **271**, 1278 (1996).
22. W. D. Martin *et al.*, *Cell* **84**, 543 (1996).
23. T. Miyazaki *et al.*, *ibid.*, p. 531.
24. G. Y. Liu *et al.*, *Immunity* **3**, 407 (1995).
25. We thank J. Rothbard, D. Kim, L. Wicker, K. Lejon, A. Ridgway, C. Taylor, and R. Kizer for secretarial assistance.