Commentary

HLA Class II Transgenic Mice: The Chance to Unravel the Basis of HLA Class II Associations with Disease

By Benjamin D. Schwartz

From the Department of Immunology, Searle/Monsanto Company, St. Louis, Missouri 63198

Though the first association between HLA and disease was established with an HLA class I molecule (1), the majority of HLA-disease associations have been with HLA class II molecules (2). Despite the awareness of such associations for nearly two decades, the pathophysiological role of HLA class II molecules in conferring susceptibility to the associated diseases remains poorly understood. One impediment to enhancing our understanding has been the inability to study the function of human class II molecules in animal models where experimental manipulations can be readily accomplished.

Two papers in this issue of *The Journal of Experimental Medicine*, by Woods et al. (3) and Yamamoto et al. (4), now document the successful construction of transgenic mice bearing chimeric or whole human DR and DQ molecules, and further demonstrate that murine T cells are capable of recognizing antigenic peptides presented by human class II molecules. In addition, the results obtained by Yamamoto et al. (4) dispel the notion that murine CD4 on murine T cells cannot function as an adequate accessory molecule during peptide presentation by human class II molecules. This concept has undoubtedly slowed progress in this much needed area.

T lymphocyte activation is initiated through recognition of a peptide-MHC molecule ligand residing on the antigen-presenting cell by the antigen-specific receptor residing on the T lymphocyte. However, optimization of the activation is achieved through a series of accessory and adhesion molecules, among which is the CD4 molecule (5). CD4 was initially identified on the surface of a subset of T lymphocytes by monoclonal antibodies, and its presence was used to study the functions of this subset. Antibodies against the 55-kilodalton CD4 glycoprotein were demonstrated to block the majority of helper T cell functions, including mixed lymphocyte reactions and induction of helper activity (6–8). These observations suggested that CD4 was therefore a marker for the helper T cell population. However, several laboratories subsequently demonstrated that the subset of cytotoxic T cells that recognized class II molecules bore CD4 (9, 10) and that monoclonal antibodies to CD4 could block the function of any T cells specific for class II molecules (11). These findings led to the currently held concept that CD4 positive T cells possess receptors that recognize peptide in the context of class II molecules, irrespective of their functions.

CD4 was demonstrated to interact with class II molecules both by functional studies and direct binding (12, 13). The two membrane-distal extracellular domains of CD4 (14, 15) bind to the β_2 domain of class II molecules (16–18) on antigen-presenting cells, whereas the intracellular domain mediates signaling together with the T cell receptor/CD3 complex on the T cell. The CD4 molecule thus is associated simultaneously with both constituents of the T cell receptor–class II molecule recognition unit involved in antigen presentation. This association leads to activation of the $p56^{lck}$ kinase and the initiation of the signaling cascade within the T cell (19–21).

Because of the critical role played by CD4 in the process of T cell activation, the possibility that a species barrier might exist between CD4 and class II raised serious concerns regarding the usefulness of transgenic mice bearing human class II molecules as models for studying HLA class II–related diseases. The studies investigating the interaction of CD4 and class II molecules across species have yielded conflicting results to date. Lamarre et al. (15) suggested that murine CD4 probably did not recognize HLA-DP, and Vignali et al. (16) demonstrated that mouse CD4 T cells reacted less well with antigen-presenting cells bearing an I-A molecule where the native β_2 domain was substituted with DRβ2. In contrast, von Hoegen et al. (22) showed that murine CD4 loss variants, transfected with either human or mouse CD4, gave functionally comparable results. Results in vivo in transgenic mice have not resolved the issue. Single DRα transgenic mice, in which the DRα chain associates with the I-Eβ chain, function normally in their ability to delete T cell receptor Vβ11 T cells (23). This result is not surprising in light of the site of interaction of CD4 with the murine I-E β_2 domain. More surprisingly, single DQβ chain transgenics were also shown capable of normally depleting Vβ11 cells, suggesting an effective interaction between human HLA-DQ β_2 domains and murine CD4 (24). In contrast, in double DQ α/β transfectants, in which the transgenic DQ molecule acted as a restricting element, anti-mouse class II antibodies which were shown not to recognize the DQ molecule, nonetheless inhibited the T cell response to DQ-presented peptides. These observations suggested that the murine CD4 might be interacting with murine class II molecules on the antigen-presenting cells rather than with the DQβ chain itself (25). Furthermore, in transgenic mice bearing human CD4, xenogeneic T cell responses to human class II molecules on exogenous cells were enhanced up to 10-fold over those of nontransgenic mice, whereas the response to allogeneic mu-

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rine class II molecules was only minimally elevated. However, even in this situation, responses to human class II molecules remained lower than responses to murine class II allogeneic molecules, suggesting that optimal T cell responses require the CD4 and class II molecule to be of the same species (26).

The aforementioned papers (24–26) raised the additional issue of whether the T cell receptors on T cells from transgenic mice bearing human class II molecules can effectively recognize those human class II molecules as antigen-presenting elements. This question was partially answered in the affirmative for DQ molecules in which DQ α/β double transgenics were shown capable of presenting streptococcal cell wall antigen to autologous T cells (25). However, data regarding the ability of DR molecules to function in a transgenic model have been lacking.

This situation has now been remedied as evidenced by the two papers by Woods et al. (3) and Yamamoto et al. (4). Woods et al. (3) have attempted to circumvent the unresolved question of cross-species interaction of murine CD4 and human DR molecules by constructing chimeric DR/I-Eα and DR/I-Eβ chains in which the α_1 and β_1 domains are from DR molecules, and the α_2 and β_2 domains are derived from I-E molecules. Thus, the murine CD4 should have optimal interaction with the β_2 domain. Using this system, these investigators demonstrated that the DR molecules influenced the T cell repertoire by depleting Vβ11-bearing T cells, and can act as restricting elements, presenting both synthetic peptides and peptides derived from processed protein antigens to antigen-reactive T cells. Thus, clearly, the murine T cell repertoire is capable of recognizing DR molecules, and can be molded to positively select such a population of T cells.

In the second paper, Yamamoto et al. (4) have attacked the question of cross-species interaction directly, and constructed a transgenic mouse expressing murine CD4 and transgenic DR molecules. Here, too, the authors were able to demonstrate the flexibility of the murine T cell receptor repertoire, and the selection of T cells bearing receptors capable of recognizing DR-presented antigens. They were further able to show that anti-murine CD4 antibodies could inhibit these responses. This result clearly indicates that the interaction between murine CD4 and human class II molecules is sufficient to support a T cell response.

Whereas both papers present impressive results that will undoubtedly influence the construction of other HLA class II transgenics, they unfortunately both beg the initial question of whether a species difference between the CD4 and class II molecules in vivo diminishes the resulting immune response. Comparison of the T cell responses in a transgenic mouse bearing DR molecules versus those T cell responses in a transgenic mouse bearing chimeric DR molecules should address the question of whether the T cell response varies with the species origin of the β_2 domain. In addition, the testing of a DR, human CD4 transgenic, murine CD4 knockout (Karr, R., X.-T. Fu, J. Strauss-Schoenberger, and J. Goelner, manuscript in preparation) will elucidate the requirements for optimal T cell responses.

With the possibility now at hand of constructing SCID mice bearing human class II transgenes, it might also be feasible to introduce human T cells from the blood or affected tissues of diseased patients into mice. A SCID/hu transgenic system of this kind has the potential to reproduce human disease in a mouse model, and to allow much needed insights into the pathophysiologic role of human class II molecules in autoimmune disease.

Nonetheless, the demonstration that human class II molecules can function as antigen-presenting molecules in vivo in a transgenic murine system paves the way to address a number of questions. Perhaps most significantly, the availability of such mice will allow the exploration of the basis for the predisposition of HLA class II molecules to particular autoimmune diseases.

Address correspondence to Dr. Benjamin D. Schwartz, Department of Immunology, Searle/Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198.

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