phenotyping on NOD splenocytes with a directly conjugated MHC class I MAb SF1-1.1 (PharMingen, San Diego, California), and demonstrated decreased background cross-reactivity with negative C57BL/6 splenocytes. Consistent with standard immunological procedures, their data show that the high background immunofluorescence of mouse splenocytes, observed with the use of FITC-conjugated goat antibodies to mouse immunoglobulin, can be eliminated with MAb SF1-1.1. When we used directly conjugated MAb SF1-1.1, we observed MHC class I data identical to that obtained by Wicker et al. As expected, we also observed decreased cross-reactivity with C57BL/6 splenocytes. Nevertheless, we observed a diminished MHC class I density with both techniques in the NOD mouse.

In humans with diabetes, lymphocytes show consistent definitive expression of MHC class I antigens. However, in NOD mice splenocytes, different antibodies to MHC class I antigens show varying degrees of defective MHC class I expression. The 9008-A antibody to H-2k8, which binds to the epitope binding region of the K8 molecule, has shown a more striking defect in MHC class I expression in NOD splenocytes than has MAb SF1-1.1 (Fig. 2A).

We prospectively monitored 201 females in our colony of NOD mice with a high incidence of diabetes from 4 to 6 weeks of age with 9008-A antibody to test the predictive power of this change in MHC class I reactivity with regard to disease penetrance. We found by flow cytometric analysis of 169 mice that MHC class I density decreased on peripheral blood lymphocytes, as reported by Gaskins et al. (Fig. 2A); the remaining 32 mice showed MHC class I expression patterns identical to those of control BALB/c mice (Fig. 2B). By 9 months of age, 100% of the 169 mice that showed modified MHC class I expression developed severe hyperglycemia and died. At 18 months of age, the 32 mice with normal lymphocyte cell surface expression of MHC class I remained normoglycemic. These results are similar to our data for human identical twins discordant for type I diabetes (1). Therefore, diminished expression of MHC class I antigens observed on the surface of peripheral lymphocytes is predictive of the progression to hyperglycemia in genetically susceptible populations of both humans and mice.

Another important technical point is that exposure of murine splenocytes to serum-containing media at room temperature
Table 1. MHC class I expression on splenic lymphocyte and macrophage cell subpopulations. MAD, mean antigen density (or mean channel fluorescence), is the density of MHC class I determinants. NOD, BALB/c, and C57BL/6 female mice were 8 to 12 weeks old. MAb to H-2K^d was purified and directly conjugated to fluorescein isothiocyanate (FITC) (CL9008-A, Accurate Chemical). It recognizes cells bearing the H-2K^d (H-2.31) antigen (Accurate Chemical). MAb H-2D^d was also purified and directly conjugated to FITC (CL9002-A, Accurate Chemical). It recognizes H-2.6^b (3). Macrophages were identified with MAb M1170.15, which reacts with polypeptides of 190 and 105 kD, which are found exclusively on macrophages. MAb M1170.15 was conjugated to phycoerythrin for two-color immunofluorescence (Coulter, Hialeah, Florida). Mouse lymphocytes were identified as cells expressing CD4-1R1 or CD8-1R1 (PharMingen). Background immunofluorescence was less than 5% for all antibodies on lymphocytes from different mice.

<table>
<thead>
<tr>
<th>Mouse strains</th>
<th>H-2K^d (CL9008-A)</th>
<th>H-2D^d (CL9002-A)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>Macrophages</td>
</tr>
<tr>
<td>BALB/c</td>
<td>99 (2.7)</td>
<td>98 (31)</td>
</tr>
<tr>
<td>NOD</td>
<td>44 (1.6)</td>
<td>93 (13.5)</td>
</tr>
<tr>
<td>BALB/c</td>
<td>97 (5.7)</td>
<td>98 (40)</td>
</tr>
<tr>
<td>NOD</td>
<td>46 (3.1)</td>
<td>94 (21)</td>
</tr>
<tr>
<td>BALB/c</td>
<td>98 (6.9)</td>
<td>100 (50)</td>
</tr>
<tr>
<td>NOD</td>
<td>66 (3.0)</td>
<td>99 (34)</td>
</tr>
</tbody>
</table>

for 3 to 5 hours before flow cytometer analysis partially corrects the defective pattern of MHC class I expression (Fig. 2C). This finding is consistent with the recent work of Ljunggren et al., which shows that the labile peptide-empty MHC class I on mutant murine lymphoma cells can be stabilized by reduced temperatures (2). Although diminished MHC class I expression in NOD mice is evident with all MHC class I antibodies tested, the apparent magnitude of the decrease varies depending on the specific MHC class I antibody used. Defective MHC class I expression is observed whether measured indirectly, with a second antibody, or with a direct assay with a fluorescent-conjugated primary antibody. Incubation of the NOD lymphocytes in serum for 3 to 5 hours at room temperature diminishes the magnitude of the defect in MHC class I expression.

With MAb SF1-1.1, Wicker et al. found MHC class I expression to be decreased in T cells but normal in B cells. However, MAb SF1-1.1 appears to be an exception; the majority of antibodies to H-2K^d or H-2D^d reveal decreased MHC class I expression on NOD antigen-presenting cells. Antibody 9008-A to H-2K^d and antibody 9002-A to H-2D^d reveal decreased MHC class I expression in T cells and in antigen-presenting cells from NOD mice (Table 1). These data then confirm our earlier conclusion: The lymphocytes of the NOD mouse have a defect in MHC class I expression.

We have also reported data (1) which suggest that this defective MHC class I expression may be controlled by a series of MHC class II–linked transporter genes. The transporters encoded by these genes regulate the delivery of cytoplasmically processed peptides into the endoplasmic reticulum for combination with MHC class I molecules. To generate these data, we rapidly prepared mRNA from NOD splenocytes and used the human RING-4 transporter probe to measure mRNA for murine MHC class II–linked transporter genes. In all instances, mRNA prepared from NOD mice whose lymphocytes showed decreased MHC class I expression also showed decreased transporter mRNA concentrations (1).

With the availability of murine probes to the two MHC class II–linked transporter genes, Ham-1 (the murine homolog of Ring-4) and Ham-2, we have found that the low transporter mRNA concentrations in NOD splenocytes reflect decreased expression of Ham-1, while Ham-2 mRNA concentrations are normal (Fig. 3A). Furthermore, Ham-1 mRNA concentrations can be rapidly restored to near-normal values by exposing splenocytes to 10% serum at room temperature for 4 to 5 hours before mRNA preparation (Fig. 3B).

Because the defective MHC class I phenotype is predictive of progression to hyperglycemia, it might therefore be expected that NOD mice with normal MHC class I surface expression that do not develop diabetes would also show normal expression of Ham-1 mRNA. Indeed, we have found that such mice have normal Ham-1 mRNA concentrations and NOD mice with decreased MHC class I expression have low Ham-1 mRNA concentrations.

Gaskins et al. conclude that macrophages from NOD mice have normal Ham-1 mRNA concentrations. However, with their methods, one would expect macrophages isolated from immunologically stimulated NOD mice and cultured in the presence of serum to show normal Ham-1 mRNA. A perplexing variety of immune stimulation protocols prevents diabetes in the NOD mouse (3–6). Future studies are sure to be directed at restoration of MHC class I expression or Ham-1 induction at a mechanism for successful treatment.

Gaskins et al. appear to confirm our NOD Southern analysis data and to demonstrate the previously described (1) Xba I DNA site of the Ham-1 (Ring-4) transporter locus. In addition, they expand our data and demonstrate that this polymorphism is rare but not unique and that this mutation does not affect the coding region.

Therefore, the NOD mouse, like humans with type 1 diabetes, shows defective MHC class I expression in lymphocytes, including antigen-presenting cells in vivo. This phenotype is correlated with low concentrations in vivo of Ham-1 mRNA and diabetes progression. We have hypothesized (1) that MHC class II–linked transporter loci control the delivery of endogenous peptides into the endoplasmic reticulum, which presumably allows correct folding of MHC class I molecules and sub-sequent expression on the cell surface. Interruption of this pathway appears to be associated with disease penetrance.

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REFERENCES AND NOTES

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